THESIS

SYNTHETIC HEXAPLOID WHEAT CONTRIBUTES FAVORABLE ALLELES FOR YIELD AND YIELD COMPONENTS IN AN ADVANCED BACKCROSS WINTER WHEAT POPULATION

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ABSTRACT

SYNTHETIC HEXAPLOID WHEAT CONTRIBUTES FAVORABLE ALLELES FOR YIELD AND YIELD COMPONENTS IN AN ADVANCED BACKCROSS WINTER WHEAT POPULATION

Wheat (*Triticum aestivum* L.) is an important crop in the Great Plains region, contributing to the global food supply as well as providing economic security. However, drought is a major abiotic stress that affects wheat production in this region as well as many other parts of the world. New sources of drought tolerance will supplement ongoing efforts to increase yield under water-limited conditions. The International Maize and Wheat Improvement Center (CIMMYT) devotes much of its effort toward the development and identification of promising lines with improved drought tolerance and other important agronomic traits. Although mostly targeted to the developing world, these lines also have the potential to be useful in the Great Plains. In addition, identifying quantitative trait loci (QTL) controlling yield and yield components under irrigated and partially irrigated conditions can aid in the development of breeding lines and cultivars improved for these traits. Our main objective was to identify QTL affecting yield and other agronomic traits under moisture stress and non-stress conditions using an advanced backcross QTL strategy.

A population of 188 BC_2F_2 and BC_2F_2-derived lines developed from a cross between the Colorado winter wheat cultivar 'Ankor' (recurrent parent) and the CIMMYT synthetic-derived spring wheat line 'Sokoll' was grown in replicated field trials in Fort Collins (2007 and 2008) and Greeley (2009) Colorado. The 22 highest and 22 lowest yielding lines from 2007 to 2009 were grown in replicated field trials in Burlington, Julesburg, and Dailey, Colorado (2010). The eight highest and eight lowest yielding lines from 2010 were grown in replicated field trials in Fort Collins (2011). At the Fort Collins and Greeley locations (except 2007), side-by-side trials ("environments") were planted; one trial was grown under moderate moisture stress ("dry") and one under fully irrigated ("wet") conditions, for a total of two environments per location per year. At the Burlington, Julesburg, and Dailey locations, three replications of a smaller number of lines were grown under rainfed ("dry") conditions with no irrigation. Nine to 11 traits were evaluated under both irrigation treatments from 2007 to 2009: grain yield, test weight, days to heading, days to physiological maturity, grain fill duration, kernel number per spike, average kernel weight, plant height, spike length, and total kernel and spike number per square meter. An additional seven to 11 agronomic traits comprising plant biomass, spike: stem ratio, harvest index, and spikelet number were also evaluated in the 2010 and 2011 environments.

Moisture stress affected grain yield in side-by-side wet and dry treatments. The 2008 dry trial in Fort Collins experienced a 14.7% reduction in grain yield, the 2009 Greeley field trials experienced a 23.2% yield reduction, and the 2011 dry trial experienced an 18.1% yield reduction. Grain yield, biomass, and kernel characteristics (average kernel number, total spike number, and total kernel number) generally had

higher mean values under wet compared to dry treatments in the different growing seasons. Test weight and average kernel weight had higher values in the wet treatments in two of three locations where there were side-by-side treatments, and spike: stem ratios had higher mean values under the dry than the wet treatment in 2011. A range of moderate to high heritability estimates for grain yield, test weight, and days to heading indicates much of the expression of these traits is genetically controlled. Heritability estimates were low to moderate for grain fill duration, plant height, and average kernel weight, indicating that environmental variation for these traits was more important than genetic variation. Heritability values were generally higher in wet than dry environments. Pearson correlation coefficients revealed positive and consistent correlations between grain yield and important yield components. Grain yield was correlated with total kernel number per m^2 in nine of 10 environments (r=0.55 to 0.97, $P \leq 0.05$) and with total spike number per m^2 for seven of 10 environments from 2007 to 2011(r=0.35 to 0.74, $P \leq 0.05$). Grain yield was positively correlated, but at a lower magnitude, with average kernel weight only in eight of 10 environments (r=0.24 to 0.57, $P \leq 0.05$).

Forty-one linkage groups spanning 1414.4 cM and covering parts of all 21 chromosomes were constructed from 141 microsatellite markers and three gene-specific markers (*Ppd-D1*, *Glu-A1*, and *Glu-D1*) in an F_2 mapping population developed from the same cross. Seventy-six of these markers were genotyped in the $BC_2F_{2:3}$ lines. The population varied for the proportion of lines carrying Sokoll alleles at these markers, from 10.0 to 47.1%, with an average of 24.7%. Single-factor QTL analysis was used to detect significant ($P \leq 0.01$) markers, the size of their effects, and the source of favorable

alleles. A total of 88 QTL were identified in the BC_2F_2-derived population. Individually, the QTL explained from 3.6 to 14.6% of the phenotypic variation. Sokoll contributed the favorable allele for 34 (38.6%) QTL (mostly on the A and B genomes), while Ankor contributed the favorable allele for 54 (61.4%) QTL. The Sokoll allele added a 235 kg/ha phenotypic effect at a QTL for grain yield located on chromosome 6B. Regions on chromosomes 1A, 1B, 2A, 2B, 2D, 3A, 4B, 5A, 5B, 5D, 7A, and 7B contained QTL for multiple traits. This co-localization of QTL for multiple traits suggests that the effects may be due to pleiotropy.

Distribution of QTL was unbalanced between irrigation treatments; 68 QTL (71.6%) were detected under full irrigation and 27 QTL (28.4%) were identified under limited irrigation. In general, QTL for most of the traits were detected in both soil moisture levels. While some QTL were detected in two to three environments, most QTL were detected only in one environment. Other markers (e.g. *Xgwm484*, *Xgwm499*, and *Xbarc108*) were consistently detected for multiple traits and under both fully and partially irrigated treatments, indicating broad adaptability and potential use of these markers marker-assisted breeding.

Mean performance of high versus low yielding lines was compared for all traits in 2010 and 2011, when the lines were grown in larger plots with more accurate yield estimates. There were significant ($P \leq 0.05$) differences between high and low lines for traits such as grain yield, test weight, and total spike number. However, there was not a significant separation in high and low means for other traits such as plant height, spike length, biomass, and spike:stem ratio. Traits with differences between high and low lines help explain the basis of yield differences in this population. The hypothesis that

biomass was a good predictor of yield was confirmed in the Dailey 2010 and Fort Collins 2011 dry and wet environments, and the hypothesis that the spike: stem ratio was a good predictor of yield was rejected in all environments and at multiple growth stages in 2010 and 2011.

The 88 QTL from the 2007-09 environments were tested with data from the 2010 and 2011. This process confirmed 28 of 88 QTL for multiple traits. The top five highest-yielding lines were also selected for their potential use in crossing and in marker- assisted breeding in winter wheat breeding programs. Line 420 was identified as the best yielding line of the 2010 and 2011 environments.

Our study indicates synthetic-derived wheat can contribute favorable alleles for yield and other agronomic traits in the Great Plains and thus offers promise for use in breeding programs.

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CHAPTER 1

LITERATURE REVIEW

The importance of wheat

Bread wheat (*Triticum aestivum*) is a globally important grain because it is a key ingredient in foods such as bread, noodles, and tortillas. Over 200 million hectares (ha) of wheat are cultivated worldwide (Reynolds et al., 2010). Wheat is the third most-produced cereal grain, and in 2007, world production was 682 million tons (FAO, 2009). It is important to improve the productivity of wheat because global demand is increasing and many regions around the world such as North Africa, the Middle East, and the Indian subcontinent depend on wheat as a major crop for survival (FAO, 2009). However, recent crop research has focused more on maize (*Zea mays*) and rice (*Oryza sativa*), whose genomes have been completely sequenced. Rice is a model genetic system in part due to its relatively small diploid genome (0.4 Gb) (Eckardt, 2008). Wheat, on the other hand, has a large, complex genome, consisting of an estimated 17 Gb and three related genomes (A, B, and D) (Eckardt, 2008). Sequencing of the wheat genome has begun, but is not complete (National Association of Wheat Growers et al., 2009).

Wheat and drought stress

The genetic understanding and improvement of wheat will be increasingly important as the world population continues to grow and if it is to meet world demands by 2050 (FAO, 2009). Wheat must be better able to combat environmental factors such as drought, salinity, and frost each year to reduce the 25% annual production loss due to these stresses (Gill et al., 2004). Drought, in particular, affects both wheat growth and yield (Ashraf et al., 1995 and Ashraf, 1998). A decrease in yield and yield components under drought stress has been reported in both durum and bread wheat (Sinha et al., 1986). Therefore, targeting environmental factors such as drought stress will be necessary as water supplies dwindle and will likely improve productivity (Alston, 2009). Drought stress related yield loss in wheat is complex because it is affected by many factors (Reynolds et al., 2006). Identifying chromosomal regions associated with drought tolerance in wheat will improve understanding of the genetic basis of drought tolerance, lead to the development of more stress-tolerant cultivars, and reduce production losses.

Whole plant responses to drought stress

During slow, long-term water deficit, plants first experience a loss of turgor pressur. Plants respond by decreasing leaf expansion and thus leaf area. A decrease in yield and yield components under drought stress has been associated with a decrease in photosynthate production (Zaharieva, 2001) and/or a decrease in photosynthate mobilization to the spike (Rebetzke et al., 2008). This is an attempt by the plant to transpire less water and conserve resources. As leaf area decreases during water deficit, root elongation increases (Taiz, 2006). Leaves can then distribute their stored assimilate to the roots. Roots will grow deep into the moist soil, a second line of defense against

drought stress. Under drought conditions, carbohydrate reserves become critical as photosynthesis is often inhibited, respiration is using up resources (Barnabas et al., 2008), and as plant roots and shoots are competing for these very resources (Taiz, 2006). Under water deficit, however, assimilates will get directed to the grain (Taiz, 2006).

In wheat, photosynthate is often stored in the stems and leaves of the plant in the form of water soluble carbohydrates (WSC). These WSC are very important under drought stress (van Herwaarden et al., 1998; Yang et al., 2001, 2007; Foulkes et al., 2002; Ehdaie et al., 2006, 2008). For example, significant increases in both grain yield and kernel size occur because of the ability of wheat to remobilize carbon from stems to grain (Austin et al., 1980; Bell and Incoll, 1990; Lópezcastaneda and Richards, 1994, Housley, 2000; Salem et al., 2007). Total WSC can comprise up to 40% dry biomass weight in wheat at harvest (Schnyder 1993, Housley 2000, Ehdaie et al., 2006), and under drought, remobilized WSC can contribute as much as 50% of total grain weight (Schnyder, 1993; van Herwaarden et al., 1998; Gebbing and Schnyder, 1999).

Stem reserve accumulation during the pre-anthesis period in wheat is considered a significant factor affecting flower and grain development under stress conditions (reviewed by Blum et al., 1994; Blum, 1998). However, drought stress during the stem elongation period decreases stem and carbon assimilation storage capacity (Barnabas et al., 2008). During the grain filling period in cereals, grain filling rate is determined by carbon assimilates both from current photosynthesis and from WSC reserves in the stem (Plaut et al., 2004; Yang and Zhang, 2006). In Colorado, Mulat (2004) found a positive correlation between stem nonstructural carbohydrate (NSC) concentration (post-anthesis) and NSC remobilization during grain filling in a dry treatment. From this finding, Mulat

concluded that the amount of nonstructural carbohydrates (NSC) able to be remobilized was determined by the amount of NSC available at the beginning of the grain filling period. When water is limiting during grain filling, photosynthesis and grain fill duration decrease, and senescence occurs earlier; however, remobilization of assimilates from the stem to the spike increases (Kiniry 1993; Gebbing and Schnyder, 1999; Takahashi et al., 2001; Plaut et al., 2004; reviewed by Yang and Zhang, 2006; Ruuska et al., 2006; Rebetzke et al., 2008). This remobilization can decrease the chance of producing small and shriveled grain or the chance of early seed abortion because more energy is invested in the formation of viable seed (Westgate and Boyer, 1986; Makela et al., 2005).

In addition, drought stress increases pollen sterility in wheat (Saini et al., 1984). This may be due to the reduction of WSC in the anthers and an increase in the activity of acidic invertase enzyme (Saini, 1977). Recent studies have confirmed that acidic invertases affect pollen sterility under drought stress (Koonjul et al., 2005).

During rapid onset of drought stress, concentrations of the hormone abscisic acid (ABA), a long-term messenger involved in mediation of drought stress signaling pathways (Bray, 2002), increase when roots sense dry soil and water loss (Barnabas et al., 2008). ABA also gets transported through the xylem to the shoot. During water deficit, solute content of the guard cells decreases, and stomata close in an attempt to reduce evaporation for the existing leaf area, a third line of defense against drought (Taiz, 2006, Barnabas et al., 2008). This loss of solutes from guard cells is triggered by ABA. Abscisic acid from the xylem is thought to influence plant reproductive structures and subsequent plant development (reviewed by Liu et al., 2005). Under drought stress, high levels of ABA in early reproductive structures are indicative of inhibition of cell division

and subsequent impairment of floret and seed development (Yang et al., 2001a). ABA is also involved in the mobilization of carbohydrate reserves under drought stress (Yang et al., 2001a).

Effect of drought and CO_2 levels on plant development

Drought stress is also affected by CO_2 levels in the atmosphere. Photosynthesis and water use efficiency (WUE) increase under high CO_2 levels because water loss is minimized (Tuba et al., 1994). Under high CO_2 levels, the rate of diffusion of CO_2 into the cell increases due to a high concentration gradient; consequently, the uptake of CO_2 through stomata and stomatal conductance increases (Reynolds et al., 2010). Under drought stress, CO_2 assimilation rates decrease because stomata close to minimize water loss (Zaharieva, 2001). Yet Veisz et al. (2007) confirmed that wheat cultivars were able to decrease the negative effects on yield and yield components when CO_2 concentration doubled under drought stress. In this way, an increase in CO_2 might be able to combat high temperatures and drought, but this finding is certainly not conclusive (Leakey et al., 2006).

Effect of temperature and CO_2 levels on plant development

The temperature optimum for wheat growth is 15 ºC (CCSP, 2009), and even a 2 ºC increase above the optimum during grain filling severely affects wheat yields (Acevedo et al., 1991). In the Great Plains region of the U.S., daily high temperatures in the summer often exceed 25 ºC (http://climate.colostate.edu/~coagmet/). To aggravate the situation, wheat is very sensitive to even short, moderate temperature increases, and grain yield and quality can decrease if high temperatures occur during just 5% of the grain filling period (Stone and Nicolas, 1994). In fact, during grain filling and at

temperatures between 17ºC and 24ºC and greater, yield losses can reach up to 4% per 1ºC increase.

Heat stress-related yield loss is caused by many factors. One major factor is the inability of the carbon assimilation processes (transpiration and photosynthesis) to work properly and efficiently (Stone, 2001). For example, transpiration rates increase under high temperatures because plants must open their stomata to cool their leaves (Barnabas et al., 2008). In addition, photosynthetic capacity of plants decreases especially at temperatures above 35°C, and Rubisco activity decreases (Crafts-Brandner and Law, 2000; Griffin et al., 2004). Also, high temperatures increase levels of ethylene in winter wheat, which can induce grain abortion and reduce kernel weight (Hays et al., 2007a). Heat stress decreases plant biomass, kernel number and size, kernels per spikelet, grain yield, harvest index, and CO_2 solubility (Blumenthal et al., 1995) and increases photorespiration (Reynolds et al., 2010).

The Intergovernmental Panel on Climate Change (IPCC) predicts an increase in global average temperature of 2 to 5 ºC during the 21st century (Tubiello et al., 2007). The IPCC also predicts an increase in CO_2 concentration from 380 ppm to greater than 550 ppm by 2050. An increase in CO_2 concentration could potentially combat or suppress the effects of high temperature (Taub et al., 2000; Bencze et al., 2004; Veisz et al., 2007), increasing biomass and yields of cereals (Veisz, 1997; Harnos et al., 1998; Reynolds et al., 2010).

Combined effect of heat and drought on plant development

Supplying sufficient food to the world currently faces many challenges. Among the greatest are increasing temperatures and decreasing water availability for crops,

which together result in an increasing incidence of drought stress (Nevo and Chen, 2010). Even in eastern Colorado, wheat must tolerate hot and dry conditions for survival (http://www.fas.usda.gov/pecad2/ highlights/2002/06/ ColoradoJune2002/index.htm). Heat and drought stress often occur at the same time and contribute to the same plant responses (Moffat, 2002; Shah and Paulsen, 2003; Tester and Bacic, 2005). In maize, barley (*Hordeum vulgare*), sorghum (*Sorghum vulgare*), and other grasses, both drought and heat stress lower plant metabolism (Rizhsky et al., 2004) and together have far more detrimental effects than either stress alone (Walter and Brunson, 1940; Craufurd and Peacock, 1993; Savin and Nicolas, 1996; Wang and Huang, 2004). Plant responses to combined heat and drought stress include: 1) decreased grain yield; 2) accelerated development (Paulsen, 1994); 3) decreased photosynthesis (Reynolds et al., 2010) and/or damage to photosynthetic machinery such as photosystem II (Paulsen, 1994); 4) increased photorespiration (Berry and Bjorkman, 1980); 5) disruptions to the respiratory process (Lin and Markhart, 1990); 6) stomatal closure leading to decreased leaf cooling (Rizhsky et al., 2002); 7) and decreased Rubsico activase activity (Law and Crafts-Brandner, 1999). Also, starch synthesis is limited during grain filling (Hurkman et al., 2003), decreasing the ability of grain to fully develop (Bhullar and Jenner, 1985) and consequently decreasing seed number and seed size (Reynolds et al., 2010). In addition, reproductive capabilities of a crop can also be reduced or impaired during meiosis and gametogenesis (Barnabas et al., 2008; Hedhly et al., 2009; Reynolds et al., 2010).

Cellular responses to drought stress

To survive under drought, plants must be able to receive and send signals to make molecular, cellular, and physiological changes (Shao et al., 2005; Barnabas et al., 2008).

These changes include increased membrane fluidity, increased activity of transporters, ABA accumulation (Bray, 2002), increased expression of dehydration-response genes (e.g., osmoprotectants), a higher interaction between reactive oxygen species/scavengers (ROS) and antioxidants, and increased activity of transcription factors.

Membrane Fluidity

Water is required for the maintenance of membrane fluidity (Blum, 2011). However, under drought stress, varying water potentials in different tissues cause structural changes in the phospholipid bilayer. To cope with these changes, plants adjust membrane structure based on water flux in and out of the cell (Chaves et al., 2003). Under drought conditions, tissue dehydration leads to an inability to maintain membrane fluidity within an optimal range (Hsiao, 2003). Fatty acid metabolism genes regulate permeability to toxic ions and repair membrane damage to maintain membrane fluidity (Torresschumann et al., 1992; Holmberg and Bulow, 1998).

Transporters

Altered regulation of molecular transport through cell and organelle membranes is a common response to abiotic stress. Expression of genes for transporters for water, sugars, peptides, and micro- and macronutrients have been found to respond to drought stress (Mohammadi et al., 2007). For example, sugar transporters move sugars through plasma membranes and the tonoplast to adjust osmotic pressure under stress. Transporters and carrier proteins regulating ions and osmolyte (potassium, phosphate, sucrose, and sorbitol) balance are also active under drought stress (Assmann and Haubrick, 1996; Mohammadi et al., 2007). Also, aquaporins are thought to control cellular transport of water under drought stress (Alexandersson et al., 2005). Aquaporins

are dynamic proteins embedded in cell membranes that may be partially responsible for explaining water movement in plants under drought stress and after stress is relieved (Kaldenhoff et al., 2008). However, Mohammadi et al. (2007) found only one aquaporin gene responsive under drought conditions in wheat.

Dehydration responsive genes

Many dehydration responsive genes encode hydrophilic polypeptides, which are thought to protect the cells against drought stress (Artus et al., 1996; Shinozaki and Yamaguchi-Shinozaki, 1999). Mohammadi et al. (2007) used a long oligonucleotide array to detect numerous dehydration-response transcripts in many metabolic pathways in hexaploid wheat. These major classes of dehydration-response genes included osmoprotectants, compatible solutes (e.g., raffinose, trehalose, and glycine betaine), proteases, glyolsyltransferases/hydrolases, signal transducer components, and ion transporters (Mohammadi et al., 2007). The osmoprotectant class will be discussed below.

Genes encoding osmoprotectants that were detected by Mohammadi et al. (2007) included genes for late embryogenesis abundant (LEA) proteins, dehydrins/dehydration stress proteins (DHNs), and actin-binding proteins (ABCs). To understand LEA genes and dehydrins, it is important to return to the hormone ABA. First, ABA triggers the synthesis of LEA proteins (Vaseva et al., 2010). The LEA proteins have a high glycine content and aid in drought adaptation and protection in vegetative stages (Vaseva et al., 2009) but especially during reproductive stages to buffer seeds from dessication (Shao et al., 2005; Barnabas et al., 2008). Second, ABA induces expression of certain DHNs under drought conditions (Vaseva et al., 2010). There are many different dehydrin genes

in hexaploid wheat (Tommasini et al., 2008). The DHNs prevent water loss due to their high hydrophilic capacity (Huseynova et al., 2007) and accumulate in the nucleus or cytoplasm (Close, 1997; Egerton-Warburton et al., 1997; Danyluk et al., 1998). Another group of osmoprotectants, the ABCs, are correlated with cytoskeleton reorganization and are also thought to have a role in signaling to potassium ion channels (Yan et al., 2005). In addition, abundant transcripts of ABCs have been found in roots of drought stressed wheat (Mohammadi et al., 2007).

ROS and antioxidants

In general, damage due to drought stress results from an excess of ROS in the chloroplasts (Al-Ghamdi, 2009); these ROS include superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) (Foyer et al.; 1994, Asada, 1997; Bartoli et al., 1999; Sgherri et al.,2000; Selote et al., 2004; Bian & Jiang, 2009). These ROS are produced in the reaction centers of photosystem I (PS I) and photsystem II (PS II) (Huseynova et al., 2007), and their presence impairs photosynthesis as electrons can no longer flow through the system (Asada, 1999). Mitochondria must also be protected against ROS under drought stress (Bartoli et al., 2004; Selote and Khanna-Chopra, 2006). The primary source of ROS in mitochondria is H_2O_2 (Moller, 2001; Rhoads et al., 2006).

Plants respond to ROS formation by building an antioxidant defense system (Al-Ghamdi, 2009). Antioxidants that respond to drought stress in plants include superoxide dismutase (SOD), catalase, and ascorbate peroxidase (APX) (Gogorcena et al., 1995; Bergmann et al., 1999). These enzymes scavenge and degrade ROS (Schwanz et al., 1996). Overcoming oxidative stress depends first on increased SOD activity then on subsequent downstream activation of antioxidant enzymes and the ascorbate-glutathione

cycle (Halliwell-Asada cycle) (Alscher et al., 2002; Blokhina et al., 2003; Selote and Khanna-Chopra, 2006). First, SOD catalyzes the conversion of superoxide radicals such as O_2^- to H_2O_2 (Bowler et al., 1992; Mascher et al., 2002). Next, the removal of H_2O_2 from the cell is aided by catalase (CAT), peroxidase (POX) and the ascorbate-glutathione cycle (Selote and Khanna Chopra, 2010). The cycle consists of ascorbate, reduced glutathione, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascrobate reductase, and glutathione reductase (Foyer et al., 1997) and is found in both photosynthetic and non-photosynthetic tissues (Asada, 1999; del-Rio et al., 2002; Rhoads et al., 2006). These enzymes determine the fate of H_2O_2 under drought conditions (Mittler and Zilinskas, 1994; Willekens et al., 1997; Luna et al., 2005; Selote and Khanna-Chopra, 2006).

The activation of antioxidant defenses depends on the level of drought stress experienced by the plant (Menconi et al., 1995), but a solid antioxidant defense system in plants decreases the amount of cellular oxidative stress (Abogadallah et al., 2010; Selote and Khanna Chopra, 2006). For example, Selote and Khanna-Chopra (2007) found that cellular antioxidant defense was better in drought-acclimated versus drought non-acclimated roots. In drought-acclimated seedlings, they discovered increased levels of antioxidants such as SOD, APX, catalase, peroxides, and ascorbate glutathione cycle components; in addition, oxidative damage to membranes and organelles decreased (Kocsy et al., 1996), H_2O_2 accumulation decreased, and relative water content (RWC) remained high (Selote and Khanna-Chopra, 2010). However, APX did not respond well and caused an increase in lipid peroxidation in non-acclimated seedlings.

Glutathione is an important antioxidant molecule (Noctor and Foyer, 1998; Mohammadi et al., 2007) that detoxifies peroxides via the enzyme glutathione-S-transferase (GST) (Mohammadi et al., 2007). Generally, glutathione has been shown to increase under drought stress (Mittler et al., 2004); however, transcript abundance of glutathione synthesis genes under drought stress decreased in wheat, rice, and oilseed rape (Lappartient and Touraine, 1997; Loggini et al., 1999; Chen et al., 2004; Sharma and Dubey, 2005; Mohammadi et al., 2007). Glutathione synthesis, therefore, depends on genotype, duration, intensity, and type of stress (Mohammadi et al., 2007). Another small molecule that protects the cell from oxidative damage under drought stress is ferritin (Barnabas et al., 2008). Ferritin sequesters intracellular iron involved in the generation of reactive hydroxyl radicals through the Fenton reaction (Bajaj et al., 1999). Calcium (Ca^{2+}) is an important signaling molecule in the cytoplasm (Bray, 2002; Kaur and Gupta, 2005). In fact, several Ca^{2+}-dependent kinases have already been shown to play a role in signaling under drought stress (Bray, 2002; Kaur and Gupta, 2005; Klimecka and Muszynska, 2007). Activation of these kinases creates a cascade leading to the activation of transcription factors (Kaur and Gupta, 2005; Nakashima and Yamaguchi-Shinozaki, 2005).

Transcription factors

Transcription factors are responsible for communication under drought stress. Two main families of transcription factors include NAC and AP2/ERFBP families, and they have been identified as contributors to the drought response in wheat and rice (Rabbani et al., 2003; Shen et al., 2003; Wang et al., 2006). The DREB/CRF transcription factors are part of the latter family and are well-known in relation to stress

signaling (Yamaguchi-Shinozaki and Shinozaki, 2006). For example, DREB1A is a well-studied and identified gene associated with drought and other stresses (Chandler and Robertson, 1994).

Breeding for Drought Tolerance

Yields in wheat and rice increased drastically during the 'Green Revolution' of the 1960s and 1970s as dwarfing traits were incorporated into the plants (Tamas, 2005). Since then steady progress in cereal productivity has been made (Trethowan et al., 2002). However, the annual percent productivity must increase to keep up with the future increase in the world population and food demand (Vandeleur and Gill, 2004). The productivity of plants under drought is affected by biotic and other abiotic stresses (Reynolds et al., 2006). Biotic stresses include pests and diseases, and abiotic stresses include heat, salinity, (Farooq and Azam, 2001; Richards et al., 2007), soil micronutrient deficiency, and mineral toxicity (Reynolds et al., 2010). For example, soils deficient in water and zinc will have difficulty in detoxifying reactive oxygen species (ROS), thus exacerbating symptoms of drought stress (Bagci et al., 2007). Developing genetic combinations of traits that address not only drought but other stresses related to drought will be vital for successful breeding in the future (Reynolds et al., 2010).

When breeding for drought tolerance in wheat, it is important to consider both morphological and physiological traits (such as carbon assimilation) adapted to a specific environment (Reynolds et al., 2007; Saint Pierre et al., 2010). Traits that have been selected to improve drought tolerance include deeper roots, decreased canopy temperature, and increased transpiration efficiency in wheat, as well as delayed senescence in sorghum and synchronous flowering in maize (Reynolds et al., 2010). It

has recently been noted (Reynolds and Tuberosa, 2008) and revealed (Reynolds et al., 2009) that confounding factors such as differing flowering times must be kept at a minimum in order to maximize the capability for gene discovery for drought tolerance.

Yield in a drought-stressed field is a common way to measure drought severity (Nevo and Chen, 2010), and the effects are often seen in the root structure. Root traits are very important in drought adaptation (Jordan et al., 1983; Jones and Zur, 1984; Otoole and Bland 1987; Sponchiado et al., 1989; Sinclair and Muchow, 2001; Manschadi et al., 2006, 2008; Reynolds et al., 2007a), as roots are the first part of the plant to sense soil water depletion (Davies and Zhang, 1991). When roots sense drought stress, they activate primary stress signaling responses (Selote and Khanna-Chopra, 2010). Acclimated roots are able to react to these responses and still retain water and keep strong root systems (Lilley and Ludlow, 1996; Sgherri et al., 2000; Selote and Khanna-Chopra, 2010). Non-acclimated roots do not respond well to drought, which decreases yield (Norouzi et al., 2008).

The ability of roots to extract water from deep within soil profiles is a key adaptation to drought stress (Lopes and Reynolds, 2010). Deep root systems have been associated with increased yields in wheat in some (Oliver-Villegas et al., 2007) but not all (Palta et al., 2011) environments. However, root traits are difficult to measure in the field (Lopes and Reynolds, 2010). Canopy temperature is an indirect selection tool for drought tolerant traits such as root depth and function (Brennan et al., 2007), and Olivares-Villegas et al. (2007) found that canopy temperature explained about 60% of yield variation under drought. Canopy temperature has been shown to correlate with stomatal conductance in wheat (Amani et al., 1996; Lu et al., 1998), with greater

conductance related to cooler temperatures (Singh et al., 1997). Canopy temperature is an important field trait because it can be rapidly screened, making it useful for field measurements (Montes et al., 2007; Oliver-Villegas et al., 2007). However, its use in the Great Plains region is often impractical because of frequently windy conditions, thus making it difficult to obtain stable measurements. In dry environments such as Australia, efforts have focused on improving canopy coverage of the soil surface early in the season, by selecting for traits related to better emergence such as long coleoptiles (Richards, 2006; Rebetzke et al., 2008) and changes in tiller number (Richards, 2010).

Another trait that breeders have selected for is "stay green," or delayed leaf senescence. This trait combats the effects the hormone ethylene has on promoting senescence and accelerating the plant life cycle (Reynolds et al., 2010). Hays et al. (2007) reported that increased ethylene was directly responsible for grain abortion and reduction in kernel weight in wheat under heat stress. The stay-green trait has been used as a visual selection criterion for sorghum breeding (Burke et al., 2010), and markers have been developed for selection against high ethylene expression in wheat (Hays et al., 2007). The maintenance of healthy, green leaves can be an indicator of tolerance to heat and/or drought stress (Olivares-Villegas et al., 2007), and the normalized difference vegetative index (NDVI) is one method providing a proxy of leaf health for photosynthesis (Araus et al., 2008). Stay-green is not only useful to determine levels of senescence but also to determine levels of soluble sugars in stems during and after grain filling in non-senescent genotypes (McBee et al., 1983).

Plants can also adapt to stresses through leaf canopy traits such as epicuticular wax, pigment composition, and leaf rolling; these increase the ability of the plant to

photosynthesize while minimizing water loss (Richards, 2006). Leaf waxiness in wheat increases transpiration efficiency, WUE, and yield in dry land conditions (Johnson et al., 1983). Leaf waxiness in rice has also shown similar results (Wassmann et al., 2009). In wheat, leaf glaucousness in the form of a bluish-green epicuticular wax has been associated with a marker useful for predicting heat and drought adaptation (Reynolds et al., 2010). Another physiological application to wheat breeding has been selecting for increased transpiration efficiency (TE), which allows cultivars to use the limited water they have more slowly and effectively (Condon et al., 2004). Carbon isotope discrimination, $\Delta^{13}C$ ratio, is a proxy for transpiration efficiency.

Selection for increased WSC reserves in stems under multiple stresses is one potential breeding focus (Conocono et al., 1998; Foulkes et al., 2002; Richards et al., 2002; Ehdaie et al., 2006, 2008). WSC-related traits will increase yields more under drought than well-watered conditions (Saint Pierre et al., 2010). It appears that WSC per unit area is a better selection criterion than WSC concentration (%WSC) for under drought conditions (Rebetzke et al., 2008; Saint Pierre et al., 2010). Regardless, WSC is controlled by many QTL of small effects, which may limit the ability of WSC to be used in marker-assisted selection (Rebetzke et al., 2008). Development of markers for stem thickness which can be used to select high-yielding lines is not out of the question (Lanning et al., 2006). In addition, other WSC-related traits such as stem length and diameter could potentially be pyramided to maximize yield increases (Saint Pierre et al., 2010). WSC has been shown to be closely associated with grain set (Pinto et al., 2010); however, an understanding of the association between WSC concentration and biomass is vital for maximizing yield gains in the future (Reynolds et al., 2007).

Maximum biomass is produced in plants under optimal water conditions, and under drought stress, biomass decreases (Clarke et al., 1991; Ashraf, 1998). One area of genetic improvement involves increasing biomass while maintaining a high harvest index (Slafer and Andrade, 1991; Calderini et al., 1999; Foulkes et al., 2007). The upper limit for harvest index in winter wheat is about 0.62 (Austin, 1980). Certain regions have approached this limit; for example 0.61 was recorded for a UK winter wheat (Spink et al., 2000). Translocations such as the 1BL.1RS wheat-rye translocation may correlate with increased biomass in modern cultivars (Carver and Rayburn, 1994; Foulkes et al., 2007). It is certain that future increases in spike biomass will depend on increases in above-ground biomass (Foulkes et al., 2007). However, above-ground biomass will be limited by factors such as water and/or nitrogen availability; therefore, it is important to focus on optimizing water and nutrient uptake in root systems (Foulkes et al., 2007). The effects of biomass on yield have been variable, as these traits have been both positively (Siddique et al., 1989; Donmez et al., 2001; Shearman et al., 2005) and negatively (e.g. Waddington et al., 1986; Austin et al., 1989; Slafer et al., 1994; Brancourt-Hulmel et al., 2003) correlated. One potential way to maximize grain yield would be to increase the stem elongation period (Bradley et al., 2005; Shearman et al., 2005). This would favor greater spike biomass, stem WSC, and crown root growth at anthesis (Foulkes et al., 2007).

Wild species

Able and Langridge (2006) note that many modern crops including wheat have been exposed to domestication and selection pressures and therefore have low diversity compared to their wild relatives. This has decreased the tolerance of crops to

environmental stresses (Nevo and Chen, 2010). One potential solution to increase crop productivity and stability is to improve the genetic backgrounds of crops to increase tolerance of abiotic stresses (Blum 1998; Ashraf et al., 2009; Nevo and Chen, 2010). Locally adapted wheat cultivars often perform better than the best synthetic wheat lines per se (Dreccer et al., 2007). However, introgression of relevant genes and QTLs from wild or wild-derived germplasm can improve performance (Xie and Nevo, 2008; Edwards et al., 2009).

Genetic resources of wild relatives have mainly been directed to introduce resistance to biotic stresses (Upadhyaya et al., 2008), while relatively few wild crop relatives have been exploited for adaptation to abiotic stresses such as drought stress (Hajjar and Hodgkin, 2007). There is evidence that alleles associated with drought tolerance in wild relatives and landraces of cultivated wheat can improve drought tolerance (Feuillet et al., 2008; Reynolds et al., 2007). In particular, *Aegilops* species are recognized as a potential source of drought tolerance alleles (Farooq et al., 1989). Synthetic hexaploid wheat is made by crossing the 'A' and 'B' genomes from tetraploid durum wheat (*Triticum turgidum*) with the 'D' genome of the diploid species *Aegilops tauschii* (also known as *Ae. squarrosa* in earlier classifications) (William et al., 1993). An untapped reservoir of genetic diversity (Feuillet et al., 2008) is represented by one-third of the synthetic hexaploid wheat genome being from a wild relative.

Crosses between elite wheat cultivars and synthetic hexaploid wheat lines have improved drought adaptations in the past (Trethowan et al., 2005). However, there are drawbacks to incorporating an exotic germplasm accession into an elite variety. First, exotic germplasm often has different maturity or vernalization requirements than their

crop relatives. Second, exotics often contain a large number of unfavorable alleles for yield and quality (Reynolds et al, 2006). For these reasons, plant breeders have avoided the use of wild species in their breeding programs. The incorporation of alleles from wild species offers potential for crop improvement because they introduce new sources of genetic diversity (Edwards et al., 2009). The challenges then are to identify favorable sources of alleles among wild accessions and successfully introgress them into a locally adapted genetic background (Reynolds et al., 2009).

The International Maize and Wheat Improvement Center's (CIMMYT) Wheat Breeding Program increasingly incorporates wild wheat germplasm and its allelic diversity into their breeding programs in the form of synthetic hexaploids (Trethowan et al., 2008; Mujeeb-Kazi, 2008; Feuillet et al., 2008). CIMMYT evaluates non-adapted genetic resources, such as landraces or wild species to search for stress-adaptive traits. CIMMYT also uses high-throughput screening tools such as infrared thermometry to discover novel genes, characterize parents for use in crosses, and to select in early generations.

DNA Molecular Markers

Simple sequence repeats (SSRs), also known as microsatellites, are molecular markers that have become available to researchers for general use (Quarrie et al., 2005). The use of SSRs is a common way to examine and detect polymorphisms in wheat. Simple sequence repeats are very informative due to their co-dominant nature. Additionally, they contain more allelic diversity per marker (Chao, 2009), and they have higher mutation rates than other marker types (Thuillet et al., 2002). SSRs continue to be

the main marker type for QTL studies in wheat, either alone or in combination with other types of markers.

Quantitative Trait Loci (QTL)

A QTL is a genomic region that is responsible for variation in a quantitative trait of interest (Doerge et al., 2002). Quantitative traits are controlled by the combined action of many genes, whereas qualitative traits are controlled by one or two major genes (Kearsey and Pooni, 19966). In past years, QTL in wheat have been examined for many different phenotypic traits, such as plant height, days to heading, test weight, grain yield, head length, and bread making quality (Narasimhamoorthy, 2006**,** Sun et al., 2009, Chu et al., 2008, Hai et al., 2008, Wang et al., 2009; El Feki, 2010). In crops like wheat and barley, segregation for genes of major agronomic effect (e.g., plant height and maturity) within experimental populations confounds the ability to identify QTLs of minor effect associated with more direct mechanisms of adaptation (Reynolds et al., 2009). The use of mapping populations with uniform flowering time (Olivares-Villegas et al., 2007) allows for easier detection of QTLs (Pinto et al., 2010) associated with complex agronomic traits and easier dissection of those traits into component loci (Marza et al., 2006).

Quantitative trait locus analysis enables the understanding of the genetic basis of complex traits such as drought tolerance (Collins et al., 2008). The major goal of QTL analysis is to discover the number, chromosomal location, and the size of effect of the loci controlling the trait. This analysis is based on the detection of associations between the variation in a phenotype and the variation in the genotype of markers located throughout the genome. Phenotypic measurements are often gathered in a field or

greenhouse setting, and genotypic marker data is gathered in the lab. Marker information is used to construct a linkage map, ideally covering all the chromosomes. Marker and phenotypic data are then combined to discover the chromosomal region controlling the trait of interest on the linkage map. Numerous methods have been developed to detect and characterize QTL, one of which is single factor analysis. This type of analysis examines each marker against each trait, not taking into account the distance from other markers on the map or the effects of loci elsewhere in the genome.

Advanced backcross (AB)-QTL analysis

Plant breeders often select plants or lines by visually examining phenotypic traits. However, phenotypic evaluation of synthetic-derived or wild materials in local environments is not always the best indicator of an accession's value in a breeding program as these materials are often unadapted to the region (Tanksley and McCouch, 1997). There is a genetic component to breeding that is equally important as the phenotypic component. The Advanced Backcross-QTL (AB-QTL) strategy (Tanksley and Nelson, 1996) is a way to unite genetic analysis and breeding. This strategy attempts to identify favorable alleles from wild species when incorporated into an adapted genetic background. Many AB-QTL studies have been conducted in crops such as tomato (*Lycopersicon esculentum*) (Tanksley et al, 1996, 2007; Bernacchi et al, 1998; Frary et al., 2003, 2004; Fulton et al., 1997, 2000, 2002), rice (Xiao et al., 1998; Li et al., 2002), pepper (Rao et al., 2003), maize (Ho et al., 2002), barley (Pillen et al., 2003, 2004) and wheat (Huang et al., 2003, 2004; Narasimhamoorthy et al., 2006; Kunert et al., 2007).

The introgression of wild wheat and derived synthetic hexaploids into common cultivars and the subsequent AB-QTL analysis will play a large role in the future in

understanding the molecular basis of drought tolerance, according to Nevo and Chen (2010). The incorporation of favorable alleles at QTL for drought tolerance into a wheat breeding program could result in better performing cultivars, which would be useful in many parts of the world suffering from drought stress. Experiments with rice have led to the successful introduction of alleles from wild species into domesticated lines, and the resulting cultivars have been released into the Asian market (Cheema, 2008). However, the selection for markers linked to quantitative traits into breeding programs has just begun.

Comparison among previous AB-QTL studies in wheat

Numerous QTL studies have been conducted in wheat for numerous traits. For example, QTL for wheat have been found for seed size, seed shape (Breseghello and Sorrells, 2007), plant height maturity, and grain yield (Kato et al., 2000; Kuchel et al., 2007; Marza et al., 2006; McCartney et al., 2005, Snape et al., 2007). Yield QTL on chromosomes 1A, 3B, and 4A have previously suggested potential genes involved in drought environments (Kirigwi et al., 2007). Pinto et al. (2010) found QTL for heat and drought stress tolerance on these same chromosomes.

Multiple AB-QTL studies have also been performed in wheat, and numerous QTL have been detected across a wide variety of traits. For example, Huang et al. (2003) used an advanced backcross population containing a German winter wheat variety 'Prinz' and a synthetic wheat line W-7984 from CIMMYT. Eleven QTL for grain yield across eight chromosomes (1A, 1B, 2A, 2B, 2D, 3B, 4D, and 5B) were detected (Huang et al., 2003). The synthetic line W-7984 contributed the favorable allele for four of these QTL. One of these QTL was found on the D-genome (2D), indicating it originated in *Ae. tauschii*.

Huang et al. (2004) also reported another AB-QTL study in a BC_2F_3 population of the German winter wheat variety 'Flair' and the synthetic wheat line XX86 from Japan. They reported nine QTL for grain yield (on 1A, 3D, 4D, 5A, 5B, 6B, and 6D), but XX86 did not contribute any favorable alleles for yield.

In an advanced backcross study by Kunert et al. (2007), two $BC_2F_{2:3}$ populations of winter wheat were used that were derived from two German winter wheat cultivars, Batis and Zentos, and two synthetic hexaploid wheat accessions, Syn022 and Syn086. The main focus of this study was to examine the contribution of synthetic hexaploid wheat to milling and baking quality; however, hectoliter weight (i.e., test weight) was calculated as well. Six QTL were found on chromosomes 3B, 4A, 6B, 7A, and 7B, but the exotic allele contributed negatively to hectoliter weight. Narasimhamoorthy et al. (2006) also evaluated test weight but found the adapted cultivar Karl92 contributed the favorable allele for the only detected QTL, on chromosome 2D.

Huang et al. (2003) detected eight QTL for ear emergence time (days to heading) on chromosomes 2A, 2D, 3B, 5A, 5B, 6A, and 7B. The synthetic-derived line contributed to earlier ear emergence time for all detected QTL, and one QTL was found on the D-genome (2D). It is possible the vernalization response gene, *Vrn-A1*, found on chromosome 5A, contributed to the control of ear emergence time on chromosome 5A (Snape et al., 1976). *Vrn* genes have been reported to affect drought adaptation (Dyck et al., 2004). In addition, the subsequent study by Huang et al. (2004) revealed five QTL for ear emergence time on chromosomes 2D, 3A, 4A, 7A, and 7D. Four synthetic XX86 alleles reduced the number of days to ear emergence (one QTL was found on chromosome 2D), and one XX86 allele increased the days to ear emergence.

Furthermore, Narasimhamoorthy et al. (2006) developed a $BC_2F_{2:4}$ population of 190 lines from a cross between Karl92, a hard red wheat, and TA 4152-4, a synthetic accession. This study detected two QTL for days to heading on chromosomes 2D and 3D. Each QTL increased days to heading, and the QTL on 2D was found to be linked with the same microsatellite locus, *Xgwm261*, which Sourdille et al. (2000) reported to be associated with the *Ppd-D1* locus, and which Korzun et al. (1998) reported to be associated with *Rht8*.

Narasimhamoorthy et al. (2006) and Huang et al. (2003 and 2004) detected QTL for tiller number per m^2. Narasimhamoorthy (2006) detected one QTL on chromosome 3B, and the synthetic allele made the favorable contribution. Huang et al. (2003) detected eight QTL on chromosomes 1B, 2A, 2D, 3B, 4D, 5D, 6D, and 7A, and each of these QTL contributed at least 9% of the total phenotypic variation. The wild allele from W-7984 contributed the favorable allele for four QTL. Huang et al. (2004) found two QTL associated with tiller number on chromosomes 1B and 7A, contributing 7.0% and 13.9% of the total phenotypic variation, respectively. The wild allele decreased tiller number on chromosome 1B but increased tiller number on chromosome 7A.

Huang et al. (2003) detected eight QTL across seven different chromosomes for. Seven had a positive effect from the wild alleles, and three were found on D-genome alleles (2D, 4D, and 7D). Three of these QTL were detected on the homoeologous positions of group seven chromosomes. These chromosomes were found across the genome on chromosomes 2A, 2D, 4D, 5B, 7A, 7B, and 7D (2003). In their subsequent study, Huang et al. (2004) detected 14 QTL for thousand grain weight (on 1B, 1D, 2A, 2D, 3A, 3B, 3D, 4B, 6A, 7A, 7D). The wild allele contributed favorably to all QTL, and

the D-genome was represented on chromosomes 1D, 2D, 3D, and 7D. In addition, Pinto et al. recently reported a QTL study that found QTL for thousand grain weight on chromosomes 3A, 4A, and 4B (2010). QTL for grain weight and plant height co-localized on 4B in the study by Pinto et al. (2010) and were consistent with previous reports (Butler et al., 2005; McCartney et al., 2005; Huang et al., 2004). In addition, this region was associated with the *Rht-B1* gene, which Marza et al. (2006) confirmed. Furthermore, Huang et al. (2003) detected five QTL for plant height on chromosomes 2B, 4B, 4D, 6A, and 7B, and the synthetic allele contributed to four QTL, one of which was located on 4D. Markers *Xgwm149* and *Xgdm61* were the two nearest markers found for the QTL on chromosomes 4B and 4D, respectively. These markers were near the *Rht-B1* and *Rht-D1* loci, respectively. In addition, Huang et al. detected 14 QTL for plant height on chromosomes 1A, 1D, 3A, 3B, 4A, 4B, 5A, 5B, 6A, 6D, 7A, and 7D in 2004, and the synthetic allele increased plant height for all QTL. Overall, many QTL were detected across multiple traits, and the wild or synthetic line contributed both positively and negatively to these traits. There is some indication the wild species *Ae. tauschii* donated some alleles because QTL for some traits were associated with the D-genome and with the synthetic line.

Goals and objectives

Based on the promising results of previous AB-QTL studies, an AB-QTL project was initiated at Colorado State University. We used wheat microsatellite markers in this study to identify QTL for yield and yield components in a BC_2F_2-derived population from a cross between a Colorado winter wheat cultivar and a CIMMYT synthetic-derived spring wheat cultivar. The long-term goals of this project are 1) to find quantitative trait

loci contributing to drought-tolerance and other agronomic traits in wheat, based on an advanced backcross population and 2) to incorporate this information and resulting germplasm into the CSU wheat breeding program.

The specific objectives of this research are:

1) To collect and analyze phenotypic data on the Ankor/Sokoll BC_2F_2-derived population grown in five Colorado environments differing in soil moisture level;

2) To gather DNA molecular marker data on the AB-QTL population and on an F_2 population derived from a cross of the same parents to develop a framework linkage map;

3) To locate and characterize QTL associated with drought tolerance and other agronomic traits using both the collected phenotypic and genotypic data;

4) To determine if there is a correlation between spike: stem ratio and biomass at various growth stages and final grain yield, based on a subset of the population; and

5) To identify promising lines for use in breeding programs based on evaluation in 10 environments.

CHAPTER 2

QTL detection for yield and yield components using molecular markers and 2007-09 field trait evaluations

INTRODUCTION

Bread wheat is an allohexaploid (AABBDD) (Feuillet et al., 2008) formed by the hybridization of the tetraploid *T. turgidum* subspp. *dicoccum* (AABB), and the diploid *Aegilops tauschii* Cosson (DD) (William et al., 1993; Feuillet et al., 2008). However, due to a domestication bottleneck and subsequent selection pressures (Able and Langridge, 2006), wheat has decreased genetic diversity compared to its wild relatives. Wild species are potentially important for crop improvement because they introduce new sources of genetic diversity (Feuillet et al., 2008; Edwards et al., 2009). However, there are drawbacks to incorporating exotic germplasm accessions into elite varieties. First, wild species often have different maturity or vernalization requirements than their crop relatives. Second, they may bring undesirable threshing characteristics. Third, undomesticated germplasm usually contains a large number of unfavorable alleles for yield and quality (Reynolds et al., 2006). For these reasons, plant breeders have avoided the use of wild species in their breeding programs, other than for single gene disease and insect resistance traits.

However, introgression of genetic material from wild species or wild-derived cultivars to locally adapted cultivars can improve performance (Xie and Nevo, 2008). The International Maize and Wheat Improvement Center's (CIMMYT) Wheat Breeding

Program increasingly incorporates wild wheat germplasm and allelic diversity into their breeding programs in the form of synthetic hexaploids (Trethowan et al., 2008; Mujeeb-Kazi, 2008; Feuillet et al., 2008). Synthetic hexaploid wheat is made by interspecific hybridization of the tetraploid durum wheat, *T. turgidum* ssp. *durum* (2n=4x=28) or emmer wheat, *T. turgidum* subspp. *dicoccum*, with the diploid wild wheat, *Ae. tauschii* (2n=2x=14), followed by chromosome doubling (Mujeeb-Kazi et al., 2008). The D-genome of *Ae. tauschii* in particular represents an untapped reservoir of diversity, and it has been known as a source of drought tolerance alleles that increase yield-related traits in wheat (Farooq et al., 1989; Trethowan et al., 2005; Reynolds et al., 2007; Feuillet et al., 2008).

Simple sequence repeats (SSRs) also known as microsatellites, are molecular markers that have become available to crop researchers for multiple uses (Quarrie et al., 2005). SSRs are a common way to examine and detect polymorphisms in wheat, are very informative due to their co-dominant nature (Chao, 2009), and have higher mutation rates than other marker types (Thuillet et al., 2002). We used wheat microsatellite markers in this study to identify quantitative trait loci (QTL) for yield and yield components in a BC_2F_2-derived population from a cross between a Colorado winter wheat cultivar and a synthetic-derived spring wheat line from CIMMYT.

Quantitative trait locus analysis contributes to understanding of the genetic basis of complex traits such as drought tolerance (Collins et al., 2008). Quantitative traits are controlled by the combined action of many genes, whereas qualitative traits more closely resemble a one-trait-one gene model (Kearsey and Pooni, 1996). In wheat, QTL have been detected for a wide range of phenotypic traits, including plant height, days to

heading, test weight, grain yield and yield components, spike length, and bread making quality (Narasimhamoorthy, 2006, Sun et al., 2009, Chu et al., 2008, Hai et al., 2008, Wang et al., 2009; El Feki, 2010).

Plant breeders often visually examine and select phenotypic traits from a wide range of germplasm. However, phenotypic evaluation of unadapted materials is not always the best indicator of a species' value in a breeding program (Tanksley and McCouch, 1997). The advanced backcross (AB)-QTL strategy (Tanksley and Nelson, 1996) is a way to unite genetic analysis and breeding and may play a large role in the future in understanding the molecular basis of drought tolerance (Nevo and Chen, 2010). This strategy successfully identifies the favorable alleles from wild species backcrossed two or more times into an adapted genetic background, and eliminates many unfavorable alleles that can interfere with phenotypic evaluation (Narasimhamoorthy et al., 2006). AB-QTL studies have been conducted in tomato (*Solanum lycopersicum*) (Tanksley et al., 1996, 2007; Bernacchi et al., 1998; Frary et al., 2003, 2004; Fulton et al., 1997, 2000, 2002), rice (*Oryza sativa*) (Xiao et al., 1998; Li et al., 2002), pepper (*Capsicum annum*) (Rao et al., 2003), maize (*Zea mays*) (Ho et al., 2002), barley (*Hordeum vulgare*) (Pillen et al., 2003, 2004) and wheat (Huang et al., 2003, 2004; Narasimhamoorthy et al., 2006; Kunert et al., 2007). While an AB-QTL study on yield and yield components has been conducted previously in winter wheat (Huang et al., 2003, 2004), our study attempts to incorporate drought tolerance alleles from unadapted species into an adapted cultivar and examines more agronomic traits than previous studies. In addition, we developed a population-specific F_2 linkage map, whereas previous research relied on the International *Triticeae* Mapping Initiative (ITMI) map for genetic distances. The ITMI map was a RIL

population from *Ae. tauschii* [DD] x Altar durum [AABB] hybridized with Opata M85 [AABBDD] bread wheat. Our study provides another comparison of similar and unique QTL among AB-QTL and other QTL studies. We predict alleles from unadapted germplasm will positively contribute to drought tolerance and agronomic traits and that novel QTL will be discovered because of this introgression.

The objectives of this study were to 1) to collect and analyze phenotypic data on the population grown in five Colorado environments differing in soil moisture level, 2) to gather DNA molecular marker data on the AB-QTL population and on an F_2 population derived from a cross of the same parents, and 3) to locate and characterize QTL associated with drought tolerance and other agronomic traits using both the phenotypic and genotypic data collected.

MATERIALS AND METHODS

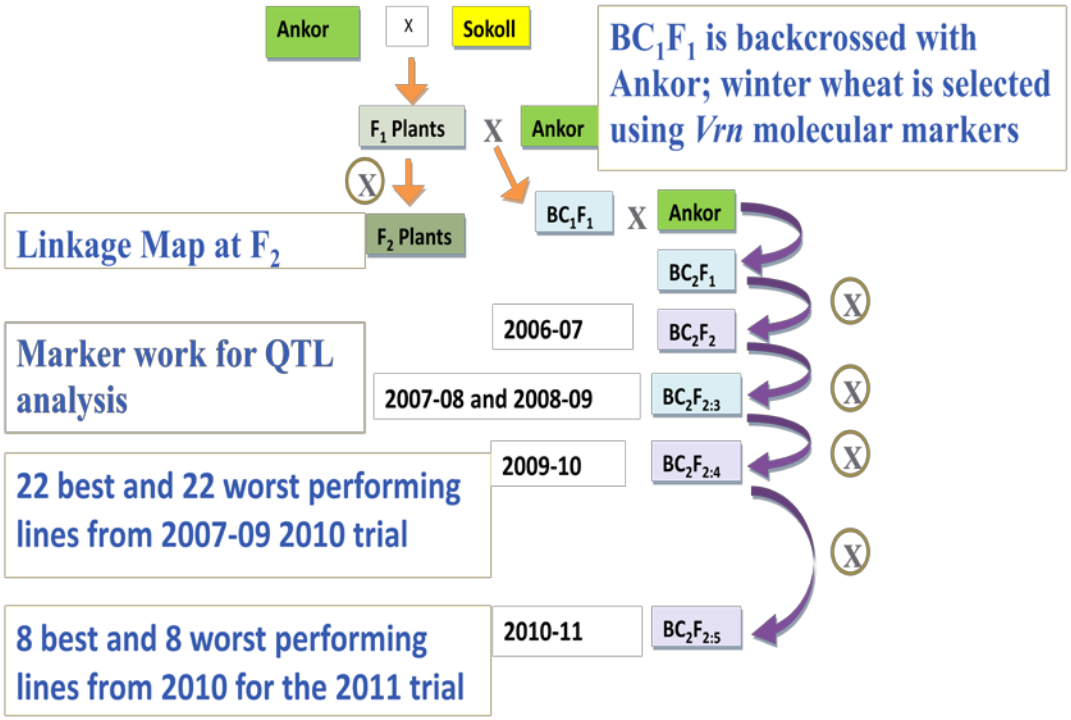
Population Development

The Ankor/Sokoll backcross population was developed to identify genomic regions of the drought tolerant spring wheat variety 'Sokoll' (developed at CIMMYT/ Mexico) that contribute to yield performance in the genetic background of the Colorado winter wheat cultivar 'Ankor.' Sokoll is a white spring wheat and was used as the donor parent for its high level of drought tolerance both pre- and post-anthesis (R. Trethowan, CIMMYT, personal communication). Sokoll was developed by crossing a synthetic hexaploid to cultivated bread wheat, and has the pedigree PASTOR [(Pfau/Seri, 14852)//(BOBWHITE, 4128)]/3/ALTAR 84/AEGILOPS SQUARROSA (TAUS), 61527//OPATA, 346421 (Singh, 2007). Ankor is a hard red winter wheat with resistance to the Russian wheat aphid (*Diuraphis noxia)* Biotype 1, and has the pedigree 'AKRON'/'HALT'//4*AKRON (Haley et al., 2004). However, it is susceptible to stem rust (*Puccinia graminis*), leaf rust (*Puccinia triticina*), stripe rust (*Puccinia striiformis*), and Hessian fly (*Mayetiola destructor*) (Haley et al., 2004).

The advanced backcross scheme is summarized in Figure 2.1. Ankor (female parent) was crossed to Sokoll (male parent) to produce F_1 seeds. Twenty-two F_1 plants were backcrossed to Ankor. Thirty-three BC_1F_1 plants were produced and genotyped for the winter-type habit as indicated by the Ankor alleles at the *Vrn-A1* (Sherman et al., 2004) and *Vrn-B1* (Blake et al., 2009) loci. Fourteen BC_1F_1 plants had the winter-type habit and were backcrossed to Ankor to produce BC_2F_1 seeds. Plants from these seeds were self-pollinated, and 228 BC_2F_2 families with sufficient seed were chosen for

planting in the 2006-07 field trials at the Agriculture, Research, Development and Education center (ARDEC) in Fort Collins, Colorado. From that harvest, the 188

Figure 2.1 Advanced Backcross Scheme.



$BC_2F_{2:3}$ lines with the most seed were planted in the 2007-08 and 2008-09 field trials.

In addition to the backcross population, an F_2 population was developed specific to the Ankor/Sokoll population to create a framework reference map. The F_2 stage provides more linkage information than a backcross population, as informative recombinants occur in both parental gametes, whereas in backcross populations only one parental gamete produces informative recombinants. An F_1 cross was made with Ankor as the female parent. One F_1 plant was self-pollinated to produce the F_2 seed. Ninety-

four F_2 seeds were planted, one seed per pot, and the leaf tissue from these plants or $F_{2:3}$ plants derived from them was used for genotyping.

DNA Extraction

Genomic DNA was extracted and purified from ground tissue of two-week old leaves of single F_2 or $F_{2:3}$ plants and from about 15 to 20 bulked $BC_2F_{2:3}$ plants. We used a method adapted from Riede and Anderson (1996) with modifications including the following: samples were incubated at 65°C for 30 min in extraction buffer [0.5 *M* NaCl, 0.1 *M* Tris pH 8.0, 0.05 *M* ethylenediaminetetra-acetic acid (EDTA), 8.4 g L^{-1} sodium dodecyl sulfate (SDS), 3.8 g L^{-1} sodium bisulfate] and mixed by inversion every 10 min for 1 h. Three hundred µL of 24:1 chloroform: isoamyl alcohol was added to each tube and mixed to form an emulsion. After centrifugation, the aqueous phase was transferred to a new tube, and DNA was precipitated by adding two volumes of 100% ethanol. Pelleted DNA was washed in 1 ml of 70% ethanol, air-dried, and re-suspended in 400 µL TE buffer (10 m*M* Tris, 1 m*M* EDTA, pH 8.0). DNA concentrations were calculated by measuring absorbance at 260 nm on a Nanodrop ND1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and the DNA quality was evaluated on a 1.0% agarose gel containing one lane of Lamba DNA/HindIII and stained with ethidium bromide. DNA was diluted to 50 ng/µL in TE (1.0 mM tris, 0.1 mM EDTA, pH 8.0).

Molecular Marker Analysis

Four hundred twenty-four SSR primer pairs were selected for screening from various primer sets, including BARC (Song et al., 2002), CFA and CFD (Guyomarc'h et al., 1998), GWM (Gatersleben Wheat Microsatellite, Roder, 1998), GDM (Gatersleben D-genome Microsatellite, Pestova et al., 2000), and WMC (Wheat Microsatellite

Consortium, Gupta et al., 2002). Gene-specific markers for *Ppd-D1* (Yan et al., 2003) and *Glu-A1* and *Glu-D1* (Liu et al., 2008) were also used for genotyping. Primers were initially screened to detect differences between parents. Ankor, Sokoll, and two lines from the F_2 generation were screened for each marker. Markers without distinct polymorphisms were not advanced for testing on the remainder of the population, while markers with distinct polymorphisms were evaluated on all 94 F_2 lines. Three hundred sixty-four SSRs were screened at Colorado State University, and 60 SSRs were screened at the United States Department of Agriculture-Agriculture Research Service (USDA-ARS) Small Grain Genotyping Center in Manhattan, Kansas.

One hundred eighty-two polymorphic markers were genotyped in the F_2 population and 76 were genotyped in the $BC_2F_{2:3}$ population. Polymerase chain reactions (PCR) were performed in 96-well micro-plates on the F_2 and $BC_2F_{2:3}$ generations according to Roder et al. (1998). The PCR reactions were conducted using a MJ PTC-100 or MJ PTC-200 thermocycler (MJ Research, Watertown, MA). These thermocyclers were set to use a touchdown (TD) thermal cycler program with different target annealing temperatures ranging from (50 to 61°C) (Williams et al., 2002). The PCR reactions were carried out in a 12 µL volume containing 2.15 µL of sterile water, 1X Thermopol reaction buffer (10 mM KCL, 10 mM $(NH_4)_2$ SO_4, 20 mM tris-HCL), 200 µM of deoxyribonucleoside-5' triphosphate (dNTPs), 125 ng of template DNA, 0.2 µM of each primer, and 0.4 unit Taq DNA polymerase. The thermocycler was set to the correct annealing temperature for each individual primer, as found in GrainGenes 2.0 (http://wheat.pw.usda.gov/GG2/index.shtml).

Amplified DNA was loaded onto either 3% biotechnology grade agarose gels (Amresco, Solon, Ohio) or 6% polyacrylamide gels for band analysis. Agarose gels were used for polymorphisms greater than 15 base pairs (bp) apart, and were electrophoresed at 120V for 2 h; however, this was adjusted based on time required to distinguish parental bands. During electrophoresis, agarose gels were stained with ethidium bromide. The gels were then digitally photographed under ultraviolet light using a gel documentation system (AlphaImager, Alpha Innotech Corp., San Leandro, CA). Polymorphisms less than 15 bp apart were run on a 6% polyacrylamide gel (BioRad, Hercules, CA) according to Laemmli (1970). The polyacrylamide gels were electrophoresed at 80 watts for approximately 2 h 30 min. Polyacrylamide gels were subsequently stained using the silver staining process as described by Bassam et al. (1991) and scanned on a Microtek flatbed scanner at 150 dpi resolution.

For 60 markers, PCR was prepared for high-throughput ABI fragment analysis at the USDA-ARS Regional Small Grain Genotyping Center, according to the ABI protocol (www.fhcrc.org/science/shared_ resources/genomics/sequencing/ BigDyeProtocol.pdf). Both types of gels were evaluated using AlphaEase software (Alpha Innotech Corp., San Leandro, CA), and ABI marker patterns were evaluated with Gene Marker (Soft Genetics, LLC, State College, PA). In the F_2 population, if an individual was homozygous for a marker allele, it was noted which parent, Ankor or Sokoll, contributed that allele. If bands from both parents were present, the individual was scored as a heterozygote. In the $BC_2F_{2:3}$ population, DNA was either scored as homozygous for the Ankor allele (A) or heterozygous (H), showing both Ankor and Sokoll alleles. Most SSR primer pairs resulted in a single polymorphic locus; however, some pairs produced two

polymorphic loci, which were scored separately. Gels scores were scored by two people and reconciled, exported to an Excel spreadsheet (Microsoft; Redmond, WA), and input into the JoinMap linkage map program for analysis (Van Ooigen, 2006). In addition, Chi-square tests were performed in JoinMap 4 software (Van Ooigen, 2006) for co-dominant markers and in Excel for dominant markers to test for the presence of segregation distortion.

Linkage map construction

A framework SSR linkage map was constructed for the F_2 population (n=94) with the regression mapping option of JoinMap 4 software (van Ooijen, 2006). The Haldane mapping function was used to calculate centiMorgan (cM) distances. When linkage groups from the same chromosome were not able to be incorporated into a single map, they were kept as distinct linkage groups and numbered in order from the distal end of the short arm according to the marker positions on the wheat consensus map in GrainGenes 2.0 (www.wheat.pw.usda.gov). Marker orders on our maps were compared to the wheat consensus map.

Field Trials

Replicated field trials were grown in five environments over three growing seasons (2007-2009) and are summarized in Table 2.1. Henceforth, the terms ‘fully irrigated’ and ‘wet’ are used interchangeably, and ‘partially irrigated’ and ‘dry’ are also used interchangeably when describing each field trial/treatment/environment. The year used to describe each trial indicates the year of harvest.

The 2007 trial consisted of short, two-row plots 0.91 meters long, and the 2008 and 2009 trials were 3.88 m long. All trials (2007 to 2009) had 23 cm spacing between

rows in a plot and 28 cm between plots. Trial dimensions in 2007 were 52 plots long by 9 plots wide. In 2008, trials were 32 plots long by 12 plots wide. In 2009, dimensions were 16 plots long by 24 plots wide. Each plot was planted to provide an approximate density of 1,400,000 seeds/ha. Trials were planted in September of each year and harvested the following July. In addition to the two parents, two Colorado cultivars, Ripper (Haley et al., 2008) and Hatcher (Haley et al., 2005) were used as checks. Trials at ARDEC in Fort Collins were irrigated with a linear overhead sprinkler system, and trials at the USDA-ARS Limited Irrigation Research Farm in Greeley, Colorado used a drip irrigation system for more precise water distribution. At both Fort Collins and Greeley, wet and dry treatments were irrigated equally after planting and in early spring;

Table 2.1 Planting locations summary for the Ankor/Sokoll population.

Year	**Environment**	**No. BC Lines**	**No. Checks**	**No. Reps**
2007	Fort Collins fully irrigated	228	4	2
2008	Fort Collins partially irrigated	188	4	2
2008	Fort Collins fully irrigated	188	4	2
2009	Greeley partially irrigated	188	4	2
2009	Greeley fully irrigated	188	4	2

however, the Fort Collins wet treatment received approximately 13.3 cm of supplemental water during stem elongation, heading, and grain filling, while the Greeley wet treatment received supplemental irrigation during grain filling. Fertilization and weed control followed practices that are typical for winter wheat in the area.

Entries for all environments were randomized using a latinized row-column design created with CycDesigN 3.0 (www.cycdesign.co.nz/). However, the 2009 Greeley environment was considered a completely randomized design due to a planting error so that entries were not planted in their assigned row-column order.

Trait Evaluation

Data were recorded for multiple traits in all environments from 2007-09 (Table 2.2). Heading date was recorded as the number of days from January 1 to when 50% of the spikes in each plot had fully emerged above the collar of the flag leaf. Days to physiological maturity was recorded as the number of days from January 1 to when 50% of the plants in each plot displayed a yellow peduncle. Grain fill duration was calculated as the days between physiological maturity and heading. Days to physiological maturity and grain fill duration were only recorded in the 2007 and 2008 environments (Table 2.2). Plant height was measured as the distance in centimeters from the ground to the tip of the awns on the head of three to five plants per plot.

Grain weight was obtained from the 2007 trial by hand harvesting, threshing, and weighing the grain. Grain weight was obtained in 2008 and 2009 field trials from a plot combine that threshed and weighed seeds. Grain weight in grams was converted to kg/ha. Test weight was measured according to standard wheat testing methods (Kansas State University, 2008). A container having the dimensions of 5.5 cm diameter x 4.5 cm length was used, and the weight was estimated in kg/hL. Test weight was only recorded in the 2008 and 2009 environments, as seen in Table 2.2.

To calculate spike length, the mean length in centimeters of a sample of 10 spikes was measured for each plot. Spikes were measured from the base of the head to the tip,

not including the awns. Kernels from this sample of 10 spikes were then threshed, and the weight in grams and number of kernels were determined for the bulk sample. This

Table 2.2. Phenotypic traits recorded in five environments for the Ankor/Sokoll BC_2F_2-derived population.

Trait	**Fort Collins 2007 (Wet)**	**Fort Collins 2008 (Dry)**	**Fort Collins 2008 (Wet)**	**Greeley 2009 (Dry)**	**Greeley 2009 (Wet)**
Grain yield	X	X	X	X	X
Test weight		X	X	X	X
Plant height	X	X	X	X	X
Days to heading	X	X	X	X	X
Days to physiological maturity	X	X	X		
Grain fill duration	X	X	X		
Kernel number per spike	X	X	X	X	X
Average Kernel weight	X	X	X	X	X
Spike length	X	X	X	X	X
Total kernel number per plot	X	X	X	X	X
Total spike number per plot	X	X	X	X	X

information was used to determine the mean kernel weight in milligrams and number of kernels per spike. Total kernel number per plot was calculated as the grain weight per plot divided by the average kernel weight. Total spike number per plot was calculated as the kernel number per spike divided by the total kernel number per plot. Total kernel and spike number were calculated per m^2 for all environments.

Statistical Analysis: Field data

All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc., 2008). To analyze phenotypic data for the five environments from 2007-09, the MIXED and GLM procedures were used. Proc MIXED was used to obtain least square means (LS means), which were then used for subsequent analysis of Ankor and BC_2-derived means and standard errors, correlations, and QTL analysis.

To account for field variability in the different environments, a spatial adjustment model similar to that employed by Butler et al. (2005) was used in the Proc MIXED

procedure. The ‘x’ and ‘y’ coordinates were assigned to all plots to adjust trait values based on the distance from the center of the adjacent plot in both dimensions. For the 2007-09 environments, the x and y coordinates were assigned, starting at 0, in 0.50 m and 4.58 m increments, respectively. Due to a mis-placement of seeds in the seed trays and consequently, a change in planting order in the Greeley 2009 dry and irrigated environments, the Proc MIXED procedure included the entry as a random effect for these environments. To maintain a consistent analysis across the five environments, the 2007 wet and 2008 dry and wet environments also included entry as a random effect. LS means were used for entries.

Means and standard errors were recorded for Ankor and the population of backcross lines, and the range was recorded for the backcross lines. Frequency distributions were determined using the SAS UNIVARIATE procedure for the following traits: grain yield, days to heading, test weight, spike length, and kernel number per spike. The Shapiro-Wilk test was used to evaluate the normality of distributions for all traits. Pearson correlation coefficients among all pairs of the phenotypic traits were obtained using the SAS CORR procedure (SAS Institute Inc., 2008). In addition, differences in performance among Ankor and the backcross lines were determined using the SAS means statement with the LSD mean separation option of the GLM procedure.

To determine heritability for all traits in each environment, mean squares, coefficients of variation, and significance levels were recorded from PROC GLM procedures. Narrow sense heritability was estimated based on variance components (Hallauer and Miranda, 1981), and the 90% confidence interval for the heritability estimate was calculated according to Knapp et al. (1985). For single environments,

heritability was calculated as $h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_G / r)$. For multiple environments, it was calculated as $h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_{GE} / n + \sigma^2_{GE} / rn)$. Furthermore, the genotype by environment interaction was ascertained using the SAS GLM procedure with combined data for all environments. Random effects under this model were entries and environments.

S*tatistical Analysis: QTL detection*

Single factor QTL analysis was performed on the BC_2F_2-derived population using phenotypic data collected from the five 2007-09 environments and the molecular marker data from the corresponding lines. This analysis was performed using the SAS GLM procedure. Each trait in each environment was analyzed for associations with all markers. The threshold significance level of $P \leq 0.01$ was used to indicate the presence of a QTL. In addition, the R^2 value (percent of phenotypic variance explained by the QTL) and the average additive effect of the Ankor and Sokoll alleles were also determined. Adjacent markers that were significant for the same trait were considered distinct QTL if they were separated by 20 cM or more (Maccaferri et al., 2008).

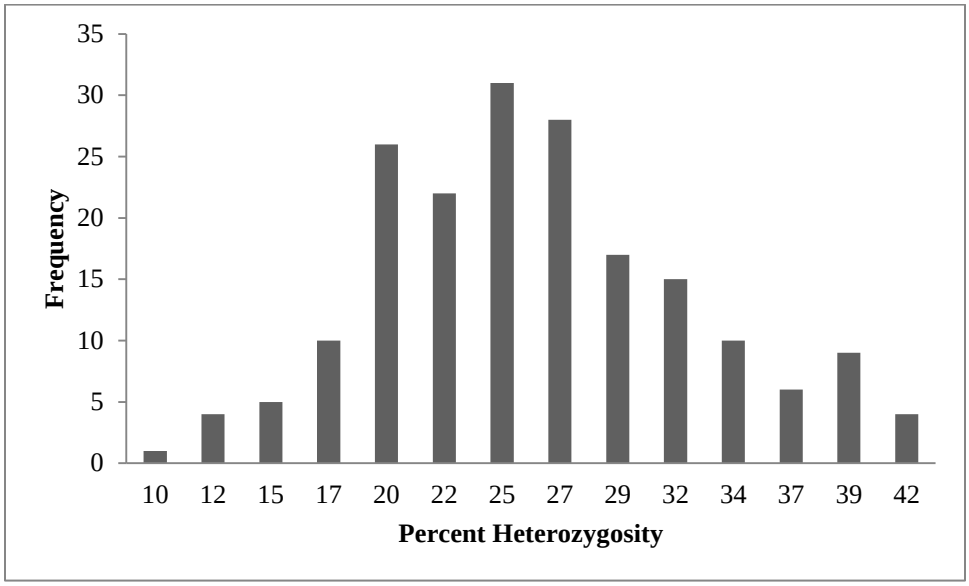
RESULTS

Molecular marker analysis

Of the 424 SSR markers screened on the parents, 314 (74%) were polymorphic. One hundred eighty-two markers with distinct, easily scorable polymorphisms were chosen to map the F_2 generation. Thirty of those markers were scored as dominant. Fifteen loci (8.2%) deviated ($P \leq 0.05$) from the expected 1:2:1 segregation ratio for co-dominant markers or 3:1 for dominant markers, and six loci (3.3%) deviated at the $P \leq 0.01$ level of significance. Of the 15 markers that showed segregation distortion, all but one had an excess of 'A' (Ankor allele) scores. Three distorted markers were located on chromosome 2A and four on chromosome 7B, all of which had an excess of A scores.

Of the 182 markers scored in the F_2 generation, 76 markers with easily scorable polymorphisms were genotyped in the $BC_2F_{2:3}$ generation for single factor QTL analysis. The frequency distribution of the percent heterozygosity of the 188 lines for the 76 markers is summarized in Figure 2.2. It is evident the percent heterozygosity was normally distributed, and the mean percent heterozygosity was 24.7%, near the expected 25% heterozygotes in this generation. Of the 76 markers, 25 deviated ($P \leq 0.05$) from the expected segregation ratio of 3:1 (75% homozygous for Ankor alleles to 25% heterozygous genotypes). Nineteen of these markers deviated at the $P \leq 0.01$ level of significance. Only marker *Xgwm219* deviated ($P \leq 0.01$) in both the F_2 and the BC_2F_2-derived generations. Segregation distortion was evident for marker *Xgwm499*, which had and had an excess of A scores. Marker *Xgwm499* is located on chromosome 5B where near *Vrn-B1*. It is possible that distortion occurred because of a strong selection for the winter-type habit of Ankor.

Figure 2.2. Percent heterozygosity of 188 lines in the Ankor/Sokoll BC_2F_2-derived population.



Linkage map construction

Forty-two linkage groups were constructed in JoinMap from 144 SSR markers (Figure 2.3). The linkage groups included regions of all 21 chromosomes. Twenty-one groups had LOD scores of 9.0 or 10.0, 16 had LOD scores between 4.0 and 8.0, and four groups a LOD score of 3.0. Chromosome assignments were based on information from GrainGenes 2.0 (www.wheat.pw.usda.gov/GG2/ index.shtml). The linkage maps spanned 1414 cM, covering 55.5% of the wheat genome relative to the SSR consensus map of Somers et al. (2004). Linkage maps for the A genome chromosomes ranged from 2.8 cM (1A) to 57.5 cM (2A) and covered a total of 530.0 cM (37.5% of the total map length). The linkage groups in the B and D genomes ranged from 5.6 cM (1B) to 107.3 cM (2B)

Figure 2.3. Linkage groups for the Ankor/Sokoll F_2 population for chromosome groups 1 to 7.

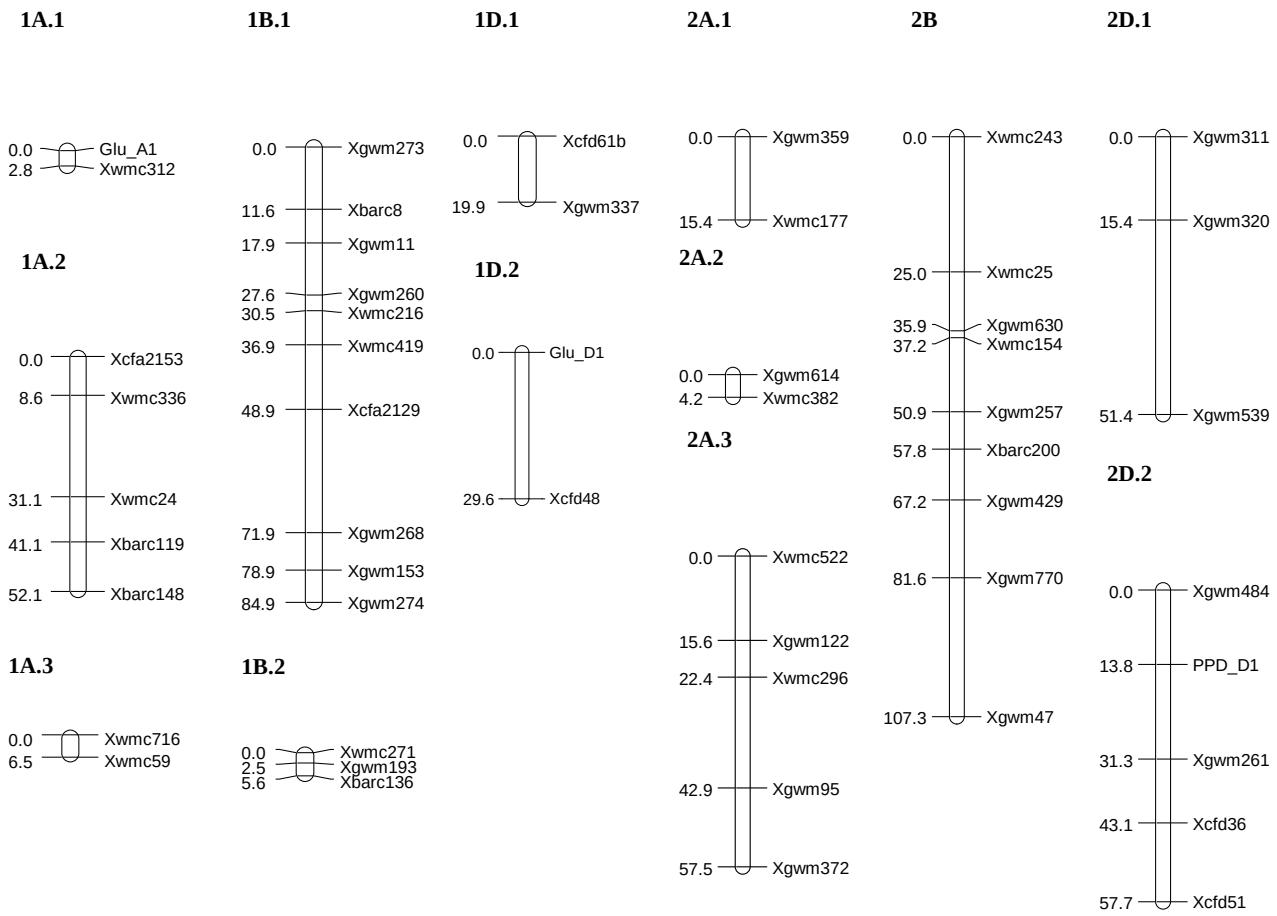


Figure 2.3. Continued.

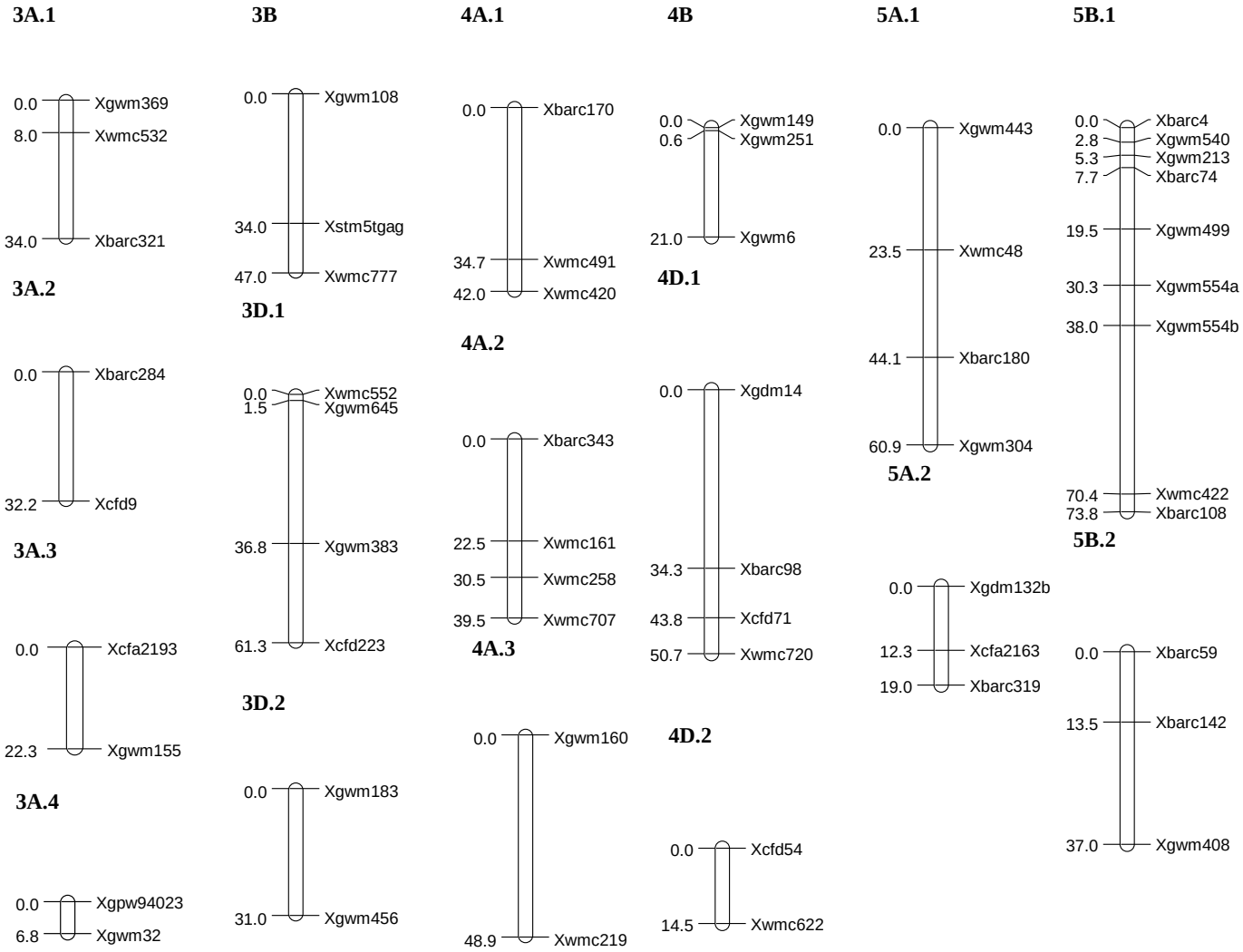
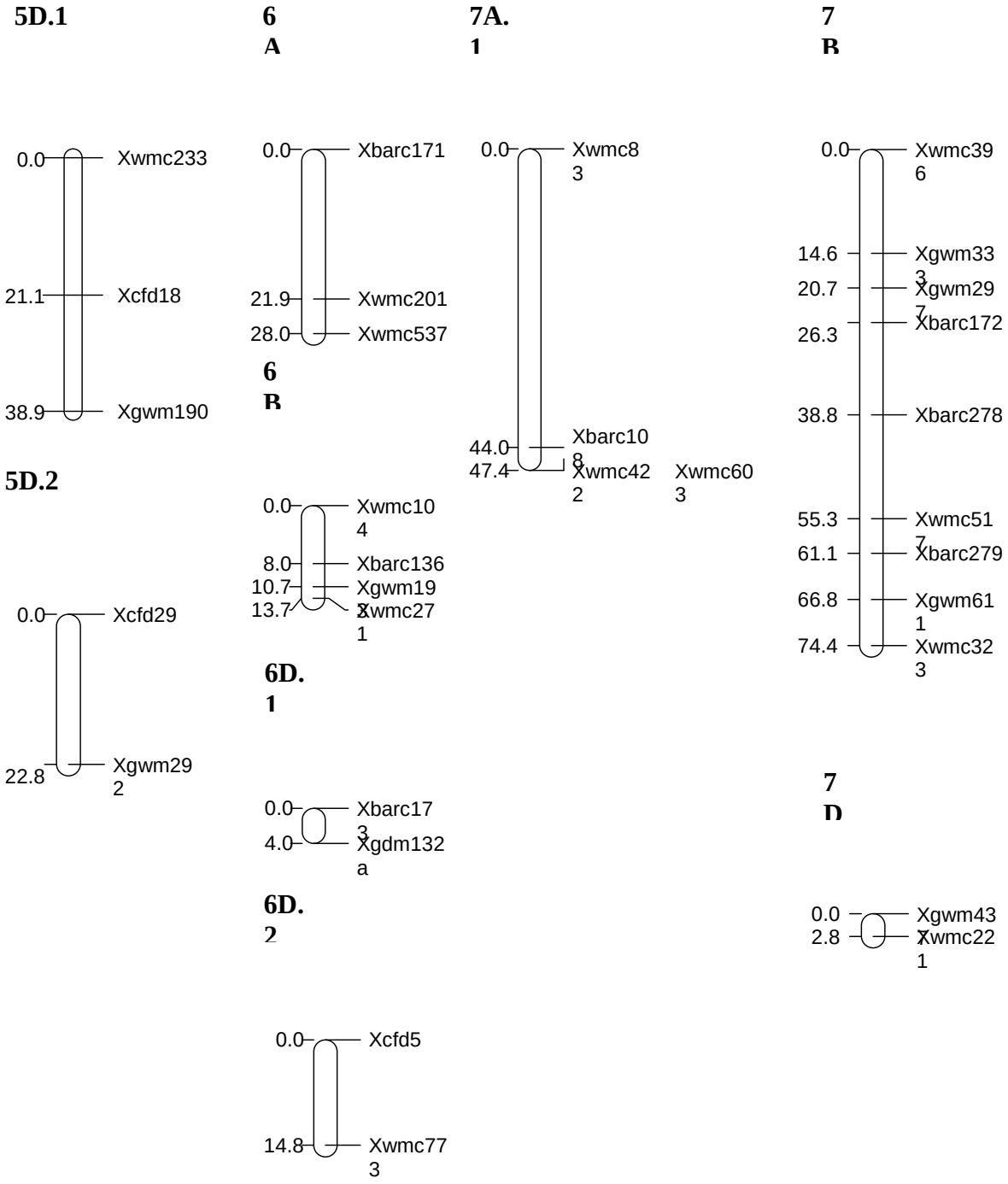


Figure 2.3. Continued.



and 4.0 cM (6D) to 57.7 cM (2D), respectively. The B genome spanned 447.0 cM (31.6% of the total map length) and the D genome spanned 437.4 cM (30.9% of the total map length). The most marker-dense chromosome regions were found on chromosomes 1A, 1B, 2A, 2B, 3A, 5B, and 5D, with 9 to 31 markers per chromosome. All chromosomes except 2B, 3B, 4B, 6A, 6B, 7B, and 7D were represented by multiple (two to four) linkage groups.

Frequency Distributions

Least squares (LS) means were obtained for each line and trait, and frequency distributions were plotted (Figures A-AT). Frequency distribution plots are informative because they reveal information about a particular trait, such as the range of phenotypic performance of all the lines in an environment, the presence of transgressive segregation, indications of the type of inheritance, and whether the trait is approximately normally distributed, an assumption in analysis of variance.

Grain yield for all environments was approximately normally distributed upon visual inspection (Appendix Figures A-E). One exception was the Greeley 2009 wet environment, as there was skewing to the left (Appendix Figure D). Days to heading (Appendix Figures K-O) and spike length (Appendix Figures AA-AE) for all environments appeared normally distributed; however, test weight for the 2008 Fort Collins dry and wet and 2009 Greeley wet environments displayed skewing to the left (Appendix Figures R-T). In addition, the distribution for kernels per spike was skewed to the left for the 2008 and 2009 dry environments (Appendix Figures AK-AO). Transgressive segregation for grain yield, test weight, days to heading, spike length, and kernels per spike was evident beyond the Ankor parent, indicating that alleles from

Sokoll improved the performance of Ankor or at least increased the trait values. The spring wheat Sokoll parent per se did not have usable mean yield values because of its poor adaptation to Colorado winters; therefore, the effect of transgressive segregation relative to Sokoll cannot be appropriately ascertained.

Fort Collins 2007 irrigated field trial

Results of the analysis of the Fort Collins 2007 irrigated field trial reveal a wide range of values for all traits as outlined in Table 2.3. In addition, the Ankor mean was much greater than the BC_2F_2 mean only for grain yield. Ankor had very comparable means to the BC_2F_2 mean for days to heading and maturity, grain fill duration, and kernel weight per spike. The maximum values of the BC_2F_2 families for grain yield (6714.4 kg/ha) and kernel weight (41.1 mg) were greater than the Ankor means (4445.8 kg/ha and 34.2 mg, respectively). For most traits, at least one family performed significantly better than Ankor.

Analysis of variance revealed that entries differed significantly for most traits (Table 2.4). Exceptions were grain fill duration and plant height. Heritability values for all traits in the Fort Collins 2007 environment were low to moderate, ranging from 0.12 for grain fill duration to 0.61 for days to heading. Grain yield, kernels per spike, and spike length all had heritability values indicating that greater than 50% of the variation was explained by genotype. The heritability of these traits was 0.59, 0.58, and 0.53, respectively (Table 2.4). Simple phenotypic correlation coefficients were estimated for all pairs of traits using the SAS CORR procedure. All 10 traits recorded in 2007 were significantly ($P \leq 0.05$) correlated with at least one other trait (Table 2.5). Grain yield was

Table 2.3. Performance of Ankor and (Ankor/Sokoll) BC_2F_2 families for eight traits in Fort Collins, 2007. Mean values are presented ± standard error.

	Ankor	BC_2F_2		
Trait†	**Mean ± SE**	**Mean ± SE**	**Range**	**C.V.**
GY (kg/ha)	4445.8 ± 637.0	4005.5 ± 621.6	1224.9 – 6714.4 (0)‡	22.8
KS (no.)	27.9 ± 2.8	30.7 ± 2.7	17.7 - 40.5 (26)	12.8
AKW (mg)	34.2 ± 1.8	34.6 ± 1.8	29.6 - 41.1 (4)	7.2
DH (no.)	144.8 ± 0.9	144.6 ± 0.9	139.5 - 149.1 (5)	1.0
DPM (no.)	180.3 ± 1.4	180.7 ± 1.3	176.4 - 185.9 (16)	1.4
GFD (days)	35.4 ± 1.5	36.0 ± 1.4	31.4 - 39.4 (15)	7.4
PH (cm)	85.6 ± 2.8	86.1 ± 2.6	76.0 - 97.9 (2)	8.6
SL (cm)	9.9 ± 0.4	10.5 ± 0.4	9.1 - 11.9 (58)	4.6
TKN (no. m^{-2})	12,995.6 ± 83.0	11,565.4 ± 196.2	3967.0 – 19,149.0 (0)	23.3
TSN (no. m^{-2})	465.2 ± 3.6	378.8 ± 6.2	142.7 – 625.0 (0)	23.2

†GY = grain yield; KS = kernels per spike; AKW = average kernel weight; DH = days to heading; DPM = days to physiological maturity; GFD = grain fill duration; PH = plant height; SL = spike length; TKN=total kernel number per 1 m^2 plot; TSN= total spike number per 1 m^2; ‡Values in parentheses indicate the number of lines significantly ($P \leq 0.05$) greater than the Ankor mean.

significantly ($P \leq 0.05$) correlated with total kernel and spike number, average kernel weight, kernel number per spike, days to physiological maturity, and spike length. These correlations with yield ranged from -0.18 for days to physiological maturity to 0.97 for total kernel number. Grain yield was strongly correlated with total kernel number (r=0.97, $P \leq 0.001$) and total spike number (r=0.74, $P \leq 0.001$). Grain yield was also

Table 2.4. Statistical analysis of traits recorded in the Ankor/Sokoll population at Fort Collins 2007 wet field trials. Analysis was conducted using Proc GLM.

Trait	**Entry mean square**	**$h^{2\dagger}$**	**C.I.‡**
Grain yield (kg/ha)	114,603.13***	0.59	0.48 , 0.68
Kernels per spike	37.00***	0.58	0.47 , 0.67
Kernel weight (mg)	7.97*	0.22	0.01 , 0.39
Days to heading	5.01***	0.61	0.50 , 0.69
Days to physiological maturity	9.56***	0.38	0.21 , 0.51
Grain fill duration	8.13^{ns}	0.12	0 , 0.31
Plant height (cm)	70.7^{ns}	0.21	0 , 0.38
Spike length (cm)	0.51***	0.53	0.40 , 0.63
Total kernel number (no. m^{-2})	$1.6x\ 10^{7}$***	0.56	0.44, 0.66
Total spike number (no. m^{-2})	14756.0***	0.46	0.31, 0.57

*, **, ***, ^{ns} Significant at the 0.05, 0.01, and 0.001 probability levels, respectively, ^{ns} = not significant.
† Narrow sense heritability
‡ 90% confidence interval of heritability estimate

positively correlated with average kernel weight (r=0.36, $P \leq 0.001$). Butler et al. (2002) also discovered positive correlations between grain yield and two-hundred kernel weight in three of four environments tested, and El Feki et al. (2010) found positive correlations with grain yield and average kernel weight in all four environments examined in his study. Days to physiological maturity was correlated with days to heading and grain fill duration (r=0.54 and -0.55, $P \leq 0.001$, respectively). These correlations indicate the longer it takes for a line to reach heading stage, the longer it will take to reach maturity,

Table 2.5. Significant Pearson correlation coefficients among 10 agronomic traits in 188 Ankor/Sokoll BC_2F_2 lines at Fort Collins 2007 wet field trials.

Trait‡	GY	KS	AKW	DH	DPM	GFD	PH	SL	TKN	TSN
GY		0.47***	0.36***		-0.18*			0.21**	0.97***	0.74***
KS			0.26**					0.40***	0.45***	-0.18*
AKW								0.26**	0.15*	
DH					0.54***	-0.38***	0.28***	-0.17*		-0.20**
DPM						-0.55***			-0.16*	-0.22**
GFD								0.15*		
PH										
SL									0.15*	
TKN										0.79***
TSN										

*, **, and ***, significant at the 0.05, 0.01, and 0.0001 levels of probability, respectively. Blank spaces indicate values were not significant.
‡ GY, grain yield; KS, kernels per spike; AKW, average kernel weight; DH, days to heading; DPM, days to physiological maturity; GFD, grain fill duration; PH, plant height; SL, spike length; TKN, total kernel number per 1 m^2 plot; TSN, total spike number per 1 m^2.

but the shorter the grain fill duration due to warmer temperatures later in the season. Days to heading was correlated with four traits ranging from -0.38 for grain fill duration to 0.54 for days to physiological maturity. The negative correlation for spike length (r=-0.17, $P \leq 0.05$) indicates as days to heading occurs later in the season, spike length decreases. This might be the result of more favorable temperature and moisture conditions occurring earlier in the season when spikelet primordia are initiated. This process occurs in parallel to the apex changing from conical form during leaf initiation to cylindrical form during spikelet primordia formation (Kirby, 1974).

Fort Collins 2008 dry and wet field trials

Overall, the 2008 field trials had the highest yields of trials from 2007 to 2009, indicating favorable growing conditions. In 2008 the $BC_2F_{2:3}$ lines experienced a 14.7% yield reduction in the dry treatment. Analysis of variance revealed Ankor had higher values than the mean of the $BC_2F_{2:3}$ lines for seven and eight of 11 traits in the dry and wet trials, respectively (Table 2.6). Ankor outperformed the $BC_2F_{2:3}$ mean for grain yield, plant height, test weight, total kernel number, and total spike number in both the 2008 wet and dry environments. The maximum value of the $BC_2F_{2:3}$ lines for grain yield was about 500 kg/ha higher than the Ankor mean and about 12.5 cm greater for plant height than the Ankor mean, indicating transgressive segregation. A greater plant height is not necessarily better, as it may increase the chance of lodging, and excessive stem length can reduce partitioning to the grain or decrease harvest index.

All Ankor and $BC_2F_{2:3}$ trait means were greater in the wet environment than their counterparts in the dry environment, except kernel weight and test weight. The average

Table 2.6. Performance of Ankor and Ankor/Sokoll $BC_2F_{2:3}$ lines for nine traits in Fort Collins, 2008. Mean values are presented ± standard error.

	2008 Fort Collins dry					2008 Fort Collins wet			
	Ankor	$BC_2F_{2:3}$				Ankor	$BC_2F_{2:3}$		
Trait†	**Mean ± SE**	**Mean ± SE**	**Range**	**C.V. ‡**		**Mean ± SE**	**Mean ± SE**	**Range**	**C.V.**
GY (kg/ha)	3840.1 ± 270.9	3174.8 ± 274.3	1758.7 - 4394.6 (3) §	21.9		4402.0 ± 270.1	3742.9 ± 259.1	2213.3 - 4849.6 (12)	12.7
KS (no.)	29.2 ± 2.9	31.5 ± 2.9	12.5 - 41.3 (0)	15.3		38.3 ± 2.6	37.1 ± 2.7	28.9 - 46.6 (0)	10.9
AKW (mg)	39.7 ± 13.5	40.3 ± 13.5	30.1 - 48.8 (1)	4.7		37.6 ± 1.4	37.6 ± 1.5	32.1 - 45.1 (8)	5.9
DH (no.)	154.9 ± 0.9	154.7 ± 0.9	152.0 - 158.2 (3)	1.1		156.8 ± 0.9	157.8 ± 0.9	154.2 - 161.4 (7)	1.2
DPM (no.)	186.5 ± 0.6	187.0 ± 0.6	184.9 - 189.7 (7)	0.9		191.8 ± 0.6	191.3 ± 0.6	189.1 - 194.6 (0)	0.8
GFD (days)	31.6 ± 1.0	32.4 ± 1.0	29.7 - 35.7 (2)	5.4		34.8 ± 0.9	33.4 ± 0.9	30.3 - 36.8 (0)	4.4
PH (cm)	77.1 ± 3.4	76.3 ± 3.4	62.3 - 88.2 (16)	11.1		85.6 ± 3.1	84.6 ± 3.1	57.6 - 95.3 (0)	6.5
SL (cm)	9.8 ± 0.3	9.7 ± 0.3	7.6 - 11.8 (34)	6.8		10.1 ± 0.3	10.2 ± 0.3	8.9 - 11.5 (56)	4.7
TW (kg/hL)	79.2 ± 0.6	78.3 ± 0.6	75.4 - 80.1 (0)	1.5		76.1 ± 0.5	75.3 ± 0.5	72.3 - 77.2 (0)	1.4
TKN (no. m^{-2})	9682.7 ± 101.0	8081.1 ± 96.8	1613.8 – 12,094.0 (0)	12.1		11,721.3 ± 61.5	9939.6 ± 96.5	6406.4 – 10586.9 (0)	6.5
TSN (no. m^{-2})	331.5 ± 2.8	257.5 ± 2.8	128.8 – 361.6 (0)	10.7		306.0 ± 3.6	268.9 ± 2.9	180.6 – 369.5 (1)	7.6

†GY = grain yield; KS = kernels per spike; AKW = average kernel weight; DH = days to heading; DPM = days to physiological maturity; GFD = grain fill duration; PH = plant height; SL = spike length; TW = test weight; TKN= total kernel number per 1 m^2; TSN= total spike number per 1 m^2.
‡ C.V., coefficient of variance.
§Values in parentheses indicate the number of lines significantly ($P \leq 0.05$) greater than the Ankor mean and were determined by SAS Proc GLM.

kernel weight was larger (Ankor by 2.1 mg and the $BC_2F_{2:3}$ mean by 2.7 mg) in the dry treatment, but the kernel number per spike was quite a bit less (9.1 and 5.6 fewer kernels for Ankor and the BC_2 population, respectively), indicating fewer but larger grains wereproduced in the 2008 dry as compared to the wet environment. This is also evident in the fact that the total kernel number per plot was greater in the 2008 wet than dry trial, yet the total spike number per plot was greater in the 2008 dry than wet trial. In addition, days to heading and days to physiological maturity both occurred a few days later in the irrigated environment. It is possible that the irrigated treatment was less stressed and thus entered into heading later, whereas the dry treatment plants may have been more stressed and started putting their resources into seed production earlier. Ankor fell in the middle of the six-day range for the $BC_2F_{2:3}$ line means for days to heading in both environments. It is expected that the range of days to heading is similar between all lines and Ankor because the lines contain on average 87.5% Ankor DNA, and they were selected for winter type alleles at two *Vrn* loci.

Analysis of variance revealed significant ($P \leq 0.05$) differences among entries for five and eight of 11 traits in the Fort Collins dry and wet treatments, respectively (Table 2.7). Grain yield, days to heading, spike length, and total kernel and spike number differed significantly ($P \leq 0.01$) among entries in the Fort Collins 2008 dry environment. In addition, the heritability values in the dry treatment were low, ranging from 0.07 (grain fill duration and kernels per spike) to 0.43 for test weight. This reveals a large amount of environmental variation and/or variation in the evaluation techniques by different people. The heritability estimates for the wet treatment traits were much higher, ranging from 0.19 (days to physiological maturity) to 0.96 (test weight). The heritability for grain

Table 2.7. Statistical analysis of traits recorded in the Ankor/Sokoll population at Fort Collins 2008 dry and wet field trials. Analysis was conducted using Proc GLM.

2008 Fort Collins dry					**2008 Fort Collins wet**			
Trait†	**MS_{entry}‡**	**h^2§**	**C.I. ¶**		**Trait**	**MS_{entry}**	**h^2**	**C.I.**
GY (kg/ha)	733325.5***	0.37	0.20 , 0.51		**GY (kg/ha)**	19005.08***	0.55	0.36 , 0.61
KS	25.3^{ns}	0.07	0 , 0.27		**KS**	23.63***	0.31	0.12 , 0.46
AKW (mg)	403.32^{ns}	0.10	0 , 0.29		**AKW (mg)**	6.22^{ns}	0.22	0 , 0.38
DH	4.18**	0.29	0.10 , 0.44		**DH**	4.91*	0.22	0.01 , 0.39
DPM	3.03^{ns}	0.17	0 , 0.35		**DPM**	2.58^{ns}	0.19	0 , 0.37
GFD	2.92^{ns}	0.07	0 , 0.16		**GFD**	3.53***	0.38	0.21 , 0.51
PH (cm)	89.4^{ns}	0.18	0 , 0.36		**PH (cm)**	37.92^{ns}	0.20	0 , 0.37
SL (cm)	0.74***	0.42	0.26 , 0.54		**SL(cm)**	0.52***	0.56	0.43, 0.65
TW (kg/hL)	1.34^{ns}	0.43	0.28 , 0.55		**TW (kg/hL)**	26.91***	0.96	0.95 , 0.97
TKN (no. m^{-2})	5.5×10^{6}**	0.34	0.16, 0.48		**TKN**	3.5×10^{6}***	0.54	0.41, 0.64
TSN (no. m^{-2})	4220.7**	0.32	0.13, 0.47		**TSN**	3040.0***	0.47	0.32, 0.58

*, **, ***, ^{ns} Significant at the 0.05, 0.01, and 0.001 probability levels and not significant, respectively
†GY=grain yield; KS=kernels per spike; AKW=average kernel weight; DH=days to heading; DPM=days to physiological maturity; GFD=grain fill duration; PH=plant height; SL=spike length; TW=test weight; TKN=total kernel number per 1 m^2; TSN=total spike number per 1 m^2;
‡Mean square entry
§Narrow sense heritability
¶ 90% confidence interval of heritability estimate

yield was 0.55, which is comparable to 0.59 for the Fort Collins 2007 irrigated trial, and average kernel weight and spike length had similar heritability values to the 2007 irrigated trial. In general, the heritability estimates for all traits in the Fort Collins 2008

wet environment were higher than in the 2008 dry environment, and heritability values for plant height, days to heading, and days to physiological maturity for the 2008 wet environment were all comparable to the dry counterparts (Table 2.7).

Pearson correlation coefficients of the 2008 dry trial reveal days to heading again was correlated with the most traits ranging from -0.80 for grain fill duration to 0.29 for plant height (Table 2.8). Days to heading was correlated (r=0.15, $P\leq$0.05) to kernels per spike in the Fort Collins 2008 dry but not the Fort Collins 2008 wet environment. Grain yield showed highly significant correlations ($P\leq$0.001) with total kernel and spike number (r=0.80 and 0.72, respectively), yet low but significant correlations with plant height, kernel number per spike, and test weight (r=0.19 to 0.23, $P\leq$0.05) and no significant correlation in either environment with average kernel weight. As total kernel and spike number, kernel number per spike, and test weight increase, it is understandable that grain yield also increased. However, as plant height increases, there should be an increase in grain yield only up to a point. After that point, taller stems can reduce partitioning to grain, thus reducing yield. Spike length was negatively correlated with grain fill duration (r=-0.20 and -0.24, $P\leq$0.01) and positively correlated with days to heading (r=0.29 and 0.41, $P\leq$0.001) in the dry and wet environments, respectively. Spike length was more strongly associated with days to heading in the wet versus the dry environment, indicating the wet environment may be better for spikelet development because conditions were more favorable than the dry environment over time. The strongest correlation in the Fort Collins 2008 wet environment was the correlation between grain yield and total kernel and spike number per plot (r=0.93 and 0.72, $P\leq$0.001). Another strong correlation was that between grain fill duration and days to

Table 2.8. Significant Pearson correlation coefficients among 11 agronomic traits in 188 Ankor/Sokoll $BC_2F_{2:3}$ lines at the Fort Collins 2008 dry and wet field trials. †

Trait‡	GY	KS	AKW	DH	DPM	GFD	PH	SL	TW	TKN	TSN
GY		0.21*								0.93***	0.72***
KS	0.22**				0.29***			0.33***	0.17*	0.21**	-0.44***
AKW		-0.48***								-0.27***	-0.22**
DH		0.15*			0.41***	-0.84***	0.32***	0.41***			
DPM		0.33***		0.26*				0.37***			-0.18*
GFD				-0.80***	-0.33***		-0.28***	-0.24***			
PH	0.23**					-0.27**					
SL		0.16*		0.29***		-0.20**	0.28***				-0.23
TW	0.19*			-0.20**	-0.25**						
TKN	0.80***	0.47***	-0.40***				0.20**				0.78***
TSN	0.72***		-0.29***		-0.24***		0.16*		0.17*	0.83***	

†Values above the diagonal refer to the fully irrigated treatment, and values below the diagonal refer to the limited irrigation treatment.
*, **, and ***, significant at the 0.05, 0.01, and 0.0001 levels of probability, respectively. Blank spaces indicate values were not significant.
‡ GY, grain yield; KS, kernels per spike; AKW, average kernel weight; DH, days to heading; DPM, days to physiological maturity; GFD, grain fill duration; PH, plant height; SL, spike length; TW, test weight; TKN, total kernel number per 1 m^2; TSN, total spike number per 1 m^2.

heading (r=-0.84, $P \leq 0.001$), and the corresponding correlation in the Fort Collins 2008 dry environment was a comparable r-value of -0.80. Lines that headed later entered the grain-filling period as temperatures were already warming up and soil moisture was becoming more limiting. Consequently, they senesced more quickly and had a shorter time to fill grains.

Greeley 2009 dry and wet field trials

Phenotypic results of the Greely 2009 dry and wet trials reveal numerous differences within and between treatments. In 2009, there was a yield reduction of 23.3% from the wet to the dry trial, suggesting the influence of moisture stress. The Ankor mean was higher than the mean of the $BC_2F_{2:3}$ lines for seven of nine traits (grain yield, kernels per spike, average kernel weight, plant height, test weight, and total kernel and spike number) in the 2009 dry environment. In contrast, the $BC_2F_{2:3}$ mean was greater than Ankor for these same seven traits in the 2009 wet environment. In the wet environment, Ankor exceeded the $BC_2F_{2:3}$ mean only for days to heading. The Ankor and $BC_2F_{2:3}$ means were comparable in the wet and dry environments for spike length, though the $BC_2F_{2:3}$ means were 0.6 cm and 0.9 cm higher than the Ankor means in the dry and wet environments, respectively. The range for grain yield in the wet environment included many lines greater than the Ankor mean (3330 kg/ha) in the wet treatment, with the highest yield at approximately 4298 kg/ha, indicating transgressive segregation. In fact, transgressive segregation was evident in most traits in both environments, as the $BC_2F_{2:3}$ ranges included values significantly greater than the Ankor mean (Table 2.9).

Analysis of variance revealed that all nine traits differed significantly ($P \leq 0.01$) among entries in the Greeley 2009 wet environment, and eight of nine traits were

Table 2.9. Performance of Ankor and (Ankor/Sokoll) $BC_2F_{2:3}$ families for nine traits in Greeley, 2009. Mean values are presented ± standard error.

	2009 Greeley dry					2009 Greeley wet			
	Ankor	$BC_2F_{2:3}$				Ankor	$BC_2F_{2:3}$		
Trait†	**Mean ± SE**	**Mean ± SE**	**Range**	**C.V.‡**		**Mean ± SE**	**Mean ± SE**	**Range**	**C.V.‡**
GY (kg/ha)	3373.4 ± 373.5	2648.2 ± 374.5	1478.8 - 3677.2 (4) ‡	16.0		3329.7 ± 319.9	3451.3 ± 319.4	1614.2 - 4298.2 (3)	13.1
KS (no.)	35.7 ± 3.0	34.9 ± 3.0	18.2 - 45.2 (0)	11.9		35.4 ± 1.2	35.7 ± 1.2	30.2 - 39.9 (0)	10.2
AKW (mg)	36.9 ± 1.4	35.0 ± 1.4	29.4 - 41.6 (1)	4.8		35.9 ± 2.5	36.0 ± 2.5	24.0 - 45.4 (2)	5.1
DH (no.)	142.5 ± 0.9	142.7 ± 0.9	140.0 - 145.0 (3)	0.7		144.8 ± 0.6	144.3 ± 0.6	141.1 - 146.8 (6)	0.1
PH (cm)	79.1 ± 5.0	77.2 ± 5.0	64.5 - 90.9 (7)	7.9		95.3 ± 2.4	99.2 ± 2.4	91.8 - 112.2 (14)	4.5
SL (cm)	9.6 ± 0.6	10.2 ± 0.6	9.1 - 11.6 (0)	8.2		9.6 ± 0.5	10.5 ± 0.5	9.1 - 12.0 (86)	6.8
TW (kg/hL)	76.1 ± 0.5	75.2 ± 0.5	73.5 - 76.9 (0)	0.8		73.9 ± 0.4	74.0 ± 0.4	71.5 - 75.6 (0)	0.8
TKN	9145.2 ± 92.0	7575.8 ± 85.4	4344.6 – 10,586.9 (0)	8,4		8675.0 ± 88.5	7575.8 ± 85.4	4173.1 – 15,119.6 (2)	7.3
TSN	256.3 ± 3.3	218.6 ± 3.0	127.9 – 431.7 (3)	11.9		242.5 ± 1.4	218.6 ± 3.0	115.8 – 402.3 (11)	9.5

†GY = grain yield; KS = kernels per spike; AKW = average kernel weight; DH = days to heading; DPM = days to physiological maturity; GFD = grain fill duration; PH = plant height; SL = spike length; TW = test weight; TKN= total kernel number per 1 m^2; TSN = total spike number per 1 m^2.
‡Values in parentheses indicate the number of lines significantly ($P \leq 0.05$) greater than the Ankor mean and were determined by SAS proc GLM.

Table 2.10. Statistical analysis of nine traits recorded in Greeley 2009 dry and wet field trials. Analysis was conducted using Proc GLM.

2009 Greeley dry					2009 Greeley wet			
Trait[†]	**MS_{entry}**‡	**h^2**§	**C.I.** ¶		**Trait**	**MS_{entry}**	**h^2**	**C.I.**
GY (kg/ha)	361253.42***	0.51	0.20 , 0.61		**GY kg/ha)**	405692.93***	0.50	0.43 , 0.65
KS	21.9*	0.20	0 , 0.39		**KS**	24.91***	0.46	0.31 , 0.58
KW (mg)	5.05***	0.43	0.28 , 0.56		**KW (mg)**	6.38***	0.47	0.33 , 0.59
DH	2.18***	0.51	0.38 , 0.61		**DH**	2.91***	0.48	0.34 , 0.59
PH (cm)	41.78^{ns}	0.10	0 , 0.30		**PH (cm)**	32.14***	0.37	0.20 , 0.51
SL (cm)	0.70***	0.00	0 , 0.21		**SL (cm)**	0.79**	0.35	0.18 , 0.49
TW (kg/hL)	1.04***	0.68	0.60 , 0.75		**TW (kg/hL)**	0.99**	0.69	0.60 , 0.75
TKN	3.0×10^6***	0.47	0.32, 0.58		**TKN**	3.4×10^6***	0.43	0.28, 0.56
TSN	4131.8***	0.35	0.17, 0.49		**TSN**	3745.2**	0.30	0.11, 0.45

*, **, ***, ^{ns} Significant at the 0.05, 0.01, 0.00, and 0.001 probability levels and not significant, respectively

[†]GY=grain yield; KS=kernels per spike; KW=average kernel weight; DH=days to heading; DPM=days to physiological maturity; GFD=grain fill duration; PH=plant height; SL=spike length; TW=test weight; TKN=total kernel number per 1 m^2; TSN=total spike number per 1 m^2.

‡Mean square entry

§ Narrow sense heritability

¶ 90% confidence interval of heritability estimate

significantly ($P \leq 0.05$) different in the Greeley 2009 dry environment (Table 2.10). Heritabilities were low to high for the Greeley 2009 dry trial, ranging from 0.00 for spike length to 0.68 for test weight, and heritability values were moderate to high for the Greeley 2009 wet trial, ranging from 0.30 for total spike number to 0.69 for test weight.

The reason for the low heritability of spike length in the dry trial is unknown; however, it is possible errors in inconsistent sampling or measurement by multiple data collectors could have led to unreliable data. For example, the biggest spikes could have been consistently chosen as opposed to the ideal of selecting spikes at random. The heritability for plant height, days to heading, and days to maturity were all comparable in

the two Greeley 2009 environments (Table 2.10). Test weight had the highest heritability in both the Greeley 2009 dry and wet and Fort Collins 2008 wet trials. The heritability for total kernel number was comparable in dry and wet environments (h^2=0.47 and 0.43, respectively), as was total spike number (h^2=0.35and 0.30, respectively).

Pearson correlation coefficients for the Greeley 2009 dry trial were low to high in magnitude, but the highest number of significant correlations was with total spike number and grain yield (Table 2.11). Grain yield was significantly ($P \leq 0.01$) correlated with four traits in the dry environment (r=0.25 for plant height to 0.97 for total kernel number). Total spike number was significantly ($P \leq 0.05$) correlated with grain yield, kernels per spike, average kernel weight, plant height, test weight, and total kernel number 2009 dry environment (r=-0.52 for kernels per spike to 0.78 for total kernel number). A negative correlation was found between test weight and both plant height and days to heading. This suggests that test weight increases when days to heading occurs earlier in the season. Earlier heading leading to a longer grain fill duration and more favorable temperature and moisture conditions.

In the 2009 wet environment total spike number was correlated with all traits. These values ranged from -0.53 for kernel weight to 0.94 for total kernel number, the strongest correlation of the 2009 wet environment (Table 2.11). Grain yield was significantly ($P \leq 0.01$) correlated with seven traits in the wet environment (r=-0.20 to 0.72), including the two main yield components, total kernel number (r=0.72, $P \leq 0.001$) and average kernel weight (r=0.24, $P \leq 0.01$). Grain yield was negatively correlated with days to heading (r=-0.20), indicating earlier heading in this population and for the conditions of this study results in a modestly higher grain yield. Days to heading and test

Table 2.11. Significant Pearson correlation coefficients among nine agronomic traits in 188 Ankor/Sokoll $BC_2F_{2:3}$ lines at Fort Collins under wet and dry conditions in the 2009.†

Trait‡	GY	KS	KW	DH	PH	SL	TW	TKN	TSN
GY		0.21**	0.24**	-0.20**		-0.18**	0.27**	0.72***	0.63***
KS					-0.17*		0.15*		-0.22**
KW					-0.28***	0.22**		-0.50***	-0.53***
DH							-0.28***	-0.18*	-0.15*
PH	0.25**			0.26**					0.19**
SL								-0.30***	-0.32***
TW	0.26**	0.15*		-0.36***	-0.26**			0.23**	0.18*
TKN	0.97***		-0.15*		0.25***		0.21**		0.94***
TSN	0.74***	-0.52***	-0.17*		0.17*		0.20**	0.78***	

†Values above the diagonal refer to the fully irrigated treatment, and values below the diagonal refer to the limited irrigation treatment.
‡ GY, grain yield; KS, kernels per spike; KW, kernel weight per spike; DH, days to heading; PH, plant height; SL, spike length; TW, test weight; TKN, total kernel number per 1 m^2; TSN, total spike number per 1 m^2.
*, **, and ***, significant at the 0.05, 0.01, and 0.001 levels of probability, respectively. Blank spaces indicate values were not significant.

weight were negatively correlated (r=-0.36 and r=-0.28, respectively; $P \leq 0.001$) in both the 2009 dry and wet treatments (Table 2.11). Spike length and kernel number per spike also showed a weak correlation of 0.22 in the 2009 wet environment. As spike length increases, there should be more spikelets and opportunities for seeds to form, thus increasing the total seed number per spike. Plant height was negatively correlated with both kernels per spike (r=-0.17, $P \leq 0.05$) and average kernel weight (r=-0.28, $P \leq 0.001$) in the wet treatment. It is difficult to understand why a decrease in plant height would correlate to an increase in average kernel weight and kernel number per spike; however,

it is possible that shorter plants translocate more photosynthate toward kernel development than biomass.

Combined phenotypic analysis for the 2007-09 environments

Results of all trials indicate a wide range of values for all traits. As stated previously, the $BC_2F_{2:3}$ lines in 2008 experienced a 15.2% yield reduction in the dry treatment; however, a more severe yield reduction of 23.3% occurred in 2009, suggesting drought stress was of greater influence during the 2009 growing season. Mean grain yield for the BC_2F_2-derived families ranged from 3174.8 kg/ha in the 2008 dry environment to 3742.9 kg/ha in the 2008 Fort Collins wet environment. Ankor means for grain yield ranged from 3840.2 kg/ha in the 2008 dry environment to 4445.8 kg/ha in the 2007 Fort Collins wet environment. Test weight values ranged from 73.9 kg/hL in the 2009 Greeley wet environment to 80.1 kg/hL in the 2008 Fort Collins dry environment for the $BC_2F_{2:3}$ lines. The greatest range for days to heading for the 90% most central lines was 5.7 days in the 2007 wet environment, and the narrowest range was 3.0 days for the 2009 dry environment. The mean range for the 90% most central lines of the dry and wet 2008 trials was 5.5 and 4.5 days, respectively. This relatively narrow range among all field trials confirms the similarity of all lines to Ankor and reduces the impact of maturity on yield measurements.

Analysis of variance revealed that eight of ten traits differed significantly ($P \leq 0.05$) in the Fort Collins 2007 and 2008 irrigated trials (Tables 2.4 and 2.7). All traits differed significantly ($P \leq 0.05$) in the Greeley field trials, except that plant height was not significant in the 2009 dry trial (Table 2.10). In addition, the heritability for grain yield was similar in the Fort Collins 2007 wet, the Fort Collins 2008 wet, and the Greeley 2009

dry and wet environments (h^2 = 0.59, 0.55, 0.51, and 0.50, respectively; see tables 2.5, 2.8, and 2.11). A combined analysis of genotype by environment (G x E) interaction for all traits in all environments revealed that G x E was highly significant ($P \leq 0.01$) for grain yield, spike length, test weight, and total kernel and spike number (Table 2.12). Test weight had the highest overall heritability (h^2= 0.88). The heritability estimates of grain yield, days to heading, and kernels per spike were also high (h^2= 0.78, 0.77 and 0.70, respectively; Table 2.12). Spike length and plant height had moderate heritability values of 0.53 and 0.50, respectively. Average kernel weight had the lowest overall heritability at 0.18.

Grain yield was significantly correlated to total kernel number (r=0.97, 0.80, 0.93, 0.97 and 0.72, $P \leq 0.001$) and total spike number (r=0.74, 0.72, 0.72, 0.74, and 0.63, $P \leq 0.001$) in all environments (Tables 2.5, 2.8, and 2.11), kernels per spike (r=0.21 to 0.47, $P \leq 0.01$) in all but the 2009 dry environment (Tables 2.5, 2.8, and 2.11), test weight in the Fort Collins 2008 dry and Greeley 2009 dry and wet environments (r=0.19. 0.26 and 0.27, $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.01$, respectively) (Tables 2.8 and 2.11). Grain yield was positively correlated with average kernel weight in both the 2007 and 2009 wet environments (r=0.36 and r=0.24, respectively), but was not significant in the other environments. Grain yield showed both positive and negative correlations for spike length in these same environments (r=0.21 and r=-0.18, respectively). Days to heading was strongly correlated to grain fill duration (r=-0.38, -0.80, and -0.84, $P \leq 0.001$) in the 2007 wet, 2008 dry, and 2008 wet environments, respectively (Tables 2.5 and 2.8). Days to heading was negatively correlated with test weight in the Greeley 2008 dry, Greeley 2009 wet, and Greeley 2009 dry field trials (r=-0.36, -0.28, and -0.20, $P \leq 0.01$, Tables 2.8

Table 2.12. Combined statistical analysis of genotype by environment interaction for all traits as calculated by Proc GLM (SAS Institute, 2008).

Trait †	**MS_{entry}‡**	**MS_{GxE}§**	**h^2¶**	**C.I.[a]**
GY (kg/ha)	1026449***	10518.24***	0.78	0.74 , 0.82
KS	56.62***	19.00^{ns}	0.70	0.63 , 0.75
KW (mg)	93.85*	83.75^{ns}	0.18	0 , 0.31
DH	10.07***	2.28^{ns}	0.77	0.73 , 0.81
DPM	6.59***	4.29*	0.35	0.19 , 0.47
GFD	5.56**	4.49**	0.19	0 , 0.34
PH (cm)	85.93***	46.51^{ns}	0.50	0.38 , 0.58
SL (cm)	1.14***	0.54***	0.53	0.42 , 0.61
TW (kg/hL)	6.36***	7.96***	0.88	0.86, 0.90
TKN	1.38 x 10^7***	4.25 x 10^{6***}	0.64	0.55, 0.70
TSN	12,037.90***	4350.00***	0.68	0.61, 0.74

*, **, ***, ^{ns} Significant at the 0.05, 0.01, 0.00, and 0.001 probability levels and not significant, respectively
† GY = grain yield; KS = kernels per spike; KW = average kernel weight; DH = days to heading; DPM = days to physiological maturity; GFD = grain fill duration; PH = plant height; SL = spike length; TW = test weight; TKN=total kernel number per plot; TSN= total spike number per plot;
‡ Mean square entry
§ Mean square of genotype by environment interaction
¶Narrow sense heritability
[a]90% confidence interval of heritability estimate

and 2.11). The highest overall correlation was that between days to heading and grain fill duration in both the 2008 wet and dry environments (*r*=-0.84 and -0.80, respectively). This indicates that as heading occurs later in the season, the shorter time there will be for the grain-fill process.

QTL Analysis

As described in Materials and Methods, adjacent markers that were significant for the same trait were considered distinct QTL if they were separated by 20 cM or more

(Maccaferri et al., 2008). Based on that criterion the results of the single factor QTL analysis are presented below.

Grain yield: From one to five QTL were detected for grain yield in each of the five environments, for a total of 12 QTL (Table 2.13). Sokoll contributed the favorable allele at five markers, in both the 2008 and 2009 wet and dry growing seasons. Markers *Xgwm603* and *Xbarc108* were found to be within the 20 cM range and were thus considered to be a single QTL. Of the 12 QTL, three were detected in two or more environments (*Xbarc108/Xgwm603*, *Xgwm268*, and *Xgwm153*). The largest effect (315.4 kg/ha) was contributed by the Ankor allele in the 2008 dry environment near the marker *Xgwm499*, located on the long arm of chromosome 5B. *Xgwm268* was the most stable QTL, as it was detected in the 2008 dry, 2008 wet, and 2009 wet environments.

Test weight: Seven QTL were found, and Ankor contributed the favorable allele at all markers. The greatest phenotypic contribution was found at the QTL associated with *Xgwm484* (R^2=7.2%) on chromosome 2D in the 2009 dry environment. *Xgwm499* was significant for both grain yield and test weight, but in different environments.

Plant height: Six QTL for plant height were found across four environments. Sokoll contributed the high-value allele at all markers except *Xwmc710* (chromosome 4B). It is also interesting to note that marker *Xwmc710* is located approximately 5.6 cM away from the reduced height gene, *Rht-B1*. It is possible *Xwmc710* and *Rht-B1* are linked and that taller plant height alleles from Ankor were favored in this chromosome region. Marker *Xbarc55* had the greatest phenotypic contribution with an R^2 percentage of 8.4%. In addition to *Xbarc55*, markers *Xbarc18*, *Xgwm630*, and *Xgwm122* were all located in the same region on 2BS and contributed 3.9%, 4.8%, and 4.2%, respectively, of the total

Table 2.13. QTL detection for agronomic and morphological traits of the Ankor/Sokoll BC_2F_2-derived population in five Colorado environments in the 2006-07, 2007-08, and 2008-09 growing seasons.

Trait	Environment†	Chromosome	Nearest Marker	*P*	% R^2	Effect‡
Grain yield	07FW	2B	*Xgwm630*	0.005	4.2	-100.70
(kg/ha)	08FD	1B	*Xgwm268*	0.008	3.8	-226.55
		3A	*Xwmc532*	0.008	3.7	41.65
		5B	*Xgwm499*	0.0003	6.9	-315.40
		5D	*Xcfd29*	0.002	5.3	-58.68
		7A	*Xbarc108*	0.0001	9.0	57.00
		7A	*Xwmc603*	0.002	5.2	45.23
	08FW	1B	*Xgwm268*	0.008	3.9	-242.11
		1B	*Xgwm153*	0.005	4.4	-245.63
		2D	*Xgwm484*	0.007	4.3	-235.60
		6B	*Xgwm193*	0.008	3.7	234.93
	09GD	3A, 1D	*Xgwm155*	0.006	4.1	182.25
		2A, 2B,2D, 6B	*Xgwm311*	0.008	3.7	-163.77
	09GW	1B	*Xgwm268*	0.009	3.7	-221.02
		1B	*Xgwm153*	0.040	4.8	-236.41
		2D	*Xgwm539*	0.001	6.2	-255.96
		7A	*Xbarc108*	0.003	4.7	40.96
		7A	*Xwmc603*	0.004	4.4	41.84
Test weight	08FW	7B	*Xwmc517*	0.0009	5.8	-0.38
(kg/hL)		7B	*Xgwm213*	0.007	4.4	-0.38
	09GD	2D	*Xgwm484*	0.0003	7.2	-0.39
		3A	*Xbarc284*	0.008	3.7	-0.27
		5B	*Xgwm499*	0.009	3.6	-0.33
		5D	*Xgwm190*	0.003	4.8	-0.30
	09GW	2A, 2B,2D, 6B	*Xgwm311*	0.001	5.7	-0.34
Plant height	07FW	2B	*Xbarc18*	0.007	3.9	1.59
(cm)		2B	*Xbarc55*	0.005	8.4	2.25
		2B	*Xgwm148*	0.004	4.4	1.77
	08FD	2A	*Xwmc522*	0.003	4.7	1.98
		2B	*Xgwm630*	0.003	3.8	1.73
	08FW	2A	*Xwmc522*	0.002	5.1	2.21
		2B	*Xbarc148*	0.005	8.2	2.26
		2B	*Xgwm122*	0.005	4.2	1.98
		4A, 5B	*Xgwm118*	0.008	3.8	0.69
	09GW	1A	*Glu-A1*	0.003	4.7	1.66

		4B	*Xwmc710*	0.007	4.0	-1.53
Days to heading	07FW	2D	*Xgwm261*	0.001	5.7	-0.84
(Julian days)		2D	*Xgwm484*	0.0001	14.0	-1.40
	08FD	1A	*Xbarc17*	0.009	3.9	0.92
	08FW	2D	*Xgwm261*	0.002	5.2	-0.79
		2D	*Xgwm484*	0.0002	7.9	-1.01
		5D	*Xgwm190*	0.003	4.7	-0.75
		5D	*Xcfd18*	0.007	4.1	-0.74
	09GD	2D	*Xgwm484*	0.008	4.1	-0.45
		5D	*Xgwm190*	0.001	5.6	-0.50
	09GW	2D	*Xgwm261*	0.0003	6.9	-0.65
		2D	*Xgwm484*	0.002	5.5	-0.59
		5D	*Xgwm190*	0.005	4.3	-0.51
Days to maturity	07FW	2D	*Xgwm484*	0.007	4.2	-0.84
(Julian days)		6A	*Xwmc553*	0.0009	5.8	-0.98
	08FW	2D	*Xgwm484*	0.002	5.6	-0.44
		5D	*Xcfd18*	0.007	4.1	-0.39
Grain fill duration	07FW	3A	*Xbarc321*	0.0009	4.6	-1.47
(Julian days)		4B	*Xwmc710*	0.0004	6.7	0.97
	08FW	5D	*Xgwm190*	0.01	3.6	0.57
Avg. kernel weight	08FW	3B	*Xbarc77*	0.005	4.3	0.80
(mg)		3D	*Xcfd9*	0.008	3.9	-0.83
		4A	*Xwmc219*	0.003	4.8	0.83
		5D	*Xgwm190*	0.003	4.7	-0.89
	09GD	2A	*Xwmc170*	0.007	3.8	-0.59
		2B	*Xbarc55*	0.008	7.3	0.83
		7B	*Xwmc323*	0.008	7.7	-0.86
	09GW	2A,2B	*Xwmc382b*	0.007	3.9	1.73
		2D	*Xgwm484*	0.007	6.2	-1.53
		4A, 5B	*Xgwm118*	0.005	4.3	-2.07
		5B	*Xgwm499*	0.0001	8.7	-2.77
		7B	*Xgwm213*	0.0004	7.2	-2.32
Kernels per spike	07FW	2D	*Xgwm261*	0.005	4.2	-1.95
		3D	*Xgwm52*	0.008	3.8	1.77
		5B	*Xgwm499*	0.0006	6.1	-2.82
		7B	*Xgwm213*	0.0008	6.6	-2.71

	08FW	2D	*Xgwm261*	0.01	3.6	-1.47
	08GW	4A, 5B	*Xgwm118*	0.003	4.9	-2.17
	09GW	5A	*Xbarc319*	0.004	8.9	1.06
		7A	*Xbarc108*	0.003	4.7	1.16
Spike length	07FW	1B, 3D	*Xgwm456*	0.005	6.5	0.32
(cm)		3A	*Xbarc284*	0.009	3.6	0.25
		3A	*Xwmc532*	0.005	4.2	0.27
		3D	*Xgwm52*	0.002	5.1	0.26
	08FD	3A	*Xwmc532*	0.004	4.4	0.26
		2A	*Xwmc522*	0.0001	7.5	0.32
	08FW	1A	*Glu-A1*	0.0009	3.8	0.22
		1B, 3D	*Xgwm456*	0.001	5.7	0.28
		2A	*Xwmc522*	0.0001	11.0	0.38
		2A	*Xgwm47*	0.005	4.2	-0.21
		2B	*Xbarc18*	0.001	5.5	-0.26
		2B	*Xbarc55*	0.0001	14.6	-0.43
		2B	*Xgwm122*	0.0001	7.8	0.32
		2D	*Xgwm484*	0.002	5.4	-0.26
		4A	*Xcfd71*	0.003	4.8	0.35
		4D	*Xbarc98*	0.003	4.6	0.34
		4D	*Xwmc720*	0.006	4.1	0.31
	09GD	2B	*Xbarc18*	0.009	3.6	-0.24
	09GW	6B	*Xgwm193*	0.006	4.0	-0.30
Total kernel #	08FD	1D	*Ppd-D1*	0.006	8.5	724.8
		5B	*Xgwm499*	0.002	3.9	-750.2
		7A	*Xbarc108*	0.001	3.7	663.1
	08FW	2B	*Xbarc18*	0.0007	6.1	-589.5
	09GD	3A, 1D	*Xgwm155*	0.005	4.2	530.3
		4A	*Xbarc343*	0.009	3.8	655.0
	09GW	1B	*Xgwm268*	0.002	5.0	-807.7
		1B	*Xgwm153*	0.002	5.7	-820.1
		5B	*Xgwm499*	0.003	4.6	-804.9
		7B	*Xgwm213*	0.0005	7.1	942.1
Total spike #	08FD	2A	*Xwmc170*	0.005	4.3	16.1
		5D	*Xcfd29*	0.0007	6.1	-28.1
	08FW	1A	*Glu-A1*	0.003	4.8	-20.3
		1B	*Xgwm153*	0.002	5.3	-22.1
		1B	*Xgwm268*	0.002	5.1	-23.1

		2B	*Xbarc18*	0.006	3.9	-17.8
		3B, 5A,6B, 7A	*Xbarc180*	0.004	8.6	27.6
		6A	*Xbarc171*	0.006	7.9	20.4
	09GD	2D	*Xgwm539*	0.004	4.5	-24.8
	09GW	1B	*Xgwm268*	0.003	4.9	-22.9
		1B	*Xgwm153*	0.001	5.7	-23.6
		5B	*Xgwm499*	0.009	3.6	21.5
		7B	*Xgwm213*	0.005	4.7	21.9

†The five environments: 07FW, 2006-07 Fort Collins fully irrigated; 08FW, 2007-08 Fort Collins fully irrigated; 08FD, 2007-08 partially irrigated; 09GW, 2008-09 Greeley fully irrigated; 09GD, 2008-09 partially irrigated field trial.
‡Phenotypic effect: calculated as the mean value of the heterozygous genotypic class minus the mean value of the homozygous genotypic class. Positive values indicate an increasing effect of Sokoll alleles, and negative values indicate an increasing effect of Ankor alleles. Units for these effects are listed under the trait name.

phenotypic variation. Marker *Xgwm148* was also detected on 2BS but was greater than 20 cM away from the marker cluster described above.

Days to heading: Four QTL were detected for days to heading. Sokoll contributed the later allele only at one QTL (*Xbarc17* on chromosome 1A), which explained 3.9% of the phenotypic variation. Markers *Xgwm261* and *Xgwm284* were detected within 20 cM of one another on chromosome 2DS; therefore, they were treated as one QTL. Marker pair *Xgwm261*/*Xgwm484* was detected in all three wet environments, and Ankor contributed the later allele. These markers are also close to *Ppd-D1*, a photoperiod sensitivity locus. In this population, the *Ppd-D1* locus revealed segregation distortion in favor of heterozygous alleles. *Xgwm484* was detected in four and *Xgwm190* in three environments (both wet and dry), revealing a wide applicability of these loci to breeding programs.

Days to physiological maturity: Three QTL were detected for days to physiological maturity in the 2007 and 2008 wet environments. *Xwmc533* on chromosome 6A explained the greatest phenotypic variation (R^2=5.8%), and Ankor contributed the later-maturing allele at all loci.

Grain fill duration: Three QTL were detected for grain fill duration on chromosomes 3A, 4B and 5D. Whereas Ankor contributed the later allele at all but one QTL for days to heading and days to physiological maturity, Sokoll contributed the higher value allele for two of three grain fill duration QTL. This is logical because the Sokoll allele was associated with fewer days to heading.

Kernel weight: Twelve QTL were found across three environments and were especially prominent under irrigated conditions. Marker *Xgwm499*, which was associated with grain yield and test weight, was also detected for average kernel weight. *Xgwm499* had the greatest phenotypic effect among the detected QTL for this trait, with an R^2 value of 8.7%. Sokoll contributed the favorable allele on chromosomes 2A, 2B, 3B, and 4A.

Kernel number per spike: Seven QTL were found across four environments. Marker *Xgwm499*, also detected for other traits and environments, was detected for kernels per spike (2007 wet) and had an R^2 value of 6.1%. Sokoll contributed the favorable allele and the highest phenotypic contribution at marker *Xbarc319* on chromosome 5A (R^2 = 8.9%). Sokoll also contributed the favorable allele for markers on chromosomes 3D and 7A. Marker *Xbarc108* was detected in the 2009 wet environment for kernel number per spike and grain yield, and *Xgwm213* was detected only in wet environments for kernels per spike (2007), test weight (2007), and kernel weight (2008).

Spike length: Thirteen QTL were detected for spike length across all five environments. Eight of these were detected in the 2008 wet environment, and only three were detected in dry environments. Multiple markers were detected across several environments, and several of these were associated with other traits. *Xbarc18, Xbarc55,* and *Xgwm122* were also associated with plant height in wet environments and were found within 20 cM of one another on chromosome 2BS. *Xwmc522* was detected for plant height and *Xwmc532* for grain yield. Marker *Xbarc55* made the greatest contribution to spike length with R^2=14.6%. Sokoll contributed favorable alleles for the majority of the QTL, including all D-genome QTL except for marker *Xgwm484* on chromosome 2D.

Total kernel number: Ten QTL were detected for total kernel number for four environments. Sokoll contributed the favorable allele for five QTL, one of which was the D-genome marker *Ppd-D1*. Marker *Ppd-D1* also made the highest phenotypic contribution to total kernel number (R^2=8.5%). Marker *Xgwm499,* detected previously for numerous traits, was also significant for total kernel number in the 2008 dry and 2009 wet environments.

Total spike number: Eleven QTL were detected for total spike number, and Sokoll contributed the favorable allele for five QTL. Two QTL were detected on D-genome markers (*Xcfd29* and *Xgwm539*), yet Ankor contributed the favorable allele for both these QTL. Marker *Xbarc180* contributed the highest percent phenotypic variation (R^2=8.6%). Markers *Xgwm153* and *Xgwm268* were detected for this trait in both the 2008 and 2009 wet environments.

DISCUSSION

Marker analysis and linkage map construction

Linkage map and segregation distortion

The desired outcome of the F_2 linkage map construction was to develop a medium-density framework map specific to the Ankor/Sokoll population covering most of the genome. This task proved difficult given the time, personnel, and resource constraints. Regardless, the map was made as complete as possible with the time and resources available. The framework linkage map included 144 markers on parts of all 21 chromosomes, and covered approximately 55.5% of the genome. Some linkage groups could not be assigned unambiguously to a chromosome. The most complete linkage groups with the desired 10 cM spacing between each marker were found on chromosomes 1A, 1B, 2B, 5B, and 7B. Chromosomes with multiple linkage groups included 1A, 1B, 1D, 2A, 2D, 3A, 3D, 4A, 4D, 5A, 5B, 5D, 6D, and 7A.

The expected segregation ratios of individuals in the F_2 generation are 25% of each parental homozygous class and 50% heterozygous for co-dominant markers. For dominant markers, the expectation was 75% homozygotes for one parent or heterozygotes to 25% homozygotes of the other parent. One-hundred eighty-two markers were tested for segregation distortion according to the χ^2 test of significance. Of these, 15 (8.2%) were significantly ($P \leq 0.05$) distorted and six were distorted at the $P \leq 0.01$ level of significance. All 15 distorted markers were co-dominant, suggesting difficulties in distinguishing heterozygotes from homozygotes. Of these markers, five (33.3%) were located on chromosome 7B, and there was an excess of ‘H’ and ‘A’ scores, indicating the

Ankor allele was favored. This could have been due to inadvertent selection in favor of the Ankor allele when the highest yielding lines were selected in 2007. Alternatively, there could be a locus in this region at which Sokoll alleles reduce viability of gametes. The expected segregation ratios of the BC_2F_2-derived lines were 75% homozygotes for the Ankor allele and 25% heterozygous genotypes. The average percentage of heterozygotes per marker in the BC_2F_2-derived individuals was 24.8%, which is very close to the expected 25%. The percentage of heterozygotes for each marker ranged from 2.1 to 63.8%. Chi square values indicated 25 (32.9%) markers were distorted at the $P \leq 0.05$ level, and 19 (25%) markers were distorted at the $P \leq 0.01$ level of significance. Of the 25 markers, five (20.0%) were located either on chromosomes 2A or 2B, and three (12.0%) were on chromosomes 4A or 4D. Chromosome 4D is where the reduced plant height gene *Rht-D1* is located. The three markers on chromosome 4D were on average 12.3% heterozygous, indicating selection toward the Ankor allele at this location. We hypothesized distorted markers would be found at or near *Vrn-A1* and *Vrn-B1*, located on chromosomes 5A and 5B, respectively, and could help explain the segregation distortion; marker *Xgwm499* located near *Vrn-B1* on chromosome 5B was distorted; however, no markers near *Vrn-A1* on chromosome 5A were distorted.

Trait Analysis, 2007-09

Correlations and Heritability, 2007-09

Kernel number has historically been the primary determinant of yield in wheat cultivars (Bingham, 1966; Sinclair and Jamieson, 2006; Tian et al., 2011), and it has been shown to be more strongly correlated with yield than kernel weight (Duggan et al., 2000; Brancourt-Hulmel et al., 2003). For example, Sayre et al. (1997) evaluated eight spring

wheat cultivars from CIMMYT and found grain yield was significantly ($P \leq 0.01$) correlated with total kernel number m^{-2} ($r=0.84$) but not with average kernel weight, kernels per spike, or total spike number m^{-2}, confirming the importance of total kernel number as a yield component. A recent review by Dolferus et al. (2011) again emphasized the importance of total kernel number rather than kernel size in determining final grain yield. In our study, grain yield was most strongly correlated with total kernel number per m^2 ($r=0.72$ to 0.97, $P \leq 0.001$) and total spike number per m^2 ($r=0.63$ to 0.74, $P \leq 0.001$) in all environments from 2007 to 2009. Grain yield was positively and significantly ($P \leq 0.05$) correlated with kernels per spike in all environments except the Greeley 2009 dry environment ($r=0.21$ to 0.47). Yield was correlated with average kernel weight only in the 2007 and 2009 wet environments ($r=0.24$ to 0.36, $P \leq 0.05$). Grain yield was also correlated with test weight in the 2009 dry and wet environments ($r=0.26$ and 0.27, respectively, $P \leq 0.01$) and plant height in the 2008 and 2009 dry environments ($r=0.23$ and 0.25, respectively, $P \leq 0.01$). These results are similar to previous studies in the Great Plains region. For example, Butler et al. (2002) found significant ($P \leq 0.05$) correlations between grain yield and kernel weight, test weight, and plant height in three of four environments over two years; El Feki (2010) detected significant ($P \leq 0.05$) correlations between grain yield and both average kernel weight and plant height in four environments over two years; and Narasimhamoorthy et al. (2006) found significant ($P \leq 0.05$) correlations between average kernel weight, test weight, and plant height in two Kansas environments. Heritability estimates varied from trait to trait, but heritability estimates for a given trait in different environments remained fairly stable. For example, heritability estimates for yield in the 2007 wet, 2008 dry and wet, and 2009

dry and wet environments were 0.59, 0.37, 0.55, 0.51, and 0.50. Heritability estimates for kernels per spike ranged from 0.07 to 0.58 and for days to heading they ranged from 0.22 to 0.61, indicating that the environmental effects varied due to factors such as soil variability for soil moisture and temperature. This is surprising, as generally days to heading has a high heritability value because it can be precisely measured and depends on a few photoperiod and vernalization genes (Griffiths et al., 2009). However, in this population, the range in days to heading was narrow, so that even small changes in relative values would reduce the heritability estimates. Plant height also had relatively low heritability values, considering this trait is easy to measure with precision; however, as with days to heading, the range of values was small. Grain yield, test weight, spike length, total kernel number, and total spike number all had highly significant ($P \leq 0.0001$) G x E interaction. Grain yield in wheat has been shown in previous studies (Huang et al., 2004; Quarrie et al., 2006) to have highly significant ($P \leq 0.001$) G x E interaction.

QTL detection and comparison among AB-QTL and QTL studies

Markers were tested for significance in five BC_2F_2-derived environments. Significant markers for yield and yield components were detected in this study mainly on chromosomes 2B, 3A, 3B, 3D, 4B, 5B, 5D, 7A, and 7B. However, apparently no novel QTL were detected for grain yield per se. Huang et al. (2003, 2004) found one AB-QTL each on chromosomes 5BS (2003) and 5BL (2004), respectively, but each QTL was located within 20 cM of the QTL in the Ankor/Sokoll population. It 2003, the synthetic W-7984 contributed the favorable allele on chromosome 5BS, indicating synthetic-derived lines can contribute to higher grain yield.

Seven QTL were detected for test weight on chromosomes 2D, 3A, 5B, 5D, and 7B. Of the previous AB-QTL studies, only Narasimhamoorthy et al. (2006) evaluated test weight, and only one QTL was detected on chromosome 2DS. In the present study, marker *Xgwm484* was also detected on 2DS; however, it was greater than 20 cM away from the QTL detected by Narasimhamoorthy et al. (2006). Of all the QTL studies, Campbell et al. (2003) discovered a test weight QTL on the long arm of chromosome 3A; however, the QTL detected in the Ankor/Sokoll population was on the short arm of chromosome 3A, indicating a potentially novel AB-QTL near marker *Xbarc284*. In addition, no AB-QTL for test weight have been detected on chromosomes 5B and 7B, indicating that markers *Xgwm499* and *Xgwm517/Xgwm213* represent new chromosomal regions associated with test weight. Both of these markers represent Ankor allele contributions. However, numerous authors (Groos et al., 2003; Quarrie et al., 2005; Marza et al., 2006) have found QTL for grain yield on chromosome 5B, near marker *Xgwm499*, suggesting test weight and grain yield could be linked to a locus or gene in that region. In fact, grain yield was correlated to test weight in the 2008 dry, 2009 dry, and 2009 wet environments (r=0.19, 0.26, and 0.27, respectively, $P \leq 0.05$). While no QTL for test weight were confirmed in the 2010-11 environments (Chapter 3), it is evident that due to the far distance apart of these two markers from previously detected QTL, there is a high probability these marker regions are novel for test weight.

Sokoll contributed positively to the QTL for plant height on chromosomes 1A, 2A, 2B, and 4A/5B, and Ankor contributed positively to the QTL on chromosome 4B for plant height. Of the AB-QTL studies, Huang et al. (2004) detected the same QTL for plant height near the distal end of the short arm of chromosome 1A as in the

Ankor/Sokoll population. Those authors also detected QTL for plant height on chromosomes 2BL and 4A/5B; however, the QTL associated with markers *Xbarc18*/*Xgwm630*/ *Xbarc55*/*Xgwm122* on chromosome 2BS and with marker *Xgwm118* on chromosomes 4A/5B in the Ankor/Sokoll population were different than those detected by Huang et al. (2004). The QTL on chromosomes 2B and 4A/5B thus represent unique QTL among the AB-QTL studies; however, among all QTL studies, no novel QTL were discovered for plant height.

Three QTL for days to heading were found on chromosomes 1AL, 2D, and 5D. Kuchel et al. (2006) found a similar QTL on chromosome 1AL using a doubled haploid population. Among the AB-QTL studies, only Narasimhamoorthy et al. (2006) reported QTL for days to heading. They detected QTL on chromosomes 2DS and 3DS. The QTL detected on chromosome 2DS by these authors is very close to the QTL detected in the Ankor/Sokoll population (*Xgwm484/Xgwm261)*. The *Xgwm484/Xgwm261* marker region is an important and consistent region, especially in wet environments. Though no novel QTL were detected for days to heading, a novel QTL was detected for days to physiological maturity on chromosome 6A, and Sokoll contributed the later allele and 5.9% of the phenotypic variation.

A total of 11 QTL were found for kernel weight primarily on chromosomes 2A, 2B, 2D, 3B, 3D, 4A, 5B, 5D, and 7B. Previous studies reported QTL on chromosomes 2A (Huang et al., 2003; McCartney et al., 2005; Sun et al., 2009; Wang et al., 2009), 2B (Hai et al., 2008 and Lin et al., 2008), 3B (Cuthbert et al., 2008 & Zhang et al., 2009c), and 3D (McCartney et al., 2005). Hai et al. (2008) reported a QTL on the long arm of chromosome 2B, which is different from the QTL detected on the short arm of

chromosome 2B for the Ankor/Sokoll population. This represents a novel QTL for which Sokoll is the parental donor of the high-value allele. In addition, Huang et al. (2003) detected QTL on chromosomes 5B and 7B and Liu et al. (2006) reported a QTL on chromosome 5B. However, these were more than 20 cM away from the QTL detected in the Ankor/Sokoll backcross population, suggesting two novel QTL. Ankor contributed the alleles for higher kernel weight for these two QTL.

A total of 13 QTL for spike length were detected on chromosomes 1A, 2A, 2B, 3A, 4A, 4D, and 6B. Three novel QTL were found on chromosomes 3A, 4D, and 6B, near markers *Xwmc532*, *Xbarc98/Xwmc520*, and *Xgwm193*, respectively. Sokoll contributed the positive allele on chromosomes 3A and 4D. The Sokoll contribution from the D-genome suggests the possibility that *Ae. tauschii* contributed the D-genome allele; however, this evidence is not conclusive, as the Sokoll pedigree consists also of the D-genome from bread wheat germplasm. Based on its pedigree, the wild diploid parent contributed approximately 4.2% of the D genome when initially crossed to Ankor.

Nine QTL were detected for total kernel number on chromosomes 1B, 1D, 2B, 3A, 4A, 5B, 7A, and 7B. Sokoll contributed the favorable allele for five QTL, one of which was the D-genome marker *Ppd-D1*. Of the nine QTL detected, four QTL were validated on chromosomes 1B, 2B, 3A, and 7A. No previous AB-QTL studies were conducted on total kernel number.

Eleven QTL were detected for total spike number on chromosomes 1A, 1B, 2A, 2B, 2D, 5B, 5D, and 7B, and Sokoll contributed the favorable allele for five of the 11 QTL. Two QTL were detected on D-genome markers (*Xcfd29* and *Xgwm539*), yet Ankor contributed the favorable allele for both these QTL. Huang et al. (2003 and 2004)

detected a QTL for tiller number on chromosome 1B; however, two markers (*Xgwm153* and *Xgwm268*) found on chromosome 1B in the Ankor/ Sokoll population were greater than 20 cM away from the QTL detected by Huang et al., indicating two novel AB-QTL. Huang et al. (2003) also detected QTL on chromosomes 2A, 2D, and 5D. The QTL found by Huang et al. (2003) on the distal end of the long arm of chromosome 2A was greater than 20 cM away from the QTL detected in the Ankor/Sokoll population. The QTL on chromosomes 2DL and 5DL detected by Huang et al. (2003) were both in similar locations to the QTL on chromosomes 2D and 5D detected in the Ankor/Sokoll population and therefore did not represent novel QTL.

In summary, a total of 88 QTL were detected for 11 traits in the present study. Distribution of QTL was unbalanced between irrigation treatments; 68 QTL (71.6%) were detected under full irrigation and 27 QTL (28.4%) were identified under limited irrigation. The greater environmental variation due to the lower heritability observed in the dry versus wet environments may have increased background noise and decreased the ability to detect more QTL in the dry environment. For 34 (39%) of these, alleles from the synthetic-derived wheat were associated with higher values, and for 54 (61%), Ankor contributed the higher value. Sokoll contributed the higher value allele for all QTL associated with markers on chromosomes 4A, 4D, and 7A and Ankor for all QTL associated with markers on chromosome 2D. Twelve novel QTL were detected compared with the other AB-QTL studies reviewed. Ankor contributed the positive allele for eight of these novel QTL on chromosomes 1B, 3A, 5B, 6B, and 7B, and Sokoll contributed the positive allele for four QTL on chromosomes 2B, 3A, 4D, and 6A.

One QTL of importance was linked to marker *Xgwm499* on chromosome 5B. *Xgwm499* was detected in exactly one environment for six different traits, and Ankor contributed the favorable allele for all traits except kernels per spike. Marker *Xgwm499* contributed to grain yield, test weight, kernel weight, and kernels per spike, the latter two of which are yield components. The significance of this marker across multiple traits could be due either to linkage or pleiotropic effects, but pleiotropy is suggested because of the relationship among the traits. It also suggests these traits should be correlated with one another. In fact each trait was correlated with grain yield in at least one environment from 2007 to 2009.

There were other noteworthy QTL detected. The QTL associated with marker *Xwmc522* was stable for the 2008 wet and dry environments for both plant height and spike length, and Sokoll contributed the positive allele across environments and traits. *Xgwm213* and *Xgwm118* were stable markers across multiple traits and multiple wet environments, indicating specificity and expression only under certain conditions. *Xbarc108*, *Xwmc532*, *Xbarc118*, and *Xgwm118* were detected for two or more traits and offer potential for use in a breeding regime using marker-assisted selection. *Xbarc108*, in particular, contributed to yield, kernels per spike, and total kernel number, three important traits for a plant breeder.

Compared to the study of Narasimhamoorthy et al. (2006), with field trials also in the Great Plains region (Kansas), our study had similar R^2 values for grain yield and days to heading, slightly lower R^2 values for kernels per spike, and much lower R^2 values for test weight; however, our study detected more QTL of smaller effect for each trait. Also, the Kansas cultivar Karl92 contributed all favorable alleles for grain yield and test

weight. Similarly, the Colorado cultivar Ankor in our study contributed all alleles for test weight; however, both Ankor and Sokoll contributed favorable alleles for grain yield in our study. In previous AB-QTL studies, the donor parent contributed the high value allele for 36.8 to 70.0% of QTL detected by Kunert et al. (2008) and Narasimhamoorthy et al. (2006), respectively. The Sokoll allele contribution of 38.9% in this study falls within the range of previous AB-QTL studies.

Conclusion

This study identified genomic regions from the synthetic-derived parent that increased yield, plant height, days to heading, kernel weight, kernels per spike, and spike length. Most of the favorable Sokoll alleles were at QTL on the A and B genomes. It is possible the D-genome from *Ae. tauschii* contributed favorable alleles to Ankor, but this cannot be stated conclusively as the pedigree of Sokoll is quite complex. This study also identified 12 novel QTL among all AB-QTL studies reviewed for test weight, days to maturity, kernel weight, and spike length on chromosomes 2A, 3A, 4A, 5B, 6B, 7A, and 7B. In addition, this study provided additional evidence that the advanced backcross QTL strategy is useful and able to enhance performance of existing cultivars. Further AB-QTL studies will assist in the contribution of positive allelic diversity in the future.

CHAPTER 3

Discovering the contribution of yield-related traits to final grain yield through the examination of high- and low-performing lines

INTRODUCTION

The production of wheat must increase by 2% per year if it is to meet world demands by 2050 (FAO, 2009). Additionally, wheat must be able to better combat the 25% annual production loss due to environmental stresses such as drought, salinity, and frost (Gill et al., 2004). Targeting these stresses will be necessary as water supplies dwindle (Alston, 2009). Drought, for example, affects both wheat growth and yield (Ashraf et al., 1995; Ashraf, 1998). A decrease in yield and yield components under drought stress has been reported in both durum and bread wheat. For example, Sharif Alhosainy (1998) and Saleem (2003) observed that water stress affected the number of spikes per m^2, kernels per spike, spikelets per spike, and final grain yield decreased in both the durum and bread wheat genotypes. Other studies have shown that the number of kernels per spike is a more important yield important yield component in defining yield in high latitudes (Peltonen-Sainio, P., 2007), while kernel weight is a more important

yield component in defining yield in certain Mediterranean environments (García Del Moral, L.F., 2003). This decrease in yield and yield components due to drought stress has been associated with a decrease in photosynthate production (Zaharieva, 2001), and/or a decrease in photosynthate mobilization to the spike (Rebetzke et al., 2008). Therefore, under post-anthesis drought conditions, carbohydrate reserves become critical as photosynthesis is often inhibited and as respiration burns up sugars (Barnabas et al., 2008).

Heat and drought stress often occur at the same time and contribute to the same plant responses (Moffat, 2002; Shah and Paulsen, 2003; Tester and Bacic, 2005). In maize, barley, sorghum, and various other grasses, both drought and heat stress lower plant metabolism (Rizhsky et al., 2004) and together have far more detrimental effects than either stress alone (Walter and Brunson, 1940; Craufurd and Peacock, 1993; Savin and Nicolas, 1996; Wang and Huang, 2004). Plant responses to combined heat and drought stress include: 1) decreased grain yield; 2) accelerated development (Paulsen, 1994); 3) decreased photosynthesis (Reynolds et al., 2010) and/or damage to photosynthetic machinery such as photosystem II (Paulsen, 1994); 4) increased photorespiration (Berry and Bjorkman, 1980); 5) disruptions to the respiratory process (Lin and Markhart, 1990); 6) stomatal closure leading to decreased leaf cooling (Rizhsky et al., 2002) and decreased Rubsico activase activity (Law and Crafts-Brandner, 1999).

When breeding for drought tolerance in wheat, it is important to consider both morphological and physiological traits (such as carbon assimilation) in specific environments (Reynolds et al., 2007; Saint Pierre et al., 2010). Selection for increased water soluble carbohydrate (WSC) reserves in stems is one potential breeding focus

(Setter et al., 1998; Foulkes et al., 2002; van Herwaarden & Richards, 2002; Ehdaie et al., 2006, 2008). Significant increases in both grain yield and kernel size occur because of the ability of wheat to remobilize carbon from stems to grain (Austin et al., 1980; Bell and Incoll, 1990; Lópezcastaneda and Richards, 1994, Housley, 2000; Salem et al., 2007). Carbon assimilates both from current photosynthesis and from WSC reserves in the stem determine grain filling rate in cereals (Plaut et al., 2004; Yang and Zhang, 2006). When water is limiting during grain filling, remobilization of assimilates from the stem to the spike increases (Kiniry 1993; Gebbing and Schnyder, 1999; Takahashi et al., 2001; Plaut et al., 2004; reviewed by Yang and Zhang, 2006; Ruuska et al., 2006; Rebetzke et al., 2008). This remobilization can decrease the chance of producing small, shriveled grain or early seed abortion, as more energy is put into the formation of viable seed (Westgate and Boyer, 1986; Makela et al., 2005). WSC have been shown to be closely associated with grain set (Pinto et al., 2010); however, an understanding of the association between WSC concentration and biomass is also vital for maximizing yield gains in the future (Reynolds et al., 2007).

Maximum biomass is produced in plants under optimal water conditions, and under drought stress, biomass decreases (Clarke et al., 1991; Ashraf, 1998). One target for genetic improvement involves increasing biomass while maintaining a high harvest index (Slafter and Andrade, 1991; Calderini et al., 1999; Foulkes et al., 2007). It is certain that future increases in spike biomass will depend on increases in above-ground biomass (Foulkes et al., 2007). However, above-ground biomass will be limited by factors such as water and/or nitrogen availability (Foulkes et al., 2007). The effects of biomass on yield have been variable, as these traits have been both positively (Siddique

et al., 1989; Donmez et al., 2001; Shearman et al., 2005) and negatively (e.g. Waddington et al., 1986; Austin et al., 1989; Slafer et al., 1994; Brancourt-Hulmel et al., 2003) correlated. One potential way to maximize grain yield would be to increase the stem elongation period (Bradley et al., 2005; Slafer et al., 2005). This would favor greater spike biomass and stem WSC (Foulkes et al., 2007).

Spike: stem ratio was thought to be a potential predictor of final grain yield, and Siddique and Whan (1993) believed spike: stem ratio at anthesis could be a better predictor of grain yield than harvest index, since the rate of increase of spike: stem ratio is unaffected post-anthesis (Siddique et al., 1989). Kumakov et al. (2000) determined that under drought, the proportion of the spike increased, whereas the proportion of the leaves stayed the same under both dry and irrigated conditions. Also, cultivars with the greatest drought tolerance had larger spikes and a greater spike: stem ratio (Kumakov et al., 2000).

The spike: stem ratio could be used to identify parental genotypes and to select at early generations for the ability to partition photosynthate (Siddique and Whan, 1993). Siddique et al. (1989) were able to detect differences in dry matter partitioning between spike and stem by the beginning of stem elongation. Studies by Siddique et al. (1989) found that more shoot dry matter allocated to the spike during the pre-anthesis period, especially early in the life cycle (Siddique and Whan, 1993), improved kernels per spike, harvest index, and final grain yield.

A major limitation in the assessment of spike: stem ratio is sampling the plants precisely at anthesis before any grain development and measuring the dry weights of the

spike and stem. This is time-consuming and laborious and therefore limits its use in breeding programs (Siddique and Whan, 1993).

The objectives of this study were 1) to determine if there is a correlation between spike: stem ratio and biomass at various growth stages and final grain yield, based on a subset of the BC_2F_2-derived population 2) to determine the traits that explain yield differences between high- and low-yielding lines 3) to validate QTL associated with drought tolerance and other agronomic traits from the 2007-09 environments and 4) to identify promising lines for use in breeding programs, based on evaluation in 10 environments.

MATERIALS AND METHODS

Population Development

As described in chapter 2, the Ankor/Sokoll backross population was developed to identify genomic regions of the drought tolerant spring wheat variety 'Sokoll' (developed at CIMMYT/ Mexico) that contribute to yield performance in the genetic background of the Colorado winter wheat cultivar 'Ankor.' Sokoll is a white spring wheat and was used as the donor parent for its potential to donate alleles promoting drought tolerance both pre- and post-anthesis (R. Trethowan, CIMMYT, personal communication).

Field Trials

Replicated field trials were grown in five environments over two growing seasons (2010-2011) and are summarized in Table 3.1. Based on yield performance of the 188 BC_2F_2-derived lines in the 2007-09 environments, the 22 highest and 22 lowest yielding lines, based on a rank summation index, were grown in 2009-10 in three rainfed environments in Eastern Colorado (Burlington, Dailey, and Julesburg). Based on mean yield performance in the three environments in 2010, the 8 highest- and 8 lowest-performing lines were grown in 2010-11 in dry and wet treatments at ARDEC in Fort Collins, Colorado. The 2010-11 trials were planted in six-row plots versus two-row plots for the previous years. The larger plot area (5.57 m^2 versus 1.98 m^2) is assumed to provide more accurate yield estimates.

Table 3.1. Planting locations summary for the Ankor/Sokoll population.

Year	Environment	No. BC Lines	No. Checks	No. Reps
2010	Burlington rainfed	44	6	3
2010	Dailey rainfed	44	6	3
2010	Julesburg rainfed	44	6	3
2011	Fort Collins partially irrigated	16	4	4
2011	Fort Collins fully irrigated	16	4	4

All trials in 2010 and 2011 had six-row plots 3.66 m long with 25.4 cm spacing between rows and plots. Trial dimensions in 2010 were 15 plots long by 10 plots wide. In 2010, trials were 20 plots long by 4 plots wide. Seed of each entry was weighed and planted to provide a plot density of 1,778,000 seeds/ha. Trials were planted in September of each year and harvested the following July. The year used to describe each trial indicates the year of harvest. Six cultivars (Ankor, Hatcher, Ripper, RonL, TAM 111, and Snowmass) were used as checks in the 2010 rainfed environments, and four cultivars (Ankor, Ripper, RonL, and TAM 111) were used as checks in 2011. Trials in Eastern Colorado received no irrigation, and trials in Fort Collins were irrigated with a linear overhead sprinkler system. Fertilization and weed control were typical for winter wheat in the area. Due to sufficient rainfall events in April, no added irrigation was applied; however, through boot stage in mid-May and through heading in early June only the wet treatment was irrigated. On June 24 (during grain filling), the dry plot was also irrigated by mistake. After the first week of July, neither the wet nor dry treatments received additional irrigation. Entries for all environments were randomized using a latinized row-column design created with CycDesigN 3.0 (www.cycdesign.co.nz/).

Trait Analysis

Data were recorded for the 2010 environments for the following traits: grain yield, test weight, harvest index, spike length, spikelet number, spike: stem ratio, kernel number per spike, kernel weight per spike, and average kernel weight (Table 3.2). In addition, above-ground biomass samples were harvested at various growth stages in the three locations. Biomass was sickled at ground level from jointing stage onward, and 10 tillers with spikes from each biomass strip were used for spike, stem, and leaf partitioning measurements. Biomass and partitioning samples were taken at Burlington and Dailey for all four growth stages: jointing, booting, heading, and harvest. At Burlington the first biomass sample at jointing was a 1 m strip. This was determined to be too bulky for transporting a large number of samples to Fort Collins; from then on, a 0.5 m biomass strip was harvested. Biomass and partitioning samples were taken at Julesburg only at harvest. The biomass and 10-tiller samples at all environments were taken from the two replicates with the most complete stands based on visual inspection. The first and second replicates were used for Burlington and Julesburg, and the second and third replicates were sampled at Dailey. The biomass samples and the 10-tiller samples were placed in a 37ºC drier for five to seven days immediately after sampling. The biomass samples were then weighed to determine total biomass dry weight.

The 10-tiller samples of leaves, stems, and spikes were collected to determine whether dry matter partitioning and growth rate at these stages were associated with grain yield, as proposed in the review by Foulkes et al. (2009). The stems and spikes from the 10-tiller samples were separated and weighed to determine the spike: stem ratio and spike growth rate. The spike: stem ratio was calculated for each plot at each location to

determine if there was a correlation with yield. Because of the distance of the sampling sites from Fort Collins, the growth stages were estimated based on weather and communication with farmers. Therefore, not all samplings took place at the exact growth stage. However, all samplings were near the intended growth stage.

Spike length was calculated by averaging the length of 10 spikes from each 10-spike sample in each environment, and spikelet number was calculated as the average number of spikelets for these same spikes. To calculate average kernel weight, 200 kernels obtained from the 10-tiller sample at harvest were counted, weighed, and divided by 200. To determine kernel weight per spike, the kernels from 10 threshed spikes were weighed and divided by 10. To determine kernel number per spike, the total kernel number from the same 10 threshed spikes was counted and divided by 10. Ten spikes were collected from most plots; however, for some plots, only nine plants were collected. This was adjusted to calculate correct averages. Harvest index was calculated as the biomass sample grain yield divided by the total biomass of the sample at harvest. Grain yield and test weight were recorded electronically on the combine at harvest.

The daily maximum temperature (T_{max}) from April to June, 2010, as well as total monthly precipitation from August, 2009 to June 2010 were gathered using the Colorado Agricultural Meteorological Network (CoAgMet, Burlington (Burlington #3) and Dailey (specifically Haxtun); T_{max} and precipitation), the National Climatic Data Center (NOAA, http://cdo.ncdc.noaa.gov/dly/ DLY, Julesburg; T_{max}), and the Community Collaborative Rain, Hail, and Snow Network (CoCoRaHS, http://www.cocorahs.org/Maps/View Map.aspx, Julesburg (Sedgwick county); precipitation).

The nearby planting of the 2011 ARDEC trial allowed for more measurements to be taken. The following traits were recorded for the 2011 dry and irrigated environments: grain yield, test weight, harvest index, spike length, spikelet number, kernel number per spike, kernel weight per spike, average kernel weight, and total kernel and spike number (Table 3.2). In addition, 1 m biomass samples were taken at all four growth stages: jointing, booting, heading, and harvest. Furthermore, spikes and stems were sub-sampled on 10 plants at the following stages: boot, heading, and harvest. All the above traits were measured as described previously in this chapter. Days to heading, days to physiological maturity, and grain fill duration were all recorded or calculated as described in Chapter 2. Plant height measurements were recorded as the number of centimeters from the ground to the tip of the spike, discounting the awns, measured on three plants. Three measurements were taken per plot and averaged. Finally, soil moisture samples were taken using a JMC Backsaver Soil Sampler (Forestry Suppliers, Inc., Jackson, MS) near boot stage (May 31), heading (June 7), about three weeks after heading (June 27), and about a month after heading (July 6). Three soil cores were taken in 30.5 cm sections for the first, second, and third depths of soil at two locations, in the first and fourth reps, in both dry and wet treatments in 2011 for a total of 12 separate samples per sampling date. The samples were placed immediately in a plastic ziplock bag so as not to lose any moisture. Samples were then weighed and transferred to an aluminum pie plate and placed in a drying oven at 105°C. Samples were dried and re-weighed four to five days later, and soil moisture percentage on a gravimetric basis was calculated.

The daily maximum temperature (T_{max}) from April to June of 2011, as well as

Table 3.2. Phenotypic traits recorded for the Ankor/Sokoll population from 2010 to 2011.

Trait	**Burlington 2010 (Dry)**	**Dailey 2010 (Dry)**	**Julesburg 2010 (Dry)**	**Fort Collins 2011 (Dry)**	**Fort Collins 2011 (Wet)**
Grain yield	X	X	X	X	X
Test weight	X	X	X	X	X
Days to heading				X	X
Days to maturity				X	X
Grain fill duration				X	X
Plant height				X	X
Kernels per spike	X	X	X	X	X
Kernel weight/spike	X	X	X	X	X
Average kernel weight	X	X	X	X	X
Spike length	X	X	X	X	X
Spikelet number	X	X	X	X	X
Biomass at jointing	X	X		X	X
Biomass at booting	X	X		X	X
Biomass at heading	X	X		X	X
Biomass at harvest	X	X	X	X	X
Spike: stem at booting	X	X		X	X
Spike: stem at heading	X	X		X	X
Spike: stem at harvest	X	X	X	X	X
Total kernel number	X	X	X	X	X
Total spike number	X	X	X	X	X

total monthly precipitation from 2010 August to 2011 June were gathered using the Colorado Agricultural Meteorologial Network (CoAgMet, Fort Collins (ARDEC); T_{max} and precipitation).

Statistical Analysis

All statistical analyses in 2010 and 2011 field trials were performed using SAS version 9.2 (SAS Institute Inc., 2008). To analyze phenotypic data for the five

environments from 2010 to 2011, the MIXED and GLM procedures were used. Proc MIXED was used to obtain least square means (LS means), which were then used for subsequent analysis of Ankor and BC_2-derived means and standard errors, correlations, and QTL detection.

To account for field variability in the different environments, a spatial adjustment model similar to that employed by Butler et al. (2005) was used in the Proc MIXED program. The 'x' and 'y' coordinates were assigned to all plots to adjust trait values based on the distance from the center of the adjacent plot in both dimensions. In 2010 and 2011, the x- and y-coordinates were assigned, starting at 0, in 1.53 and 4.88 m increments, respectively. The spatial adjustment model for each environment provided appropriate LS means for each trait.

Means and standard errors were recorded for Ankor and the backcross population, and the range was recorded for the backcross lines using the LSmeans. Frequency distributions, normality of distributions, and correlations were determined as described in Chapter 2. Furthermore, the eight highest and eight lowest performing lines from the five environments from 2007 to 2009 were used to determine if there were significant differences between the high and low means for all traits in 2010 and 2011 using the contrast statement in the Proc MIXED procedure. The top five lines were identified based on yield for potential use in a breeding program.

Statistical Analysis: QTL Confirmation

Significant ($P \leq 0.01$) markers detected in at least one of the 2007 wet, 2008 dry and wet, and 2009 dry and wet environments for 11 traits were tested in the 2010 Burlington, Julesburg, and Dailey and 2011 Fort Collins dry and wet environments. A

less stringent threshold of significance ($P \leq 0.05$) was used to validate markers because the number of lines was decreased to 44 lines in 2010 and 16 lines in 2011, compared to the 2007-09 trials (≥188 lines), thus decreasing the power of detection of QTL.

RESULTS

Trait Analysis

Temperature and Precipitation

The weather data from Burlington, Dailey, and Julesburg reveals Dailey was on average slightly cooler than the other two locations from April to June (Figure 3.8). Julesburg was the warmest location, having both the hottest April and June maximum temperatures (Figures 3.1 to 3.3). From boot stage until harvest, there was an overall increase in maximum daily temperature from a low of 5.6 °C to a high of 37.4 °C in Dailey (Figure 3.2). In Burlington, the range of maximum daily temperature was about 7 to 36 °C (Figure 3.1), a less dramatic temperature change from mid-May to June. In Julesburg, the range from mid-May to June was 8.8 to 37.2 °C (Figure 3.3), intermediate between Burlington and Dailey. In addition, during the 11 days before heading in Burlington, temperatures increased from a low of about 14.0 °C to a high of 34 °C. During the third week of May before heading at Burlington, temperatures decreased slightly. In the 11 days before heading in Dailey, temperatures increased from a low of 5.6 to 33.7 °C. Around June 1 right before heading temperatures decreased slightly. Finally, in the 11 days before heading in Julesburg, temperatures increased from 8.8 to 32.8 °C.

The total monthly precipitation in Dailey was low from November (0.15 cm) to February (0.20 cm) and then increased from March (1.14 cm) until June (2.21 cm). The low winter precipitation may have affected early vegetative growth and thus low stand counts. Burlington and Julesburg experienced much greater total rainfall from August

Figure 3.1. Daily maximum temperature gathered from CoAgMet for Burlington, April to June 2010.

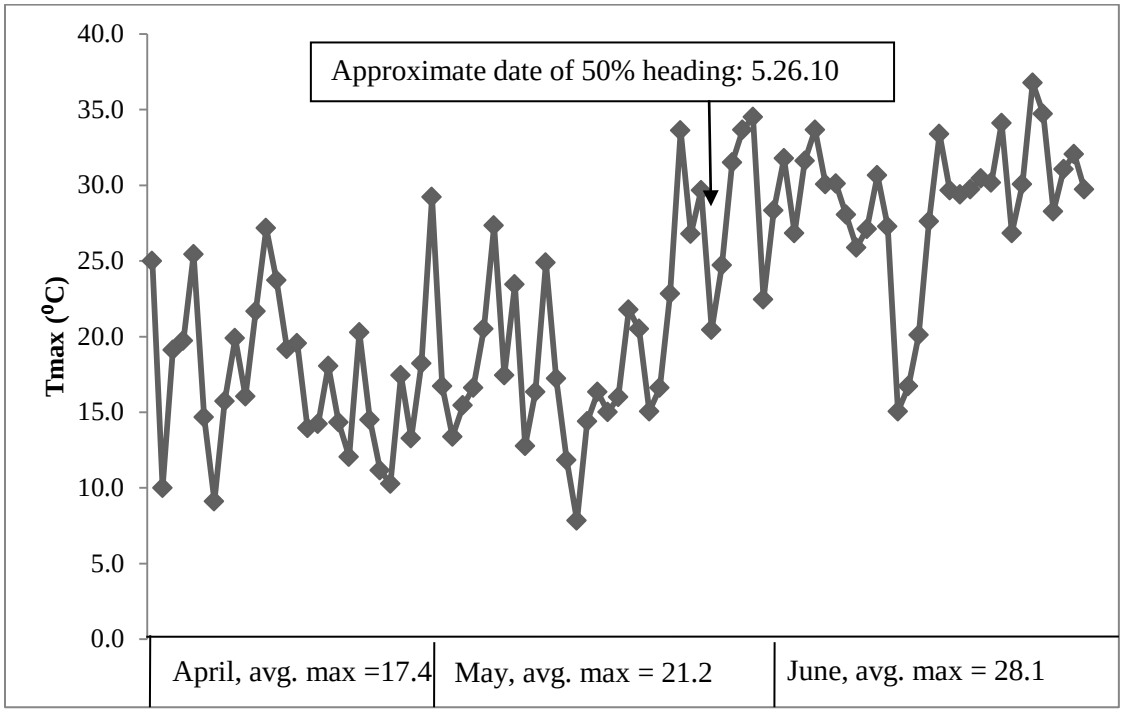


Figure 3.2. Daily maximum temperature gathered from CoAgMet for Dailey, April to June 2010.

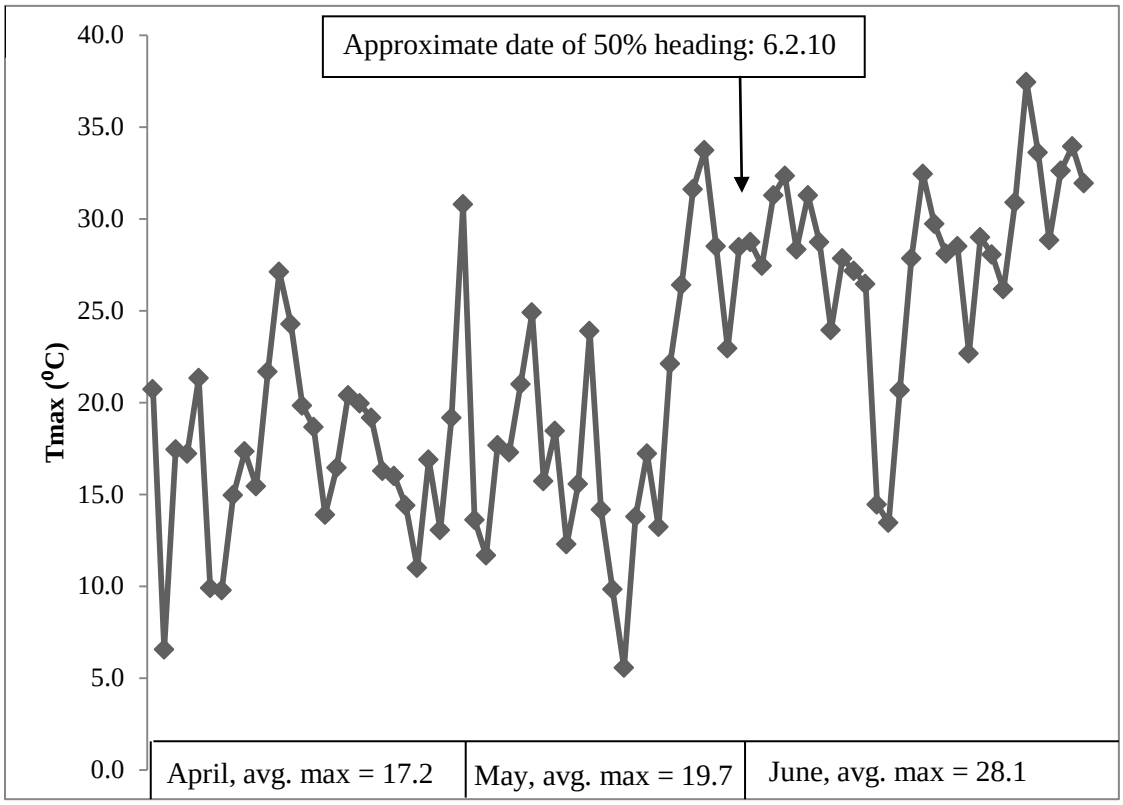


Figure 3.3. Daily maximum temperature gathered from NOAA for Julesburg, April to June 2010.

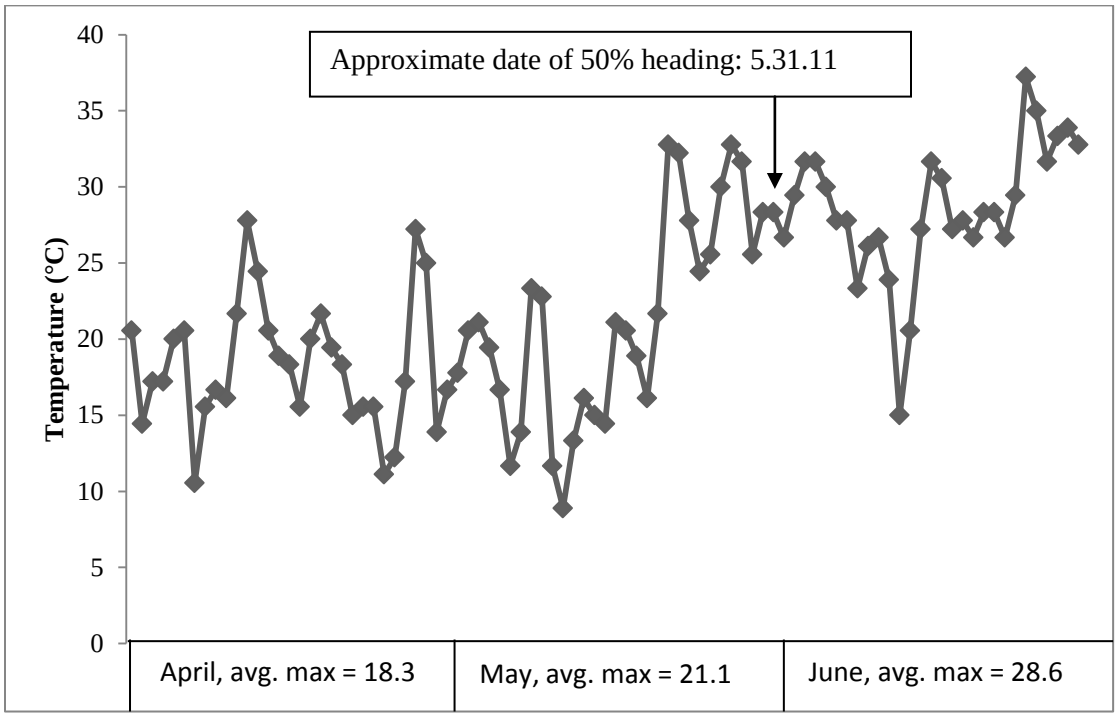


Figure 3.4. Total Monthly Precipitation gathered from CoAgMet for Burlington, August 2009 to June 2010.

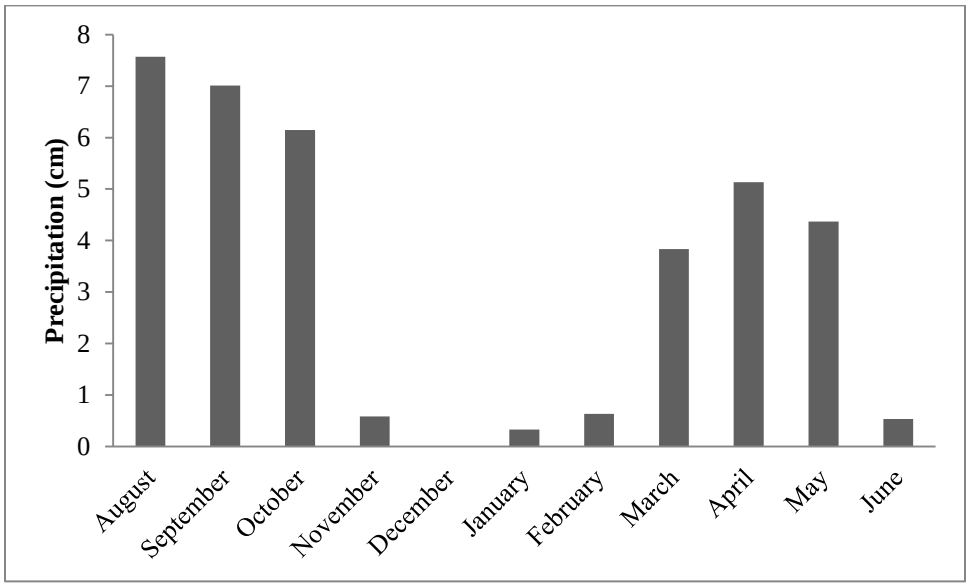


Figure 3.5. Total Monthly Precipitation gathered from CoAgMet for Dailey, August 2009 to June 2010.

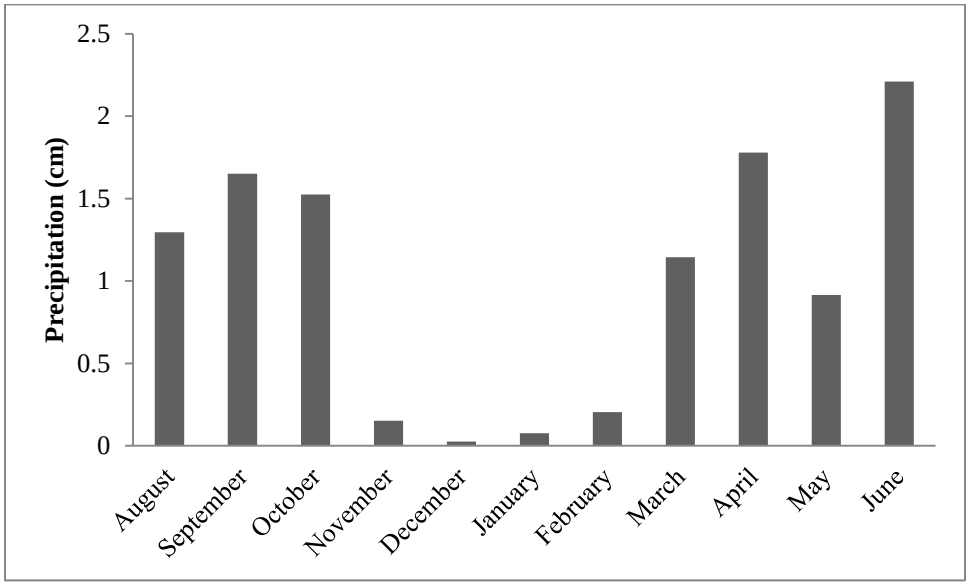
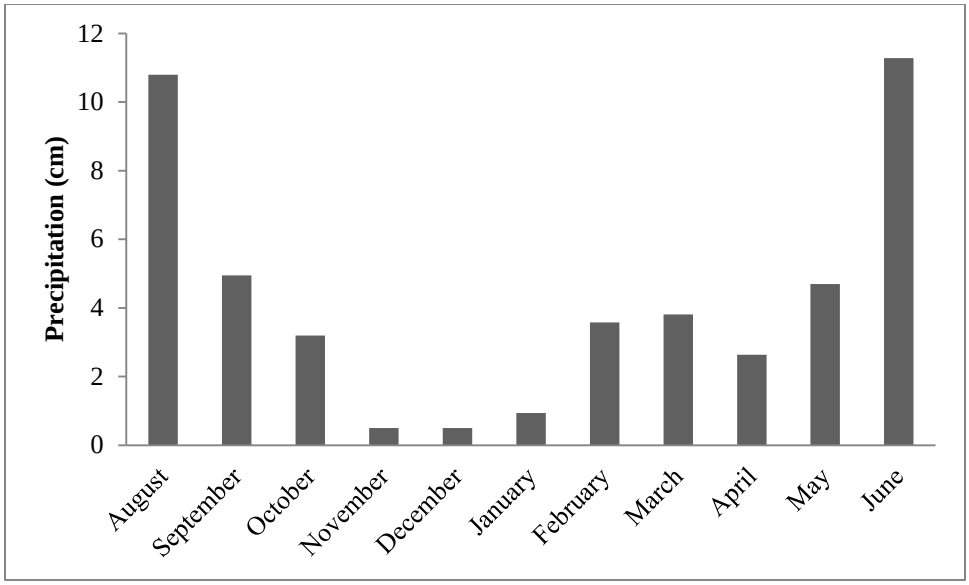


Figure 3.6. Total Monthly Precipitation gathered from CoCoRaHS for Julesburg, August 2009 to June 2010.



to October of 2009 (20.7 and 19.0 cm, respectively) and from March to June of 2010 (13.9 and 22.4 cm, respectively) than Dailey (4.5 and 6.9 cm, respectively) (Figures 3.4 to 3.6). The average monthly precipitation in Dailey from August to October 2009 was around 1.49 cm and from March to June 2010 was approximately 1.51 cm, whereas in Burlington, these means were 6.91 cm and 3.47 cm, respectively.

The weather records from Fort Collins reveal variable temperatures and levels of precipitation in the months leading up to harvest (Figure 3.6 and 3.7). At the beginning of May, there were three consecutive days of temperatures greater than 28°C, with the highest of those temperatures being 29.5°C on May 8. Three days later, the temperature high dropped to 6.5°C. From this low on May 11, temperature highs trended upward and ranged from about 14°C to 24°C for the rest of May and into heading in early June. For the month of June, temperatures continued to increase with highs of 32.7°C in early June to 35.6°C at the end of June (Figure 3.7).

The total monthly precipitation at ARDEC in Fort Collins was low until April, 2011. Precipitation was greater than 1 cm for the months of August, October, and November, 2010, with the highest precipitation in October (1.85 cm). The low winter precipitation in December and January, 2010 may have affected early vegetative growth, leading to poor tillering and a uniformly low stand density in both environments. Precipitation did not reach above 1 cm from November to March and finally surpassed the 1 cm mark in the months of April, May, and June. In May and June, the wet treatment was irrigated seven and the dry treatment one time in late June. Precipitation levels for April, May, and June were 3.8, 9.0, and 5.1 cm, respectively (Figure 3.8). In 2011, soil moisture content was estimated using a soil probe in three 30.5 cm depths at

Figure 3.7. Daily maximum temperature gathered from CoAgMet for Fort Collins, April to June 2011.

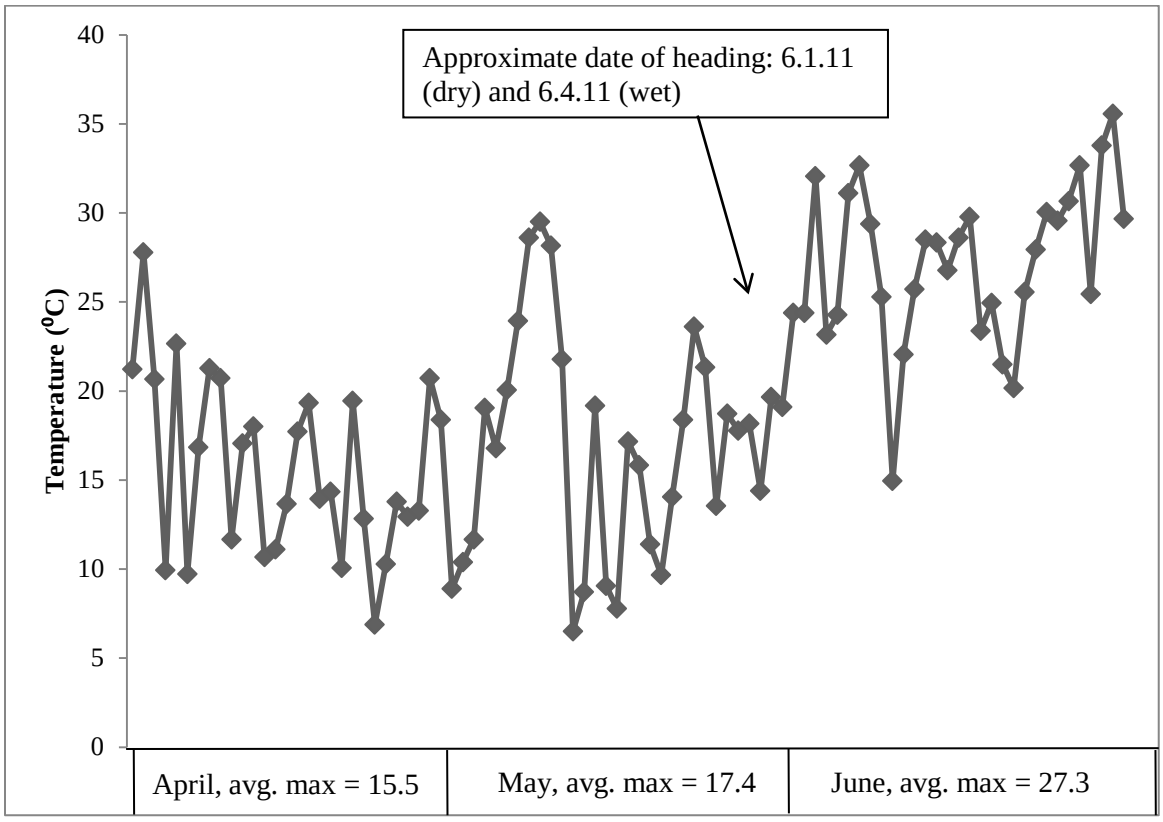
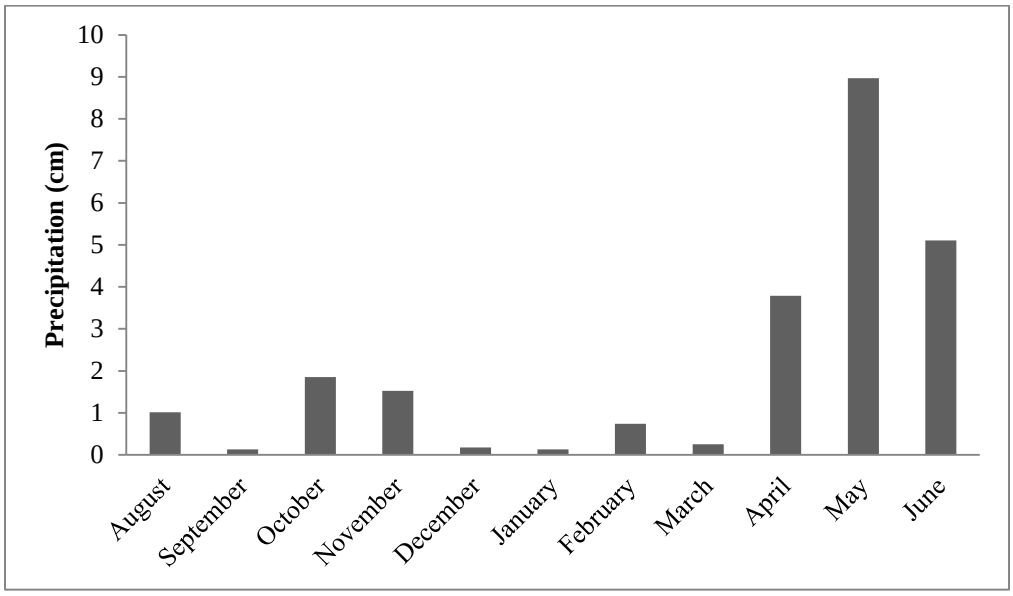


Figure 3.8. Total Monthly Precipitation gathered from CoAgMet for Fort Collins, August 2010 to June 2011.



four dates, beginning around heading and ending before harvest. Soil moisture percentages were similar in dry and wet environments for the first three samples (Table 3.3). It was not until the July 6 soil sampling that the dry treatment became markedly drier than the wet treatment.

Table 3.3. Mean gravimetric soil moisture percentage per 30.5-cm increment of soil in the Fort Collins 2011 dry and wet environments.

		Percent soil moisture in 30.5-cm increments		
Date	**Environment**	**Top**	**Middle**	**Bottom**
31-May	Dry	18.8	19.4	22.4
	Wet	18.1	18.7	15.4
7-Jun	Dry	15.5	15.8	15.5
	Wet	14.9	16	17.5
27-Jun	Dry	13.3	13.1	16.4
	Wet	13.5	11.1	15.4
6-Jul	Dry	12.2	12.6	15.2
	Wet	15.3	14.5	16.7

Frequency Distributions

Least squares means were obtained for each line and trait, and frequency distributions were plotted (Appendix Figures F-J, P, Q, V-Z, AF-AJ, and AP-AT). Additionally, the Shapiro-Wilk test of the SAS UNIVARIATE procedure was used for a statistical determination of normality. None of the environments in 2010 deviated from normality ($P \geq 0.05$) for grain yield, test weight, and spike length; however, kernel number per spike was normal only for the 2010 Burlington environment.

In the 2011 dry and wet environments, grain yield was statistically normal ($P=0.17$ and 0.11, respectively, Appendix Figures I and J). Days to heading and test

weight were also statistically normal. Both wet and dry treatments appeared and were statistically normal ($P \geq 0.05$) for kernel number per spike (P=0.42 and 0.82, respectively).

For all environments, transgressive segregation for grain yield, test weight, spike length, and kernels per spike was evident beyond the Ankor parent, indicating that alleles from Sokoll improved the performance of Ankor.

Burlington 2010 Rainfed Field Trial

Overall, Burlington had more favorable growing conditions, which are reflected by the final grain yield and biomass weights at each growth stage. Table 3.4 reveals that the Ankor mean exceeded the $BC_2F_{2:4}$ range for 10 of 18 traits. This $BC_2F_{2:4}$ population consisted of the 22 high and 22 low performing lines; therefore, ranges are more meaningful than population means. The Ankor mean exceeded the $BC_2F_{2:4}$ range for grain yield and average kernel weight, an important yield component. For all other traits except total kernel number, the $BC_2F_{2:4}$ ranges exceeded that of the Ankor mean, indicating some of the $BC_2F_{2:4}$ lines performed better numerically than Ankor, although the difference was not always significant. For example, the Ankor mean was near the middle of the range for all biomass samples, indicating transgressive segregation. The mean harvest index for Ankor and the $BC_2F_{2:4}$ lines were identical, but the $BC_2F_{2:4}$ range exceeded the Ankor means. In addition, all spike: stem ratios from boot, heading, and harvest were all very similar between Ankor and the $BC_2F_{2:4}$ means, yet, transgressive segregation was still evident.

Burlington was the highest-yielding environment in 2010 for the $BC_2F_{2:4}$ population. The Burlington mean was 5428.7 kg/ha, followed by 3954.0 and 3512.1

Table 3.4. Performance of Ankor in comparison to the Ankor/Sokoll $BC_2F_{2:4}$ means and ranges at the 2010 Burlington rainfed trial. Mean values are presented ± standard error.

	Ankor	**$BC_2F_{2:4}$**		
Trait	**Mean**	**Mean**	**Range**	**No. Lines > than Ankor†**
Grain Yield (kg/ha)	5428.7 ± 202.4	4967.1 ± 200.7	4229.4 – 5576.4	0
Test Weight (kg/hL)	80.9 ± 10.5	78.0 ± 2.8	74.0 – 80.9	0
Biomass Sample 1 (g)	58.1 ± 7.0	53.0 ± 7.0	38.3 - 68.3	0
Biomass Sample 2 (g)	174.2 ± 16.8	185.6 ± 16.7	154.7 - 223.0	0
Biomass Sample 3 (g)	208.2 ± 21.2	199.4 ± 21.1	147.7 - 246.6	0
Biomass Sample 4 (g)	240.5 ± 22.1	235.4 ± 22.0	158.5 - 270.2	0
KwtS4 (g)	76.0 ± 7.6	72.9 ± 7.5	47.8 - 83.8	0
Harvest Index	0.31 ± 0.03	0.31 ± 0.03	0.26 - 0.37	0
Spike Length (cm)	10.6 ± 0.3	10.1 ± 0.3	9.2 - 11.0	0
Spikelet Number	15.5 ± 0.5	15.6 ± 0.5	14.8 - 17.0	0
Kernel Number/Spike	30.4 ± 2.9	31.8 ± 2.9	26.6 - 36.7	0
Kernel Weight/Spike (g)	0.74 ± 0.09	0.90 ± 0.08	0.72 - 1.11	3
Average Kernel Weight (mg)	25.2 ± 1.5	28.5 ± 1.5	24.6 - 31.0	6
B2Spike:Stem (g)	0.08 ± 0.02	0.07 ± 0.02	0.02 - 0.12	0
B3Spike:Stem (g)	0.54 ± 0.06	0.54 ± 0.05	0.46 - 0.67	0
B4Spike:Stem (g)	2.05 ± 0.14	2.07 ± 0.14	1.77 - 2.29	0
Total Kernel Number‡	21,538.1 ± 287.1	17,507.5 ± 230.6	14,288.6 – 21,592.8	0
Total Spike Number‡	708.5 ± 11.5	554.1 ± 9.1	408.9 – 672.6	0

† Number of lines significantly ($P \leq 0.05$) greater than Ankor for each trait.
‡ Values are per 1 m^2

kg/ha for Julesburg and Dailey, respectively. The mean $BC_2F_{2:4}$ test weight for Burlington was also the highest of the three environments at 80.9 kg/hL. Test weight for Julesburg and Dailey were similar to each other, at 73.4 kg/hL and 70.0 kg/hL, respectively. Burlington also had the highest biomass dry weight at harvest with a $BC_2F_{2:4}$ line mean of 235.4 g (compared to 156.8 and 159.9 g for Julesburg and Dailey, respectively). Harvest index was consistent across all three environments (0.31, 0.31, and 0.33 at Burlington, Dailey, and Julesburg, respectively).

Simple Pearson correlation coefficients for the Burlington 2010 environment were calculated for all pairs of 18 traits (Table 3.5). The hypothesis that the spike: stem ratio correlates or can be predictive of grain yield was rejected in this environment. Grain yield was not significantly ($P \leq 0.05$) correlated with any of the spike: stem ratios at boot, heading, or harvest growth stages. More biomass would appear indicative of higher grain yield at all growth stages; however, biomass was only correlated with grain yield at boot stage ($r=0.30$). The final grain yield might be expected to increase as the spike: stem ratio increased, because more photosynthate would go towards the developing spike tissues. However, this correlation was not observed in Burlington wheat plants. It is possible that the conditions were ideal enough (lack of water stress) to provide sufficient resources for growth of both biomass (stem) and grain. Therefore, not all the photosynthate would be directed toward the grain and could be distributed to stem biomass as well. Total kernel weight at harvest from the half-meter biomass strip samples was positively correlated ($P \leq 0.05$) with five traits: final grain yield ($r=0.40$), dry biomass at boot ($r=0.36$) and harvest ($r=0.78$), harvest index ($r=0.37$), and total kernel number ($r=0.36$). There was an expected correlation between the above ground biomass weight and the total kernel weight from the above ground biomass sample ($r=0.78$, $P \leq 0.001$) at harvest. It is interesting that final grain yield was correlated with the kernel number from the biomass sample at harvest ($r=0.40$, $P \leq 0.001$), yet there was no correlation with above ground biomass at harvest and final grain yield which refutes our hypothesis that biomass could be a predictor of grain yield. The highest correlation of 0.82 was between kernels per spike and kernel weight per spike. It is expected that the more kernels there are in a spike, the greater the kernel weight per spike will be.

Table 3.5. Pearson correlation coefficients among 18 field traits in 44 Ankor/Sokoll $BC_2F_{2:4}$ lines in Burlington, 2010.

	Trait†															
Trait†	TW	AGB1	AGB2	AGB3	AGB4	KWB4	HI	SL	SpN	KS	KWS	AKW	B2:SS	B3:SS	B4:SS	TKN	TSN
GY			0.30*			0.40**										0.71***	0.35*
TW																	
AGB1			0.30*		0.40**								0.50**				0.32*
AGB2						0.36*											0.31*
AGB3					0.30*												
AGB4						0.78***											
KWB4							0.37*									0.36*	
HI															0.32*		
SL									0.39**	0.30*							
SpN										0.52**	0.38*						
KS											0.82***						-0.66***
KWS												0.32*					-0.81***
AKW																-0.63***	
B2:SS														0.33*			
B3:SS																	
B4:SS																	
TKN																	0.53**

*, **, ***, Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. Blank spaces indicate the correlation was not significant.
GY=grain yield, TW=test weight, AGB1=biomass at jointing, AGB2=biomass at booting, AGB3=biomass at heading, AGB4=biomass at harvest, KWB4=kernel weight from biomass at harvest, HI=harvest index, SL=spike length, SpN=spikelet number, KS=kernels per spike, KWS=kernel weight per spike, AKW=average kernel weight, B2:SS=spike: stem ratio at booting, B3:SS=spike: stem ratio at heading, B4:SS=spike: stem ratio at harvest, TKN=total kernel number per 0.5 m biomass strip, TSN=total spike number per 0.5 m biomass strip.

One interesting correlation was between the biomass at jointing and with spike: stem ratio at boot stage (r=0.50). This fairly strong correlation would suggest that the biomass at an earlier stage is somewhat predictive of the spike: stem ratio at a later developmental stage, with greater biomass indicating more photosynthate is going to be available to the spike for future production of seed.

2010 Dailey rainfed field trial

Dailey was the most stressed of the three rainfed environments as seen by the sparse tiller stands, the consistently low biomass at all growth stages, the final grain yield, and the lowest rainfall of any of the three environments. Ankor performed better than the $BC_2F_{2:4}$ mean for 10 of 18 traits (Table 3.6). The $BC_2F_{2:4}$ mean was greater than the Ankor mean in the biomass samples at boot, heading, and harvest, the spike: stem ratios at boot and heading, kernels per spike, and kernel weight per spike; however, Ankor was superior for final grain yield, test weight, spike length, spikelet number, and total kernel number. The harvest index for Ankor was 0.34, which was higher than the mean harvest index of 0.31 for the $BC_2F_{2:4.}$ However the $BC_2F_{2:4}$ range exceeded the Ankor mean for harvest index. Two $BC_2F_{2:4}$ lines were significantly ($P\leq0.05$) greater than Ankor for grain yield. Ankor was significantly ($P\leq0.05$) greater than seven $BC_2F_{2:4}$ lines for the spike: stem ratio at heading and four $BC_2F_{2:4}$ lines for kernel weight per spike. It is important to note that transgressive segregation was apparent in all traits at Dailey, with some $BC_2F_{2:4}$ lines performing much better than the Ankor mean.

Pearson correlation coefficients for the Dailey 2010 field trial reveal more significant correlations than for the Burlington environment. Grain yield was correlated with both key yield components, average kernel weight (r=0.31) and total kernel number

Table 3.6. Performance of Ankor in comparison to the $BC_2F_{2:4}$ means and ranges at the 2010 Dailey rainfed trial. Mean values are presented ± standard error.

	Ankor	**$BC_2F_{2:4}$**		
Trait	**Mean**	**Mean**	**Range**	**No. Lines > than Ankor†**
Grain Yield (kg/ha)	3312.1 ± 137.4	3252.2± 135.7	3142.4 - 4477.5	2
Test Weight (kg/hL)	73.4 ± 1.9	73.0 ± 1.9	70.1 – 74.9	0
Biomass Sample 1 (g)	36.5 ± 8.4	38.3 ± 8.5	21.1 - 61.8	1
Biomass Sample 2 (g)	108.7 ± 17.5	109.0 ± 17.5	70.5 - 162.8	0
Biomass Sample 3 (g)	157.2 ± 17.8	159.9 ± 18.0	119.8 - 206.8	0
Biomass Sample 4 (g)	254.5 ± 43.5	221.6 ± 42.5	107.9 - 322.2	0
KwtS4 (g)	52.7 ± 5.3	49.0 ± 5.3	27.4 - 68.6	0
Harvest Index	0.34 ± 0.03	0.31 ± 0.04	0.23 - 0.42	0
Spike Length (cm)	10.1 ± 0.3	10.0 ± 0.3	8.8 - 11.0	1
Spikelet Number	17.0 ± 0.4	16.8 ± 0.4	15.19 - 18.8	0
Kernel Number/Spike	27.3 ± 3.3	31.8 ± 3.26	25.9 - 39.3	1
Kernel Weight/ Spike (g)	0.74 ± 0.07	0.82 ± 0.07	0.53 - 1.13	4
Average Kernel Weight (mg)	26.8 ± 1.5	25.9 ± 1.5	21.3 - 30.6	0
B2 Spike:Stem (g)	0.01 ± 0.01	0.02 ± 0.01	0.00 - 0.03	0
B3Spike:Stem (g)	0.55 ± 0.04	0.64 ± 0.11	0.54 - 0.79	7
B4Spike:Stem (g)	2.95 ± 0.11	2.60 ± 0.11	1.88 - 3.05	0
Total Kernel Number‡	12,380.5 ± 56.9	12,614.7 ± 201.4	9574.6 – 14,743.0	0
Total Spike Number‡	453.1 ± 2.8	400.1 ± 7.5	271.5 – 503.4	0

† Number of lines significantly ($P \leq 0.05$) greater than Ankor for each trait.
‡ Values are per $1m^2$

(r=0.68) (Table 3.7). Grain yield was correlated with the spike: stem ratio only at harvest (r=0.41). The hypothesis that biomass was correlated with grain yield was upheld in Dailey. Grain yield was correlated with all four biomass sample dry weights, indicating that as biomass increased, grain yield increased. It also indicates that because the biomass samples at later growth stages had stronger correlations, they were more

Table 3.7. Pearson correlation coefficients among 18 field traits in 44 Ankor/Sokoll $BC_2F_{2:4}$ lines in Dailey, 2010.

	Trait†															
Trait†	TW	AGB1	AGB2	AGB3	AGB4	KwtB4	HI	SL	SpN	KS	KWS	AKW	B2:SS	B3:SS	B4:SS	TKN	TSN
GY	0.56***	0.39**	0.44**	0.61***	0.33*	0.74***					0.34*	0.31*			0.41**	0.68***	0.34*
TW				0.37*		0.40**					0.43**	0.46**		-0.31*			
AGB1			0.51**	0.40**		0.46**			0.31*							0.53**	0.34*
AGB2				0.34*	0.43**	0.35*										0.58***	0.30*
AGB3					0.39**	0.80***				0.38*						0.56***	
AGB4						0.39**										0.32*	0.36*
KwtB4							0.39**								0.35*	0.53**	
HI																	
SL									0.45**	0.37*	0.33*						-0.37*
SpN																	
KS											0.78***				0.33*		-0.60***
KWS												0.56***			0.38*		-0.71***
AKW																-0.39**	0.33*
B2:SS																	
B3:SS																	
B4:SS																	
TKN																	0.65***

*, **, ***, Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. Blank spaces indicate the correlation was not significant.
GY=grain yield, TW=test weight, AGB1=biomass at jointing, AGB2=biomass at booting, AGB3=biomass at heading, AGB4=biomass at harvest, KWB4=kernel weight from biomass at harvest, HI=harvest index, SL=spike length, SpN=spikelet number, KS=kernels per spike, KWS=kernel weight per spike, AKW=average kernel weight, B2:SS=spike: stem ratio at booting, B3:SS=spike: stem ratio at heading, B4:SS=spike: stem ratio at harvest, TKN=total kernel number per 0.5 m biomass strip, TSN=total spike number per 0.5 m biomass strip.

predictive of final grain yield. Grain yield was also highly correlated with test weight (r=0.64). Biomass samples at all growth stages were correlated with the kernel weight of the biomass sample at harvest, indicating that larger biomass early on in development is somewhat predictive of grain yield. The highest correlations were between the biomass sample at harvest and its corresponding kernel weight at harvest (r=0.80) and between kernels per spike and kernel weight per spike (r=0.78). This latter correlation was also high at Burlington (r=0.82).

2010 Julesburg rainfed field trial

Initial phenotypic analysis of the 2010 Julesburg rainfed field trial indicated seven $BC_2F_{2:4}$ lines performed significantly ($P \leq 0.05$) better than Ankor for both grain yield and test weight, and two lines performed significantly better than Ankor for harvest index. Ankor had a higher mean than the $BC_2F_{2:4}$ lines in five of thirteen measured traits (Table 3.8). Ankor and the $BC_2F_{2:4}$ lines had comparable means for test weight, harvest index, kernel weight per spike, and average kernel weight. The spike: stem ratio at harvest was much greater in the $BC_2F_{2:4}$ lines (mean of 2.27) than for Ankor (1.88). The $BC_2F_{2:4}$ mean for grain yield was about 42 kg/ha greater than the Ankor mean, whereas Ankor outperformed the $BC_2F_{2:4}$ mean by about 60 kg/ha at Dailey and about 462 kg/ha at Burlington. Ankor fell within the $BC_2F_{2:4}$ range for all traits.

Pearson correlation coefficients reveal many significant correlations (Table 3.9). Grain yield was correlated to seven traits, ranging from 0.34 for kernel weight per spike to a strong correlation of 0.69 for test weight. As in Dailey, final grain yield was correlated with the spike: stem ratio at harvest, suggesting that a higher spike: stem ratio indicates a higher grain weight at harvest. A study by Kumakov et al. (2000) examined

Table 3.8. Performance of Ankor in comparison to the $BC_2F_{2:4}$ means and ranges at the 2010 Julesburg rainfed trial. Mean values are presented ± standard error.

	Ankor	**$BC_2F_{2:4}$**		
Trait	**Mean**	**Mean**	**Range**	**No. Lines > than Ankor†**
Grain Yield (kg/ha)	3954.0 ± 100.4	3995.9 ± 102.9	3142.4 - 4477.5	7
Test Weight (kg/hL)	73.5 ± 2.1	73.4 ± 2.1	70.5 – 76.7	7
Biomass Sample 4 (g)	175.1 ± 15.8	156.8 ± 16.0	114.9 - 199.6	0
KwtS4 (g)	56.7 ± 6.1	51.9 ± 6.2	34.4 - 72.2	0
Harvest Index	0.32 ± 0.02	0.33 ± 0.02	0.28 - 0.40	2
SL (cm)	10.6 ± 0.4	9.8 ± 0.4	8.8 - 10.6	0
Spikelet Number	17.2 ± 0.6	16.0 ± 0.	14.5 - 16.9	0
Kernel Number/Spike	31.7 ± 3.	32.3 ± 3.4	21.8 - 37.6	0
Kernel Weight/Spike (g)	0.86 ± 0.09	0.88 ± 0.09	0.48 - 1.09	0
Average Kernel Weight (mg)	27.1 ± 1.4	27.2 ± 1.4	23.9 - 30.5	0
B4 Spike:Stem (g)	1.88 ± 0.20	2.27 ± 0.21	1.67 - 2.78	0
Total Kernel Number‡	14,612.5 ± 157.9	14,702.1 ± 151.4	12,385.8 – 17,240.9	0
Total Spike Number‡	461.7 ± 4.2	461.2 ± 10.2	395.0 – 753.4	1

† Number of lines significantly ($P \leq 0.05$) greater than Ankor for each trait.
‡ Values are per 1 m^2

accumulation of dry matter in leaves, stems, and spikes of four cultivars over the course of 12 years. They determined that in both rainy and dry years, the proportion of leaves was the same. Therefore, the higher spike: stem ratio at harvest in our study would most likely be due to a higher grain weight, not a lower leaf weight. Grain yield was also correlated to both average kernel weight (r=0.47, $P \leq 0.01$) and total kernel number (r=0.60, $P \leq 0.001$). Unlike Dailey, however, grain yield was not correlated to the final biomass at harvest. Similar to Dailey, the kernel weight from the biomass sample at harvest was correlated to grain yield, test weight, harvest index, and all the biomass

Table 3.9. Pearson correlation coefficients among 13 field traits in 44 Ankor/Sokoll $BC_2F_{2:4}$ lines in Julesburg, 2010.

	Trait†											
Trait†	TW	AGB4	KWB4	HI	SL	SpN	KS	KWS	AKW	B4:SS	TKN	TSN
GY	0.69***		0.39**	0.38*				0.34*	0.47**	0.34*	0.60***	
TW			0.34*	0.41**		0.30*		0.45**	0.49**			
AGB4			0.83***			-0.34*						
KWB4				0.65***					0.35*			
HI									0.37*			
SL						0.56***						
SpN												
KS								0.87***				-0.81***
KWS									0.52**			-0.83***
AKW											-0.37*	-0.34*
B4:SS												
TKN												0.45**

*, **, ***, Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. Blank spaces indicate the correlation was not significant.
GY=grain yield, TW=test weight, AGBS4=biomass at harvest, KWB4=kernel weight from biomass at harvest, HI=harvest index, SL=spike length, SpN=spikelet number, KS=kernels per spike, KWS=kernel weight per spike, AKW=average kernel weight, B4:SS=spike: stem ratio at harvest, TKN=total kernel number per 0.5 m biomass strip, TSN=total spike number per 0.5 m biomass strip.

samples taken. Julesburg had the highest correlation between kernels per spike and kernel weight per spike (r=0.87); however, this correlation was consistently high across Burlington and Dailey as well (r=0.82 and 0.78, respectively). The correlation between the biomass sample and its corresponding kernel weight at harvest was 0.83 at Julesburg, similar to the correlations at Dailey and Burlington (r=0.80 and 0.78).

2011 Fort Collins Dry Trial

The 2011 trials in Fort Collins included the eight highest and eight lowest yielding $BC_2F_{2:5}$ lines, in contrast to the 2010 trials, where the 22 highest and 22 lowest lines were evaluated. Analysis of the 2011 Fort Collins dry field trial indicated Ankor had a higher mean than the $BC_2F_{2:5}$ lines in seven of the 22 measured traits (Table 3.10). Ankor and $BC_2F_{2:5}$ lines had very similar means for grain yield (4614 and 4654 kg/ha, respectively). The maximum of the range of $BC_2F_{2:5}$ lines was 5097 kg/ha, indicating transgressive segregation. Ankor and $BC_2F_{2:5}$ lines were also similar for days to heading, three of four biomass samples, harvest index, and spike length. The spike: stem ratio at harvest was greater in the $BC_2F_{2:5}$ lines than for Ankor. Ankor fell within the $BC_2F_{2:5}$ range for all traits. In the Fort Collins 2011 dry trial, at least one $BC_2F_{2:5}$ line performed significantly ($P\leq0.05$) better than Ankor for 18 of 22 traits. Six $BC_2F_{2:5}$ lines performed significantly ($P\leq0.05$) better than Ankor for kernels per spike, two $BC_2F_{2:5}$ lines had higher grain yield, and five lines were better for test weight.

Pearson correlation coefficients revealed significant and/or stronger correlations not found in previous years (Table 3.11). Grain yield was correlated (r=0.49 to 0.79, $P\leq0.05$) to six traits, including total kernel number and average kernel weight. The

Table 3.10. Performance of Ankor in comparison to the $BC_2F_{2:5}$ means and ranges at the Fort Collins 2011 dry trial. Mean values are presented ± standard error.

	Ankor	**$BC_2F_{2:5}$**		
Trait	**Mean ± SE**	**Mean ± SE**	**Range**	**No. Lines > Ankor†**
Grain Yield (kg/ha)	4613.8 ± 200.4	4653.6 ± 204.2	4099.1 - 5097.4	2
Test Weight (kg/hL)	58.9 ± 1.0	59.7 ± 1.0	58.1 - 61.5	5
Days to Heading (Julian days)	152.3 ± 0.4	152.6 ± 0.4	150.9 - 153.7	2
Days to Maturity (Julian days)	191.8 ± 0.5	192.9 ± 0.5	192.2 - 193.7	1
Grain Fill Duration (Julian days)	39.43 ± 0.6	40.22 ± 0.6	38.5 - 42.0	2
Biomass Sample 1 (g)	43.9 ± 7.6	43.5 ± 7.9	33.3 - 54.0	1
Biomass Sample 2 (g)	75.9 ± 18.8	76.8 ± 18.9	58.2 - 94.4	1
Biomass Sample 3 (g)	93.1 ± 14.7	93.7 ± 14.8	64.7 - 128.8	2
Biomass Sample 4 (g)	216.6 ± 23.4	227.8 ± 23.4	171.6 - 290.1	2
KwtS4 (g)	82.1 ± 9.5	87.1 ± 9.5	66.9 - 111.5	3
Harvest Index	0.43 ± 0.02	0.44 ± 0.02	0.41 - 0.48	1
Spike Length (cm)	9.2 ± 0.8	9.1 ± 0.8	8.6 - 12.5	1
Spikelet Number	15.8 ± 0.6	15.5 ± 0.6	14.6 - 17.6	0
Kernel Number/Spike	35.5 ± 2.5	41.3 ± 2.5	35.4 - 47.8	5
Kernel Weight/ Spike (g)	1.14 ± 0.09	1.37 ± 0.09	1.11 - 1.66	6
Average Kernel Weight (mg)	32.9 ± 1.2	33.1 ± 1.2	29.8 - 36.1	1
B2Head:Stem (g)	0.79 ± 0.33	0.72 ± 0.33	0.43 - 1.14	1
B3Head:Stem (g)	1.34 ± 0.10	1.24 ± 0.11	0.77 - 1.81	0
B4Head:Stem (g)	2.31 ± 0.17	2.65 ± 0.17	2.37 - 3.07	2
Plant Height (cm)	80.3 ± 1.0	82.0 ± 1.1	77.5 - 84.4	3
Total Kernel Number‡	14,032.6 ± 91.8	14,076.0 ± 251.1	12,275.9 – 15,430.0	0
Total Spike Number‡	395.3 ± 2.2	343.9 ± 11.1	274.3 – 422.2	0

† Number of lines significantly ($P \leq 0.05$) greater than Ankor for each trait.
‡ Values are per $1m^2$

Table 3.11. Pearson correlation coefficients among 22 field traits in 16 Ankor/Sokoll $BC_2F_{2:4}$ lines in the Fort Collins 2011 Dry environment.

	Trait†																			
Trait†	TW	DH	DPM	GFD	AGB1	AGB2	AGB3	AGB4	KWB4	HI	SL	SpN	KS	KWS	AKW	B2:SS	B3:SS	B4:SS	PH	TKN	TSN
GY					0.71***	0.76***	0.79**	0.49*	0.53*											0.55*	
TW																					0.60*
DH				-0.90***																	
DPM																					
GFD																					
AGB1						0.51*															
AGB2							0.79***				0.50*										
AGB3												0.78***					0.61*				
AGB4											0.58*	0.75***			0.50*					0.89***	0.66*
KWB4															0.53*					0.94***	0.66*
HI																					
SL												0.50*		0.58*							
SpN													0.78***	0.75***					0.60*		
KS														0.88***					0.63*		-0.53*
KWS															0.71*				0.61*		
AKW																					
B2:SS																					
B3:SS																					
B4:SS																					
PH																					
TKN																					0.83***

*, **, ***, Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. Blank spaces indicated the correlation is not significant.
GY=grain yield, TW=test weight, DH=days to heading, DPM=days to physiological maturity, GFD=grain fill duration, AGB1=above ground biomass at jointing, AGB2=above ground biomass at booting, AGB3=above ground biomass at heading, AGB4=above ground biomass at harvest, KWB4=kernel weight from biomass at harvest, HI=harvest index, SL=spike length, SpN=spikelet number, KS=kernels per spike, KWS=kernel weight per spike, AKW=average kernel weight, B2:SS=spike: stem ratio at booting B3:SS =spike: stem ratio at heading, B4:SS =spike: stem ratio at harvest, PH=plant height, TKN=total kernel number per 0.5 m biomass strip, TSN=total spike number per 0.5 m biomass strip.

hypothesis that grain yield was correlated with biomass was confirmed in this environment, as grain yield was correlated with every biomass sample taken (r=0.71, 0.76, 0.79, and 0.49, $P \leq 0.05$); however, the hypothesis that grain yield was correlated with the spike: stem ratio was rejected at all sampled growth stages. Days to heading was significantly ($P \leq 0.001$) and negatively correlated with grain fill duration (r=-0.90). This indicates that the later the heading date, the shorter the grain fill duration. Later heading in Colorado usually coincides with higher temperatures, which hasten senescence and therefore shorten the grain fill period. Spikelet number was positively correlated with the biomass sample weight at both heading and harvest (r=0.78 and 0.75, $P \leq 0.001$) at the time when grain filling is important. Spikelet number was also correlated with kernel number per spike (r=0.78) and kernel weight per spike (r=0.75). This is logical as the greater the number of spikelets, the greater chance for grains to form and thus the greater total kernel weight. Spike length was also correlated with kernel weight per spike (r=0.58), indicating that the longer the spike length, the greater the chance for kernels to form and have a greater kernel weight. Also, the total kernel number and the total spike number were correlated to each other and each with the biomass samples at heading and harvest.

2011 Fort Collins Wet Trial

Analysis of the 2011 Fort Collins wet field trial indicates Ankor had a higher mean than the $BC_2F_{2:5}$ lines in eight of the 22 measured traits (Table 3.12). Ankor and $BC_2F_{2:5}$ lines had very similar means for traits such as harvest index (0.46 and 0.45, respectively). Mean grain yield for Ankor was approximately 125 kg/ha greater than the mean of the $BC_2F_{2:5}$ lines (5806 and 5682 kg/ha, respectively). However, the maximum

Table 3.12. Performance of Ankor in comparison to the $BC_2F_{2:5}$ means and ranges at the Fort Collins 2011 wet trial. Mean values are presented ± standard error.

	Ankor	**$BC_2F_{2:5}$**		
Trait	**Mean ± SE**	**Mean ± SE**	**Range**	**No. Lines > Ankor†**
Grain Yield (kg/ha)	5805.6 ± 131.9	5682.1 ± 134.9	5262.1 - 6199.9	3
Test Weight (kg/hL)	61.9 ± 0.3	61.6 ± 0.3	61.0 - 62.4	1
Days to Heading (Julian days)	155.6 ± 0.39	155.4 ± 0.4	153.4 - 156.8	1
Days to Maturity (Julian days)	193.9 ± 0.4	194.4 ± 0.4	193.7 ± 195.6	5
Grain Fill Duration (Julian days)	38.2 ± 0.5	39.1 ± 0.5	37.5 - 41.0	8
Biomass Sample 1 (g)	59.7 ± 8.1	51.4 ± 8.2	40.7 - 63.1	3
Biomass Sample 2 (g)	112.7 ± 16.2	106.9 ± 16.2	87.1 - 134.9	1
Biomass Sample 3 (g)	151.7 ± 17.7	127.3 ± 17.9	97.6 - 169.2	1
Biomass Sample 4 (g)	218.3 ± 30.8	272.8 ± 31.5	200.7 - 316.4	6
KwtS4 (g)	83.2 ± 11.2	103.4 ± 11.5	83.0 - 124.1	5
Harvest Index	0.46 ± 0.02	0.45 ± 0.02	0.40 - 0.49	1
Spike Length (cm)	8.7 ± 0.2	9.6 ± 0.2	9.2 - 10.4	14
Spikelet Number	15.2 ± 1.3	17.3 ± 1.4	16.2 - 20.8	1
Kernel Number/Spike	39.4 ± 2.3	46.8 ± 2.4	42.5 - 53.9	10
Kernel Weight/ Spike (g)	1.37 ± 0.10	1.67 ± 0.10	1.38 - 2.04	5
Average Kernel Weight (mg)	35.0 ± 0.9	35.7 ± 0.9	33.3 - 37.4	3
B2Head:Stem (g)	0.31 ± 0.19	0.43 ± 0.19	0.26 - 0.74	5
B3Head:Stem (g)	0.83 ± 0.04	0.90 ± 0.05	0.51 - 1.24	0
B4Head:Stem (g)	2.61 ± 0.27	2.57 ± 0.27	2.02 - 3.48	1
Plant Height (cm)	88.4 ± 1.6	89.8 ± 1.6	85.9 - 94.2	1
Total Kernel Number	16,599.9 ± 88.9	15,934.9 ± 236.0	14,547.1 – 17,862.3	5
Total Spike Number	420.0 ± 9.4	342.1 ± 8.6	285.4 – 400.5	0

† Number of lines significantly ($P \leq 0.05$) greater than Ankor for each trait.
‡ Values are per 1 m^2

yield value of the $BC_2F_{2:5}$ lines grain yield value was 6200 kg/ha, indicating transgressive segregation. Ankor was greater than the $BC_2F_{2:5}$ lines for test weight and three of four biomass samples taken. However, transgressive segregation was evident in all traits for the wet treatment. The mean spike: stem ratio at boot and heading stages was greater for the $BC_2F_{2:5}$ lines, but the spike: stem ratio at harvest was greater for Ankor. In the Fort Collins 2011 wet trial, one or more $BC_2F_{2:5}$ lines performed significantly ($P \leq 0.05$) better than Ankor for 18 of 22 traits. Ten $BC_2F_{2:5}$ lines had significantly ($P \leq 0.05$) more kernels per spike, eight lines had longer grain fill duration, 14 had longer spikes and three had higher grain yield.

Pearson correlation coefficients in the Fort Collins 2011 wet environment revealed more correlations than in the 2011 dry environment (Table 3.13). Grain yield was significantly ($P \leq 0.05$) correlated with test weight (r=0.58), as well as the biomass samples at jointing, booting, and heading (r=0.58, 0.73, and 0.61, respectively), lending further support to the correlation between biomass and grain yield. Days to heading was significantly ($P \leq 0.001$) and negatively correlated again with grain fill duration (r=-0.72), indicating the potential effect of hot temperatures later in the summer and the ability to fill grains effectively. Days to heading was also negatively correlated with average kernel weight (r=-0.24, $P \leq 0.05$), revealing that as heading occurred later in the season, final kernel weight decreased. Total kernel number and total spike number were significantly ($P \leq 0.001$) correlated to each other (r=0.84); in addition, total kernel number was correlated with the biomass sample at harvest, the final kernel weight at harvest, and harvest index (r=0.91, 0.96, and 0.39, $P \leq 0.001$, respectively) and total spike number was also correlated with these same traits (r=0.74, 0.80, and 0.42, $P \leq 0.001$, respectively).

Table 3.13. Pearson correlation coefficients among 22 field traits in 16 Ankor/Sokoll $BC_2F_{2:4}$ lines in the Fort Collins 2011 Wet environment.

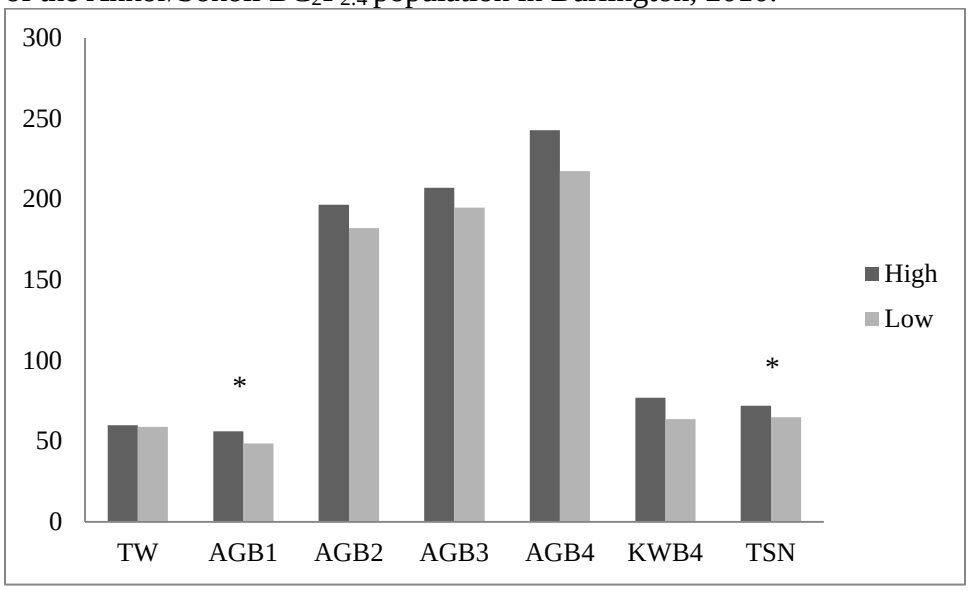
	Trait†																				
Trait†	TW	DH	DPM	GFD	AGB1	AGB2	AGB3	AGB4	KWB4	HI	SL	SpN	KS	KWS	AKW	B2:SS	B3:SS	B4:SS	PH	TKN	TSN
GY	0.58*				0.58**	0.73***	0.61**				-0.49*				0.57**			0.53*			
TW			-0.47*		0.50*						-0.76***		0.51*								
DH			0.28*	-0.72***	-0.33**										-0.24*	-0.49***					
DPM				0.47***	-0.33**	-0.26*					0.26*	0.25*							0.26*		
GFD																0.30**					
AGB1						0.61***					-0.23*				0.34**						
AGB2												-0.35**			0.28*						
AGB3											-0.27*		-0.31**				-0.42***				
AGB4									0.96***	0.64***	0.25*		0.30**	-0.64***				0.22*		0.91***	0.74***
KWB4										-0.42***	0.24*		0.30**	-0.42***				0.25*		0.96***	0.80***
HI														1.00***						0.39***	0.42***
SL													0.64***				0.33**		0.46***	0.30**	
SpN													0.23*						0.24*		
KS																	0.23*		0.38***	0.28*	-0.26*
KWS																				-0.39***	-0.42***
AKW																					
B2:SS																			-0.30**		
B3:SS																					
B4:SS																				0.28*	
PH																					
TKN																					0.84***

*, **, ***, Significant at the 0.05, 0.01, and 0.001 probability levels, respectively. Blank spaces indicated the correlation is not significant.
GY=grain yield, TW=test weight, DH=days to heading, DPM=days to physiological maturity, GFD=grain fill duration, AGB1=above ground biomass at jointing, AGB2=above ground biomass at booting, AGB3=above ground biomass at heading, AGB4=above ground biomass at harvest, KWB4=kernel weight from biomass at harvest, HI=harvest index, SL=spike length, SpN=spikelet number, KS=kernels per spike, KWS=kernel weight per spike, AKW=average kernel weight, B2:SS=spike: stem ratio at booting B3:SS =spike: stem ratio at heading, B4:SS =spike: stem ratio at harvest, PH=plant height, TKN=total kernel number per 0.5 m biomass strip, TSN=total spike number per 0.5 m biomass strip.

Eight high and eight low means comparison, 2010

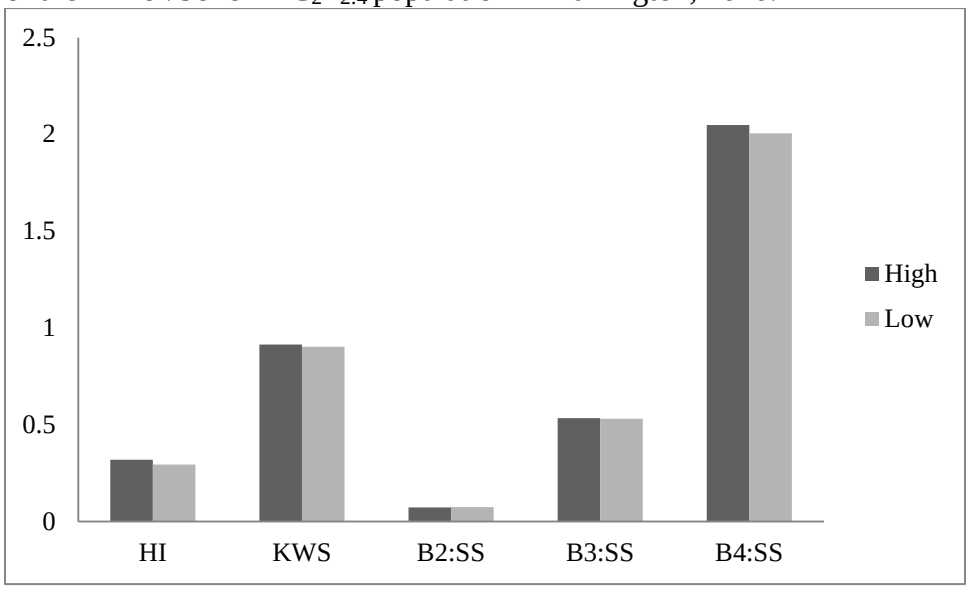
The means of the eight highest and eight lowest lines based on performance from 2007 to 2009 were compared for most traits in the 2010 field trials (see Figures 3.9 to 3.20). In all three environments, grain yield was significantly ($P \leq 0.001$) higher in the high versus the low lines. In Burlington and Dailey, the eight high lines had a greater total estimated spike number than the eight low lines means for total estimated spike number ($P \leq 0.05$ and 0.01, respectively). In addition, test weight was significant higher ($P \leq 0.001$) in the high set of lines in both Dailey and Julesburg. Burlington and Dailey both had one biomass sample that was significantly different between the groups of lines. Dailey was the only environment to have any significant difference between high and low lines in spike: stem ratio at harvest. It is a bit surprising that kernel number, kernel weight, biomass, and spike: stem ratios did not show more significance, as it seems logical that larger values for these traits would be associated with the higher yielding lines.

Figure 3.9. Means of seven traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Burlington, 2010.



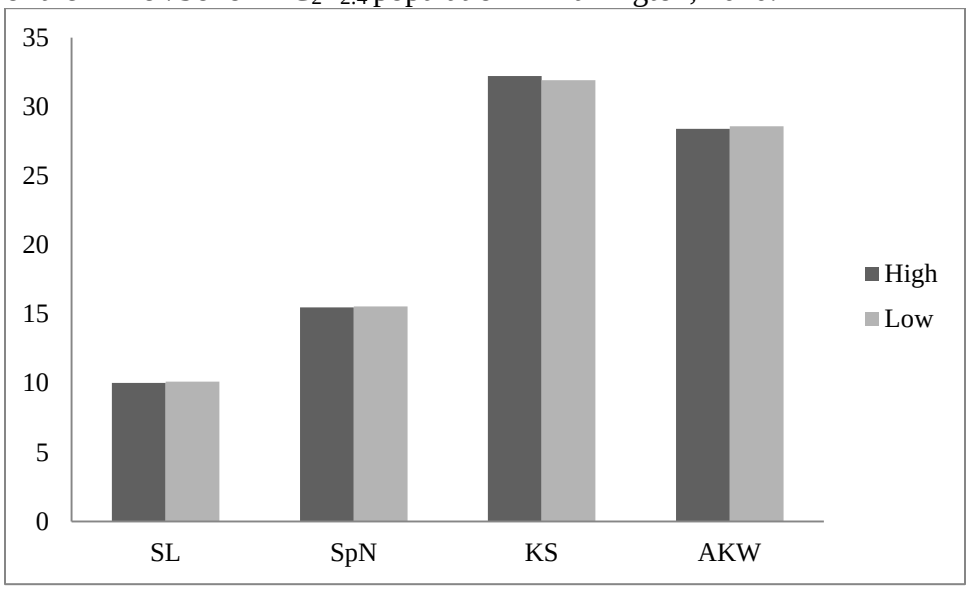
TW=test weight; AGB1=above ground biomass sample at jointing; AGB2=above ground biomass sample at booting; AGB3=above ground biomass sample at heading; AGB4=above ground biomass sample at harvest; KWB4=kernel weight of sample 4; TSN=total spike number ($x10^3$) per m^2.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.10. Means of five traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Burlington, 2010.



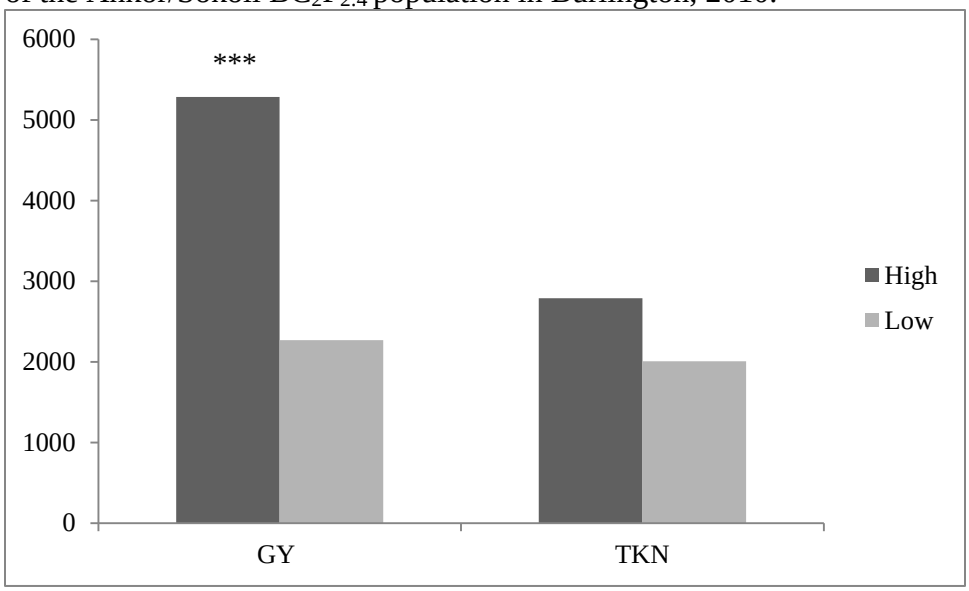
HI=harvest index; KWS= Kernel weight per spike, B2:SS=spike to stem ratio at booting; B3:SS=spike to stem ratio at heading; B4:SS=spike to stem ratio at harvest.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.11. Means of four traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Burlington, 2010.



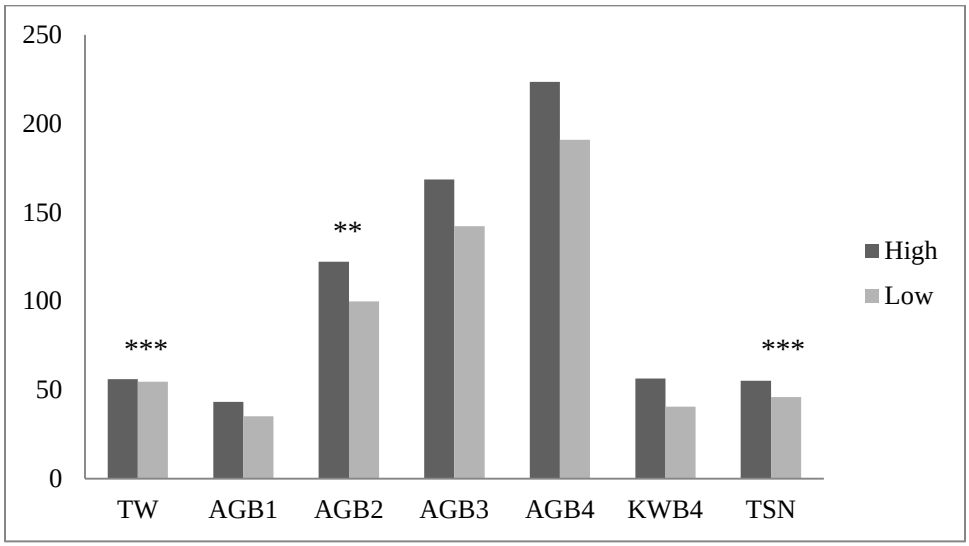
SL=spike length; SpN=spikelet number per spike; KS=kernel number per spike; AKW=average kernel weight.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.12. Means of two traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Burlington, 2010.



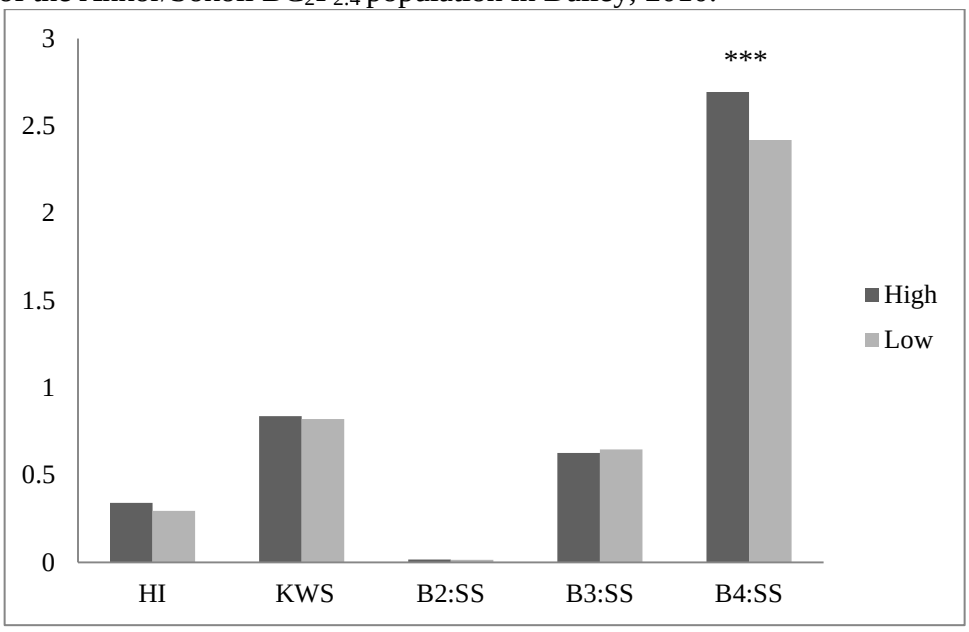
GY=grain yield; TKN=total kernel number ($x10^3$) per m^2.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.13. Means of seven traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Dailey, 2010.



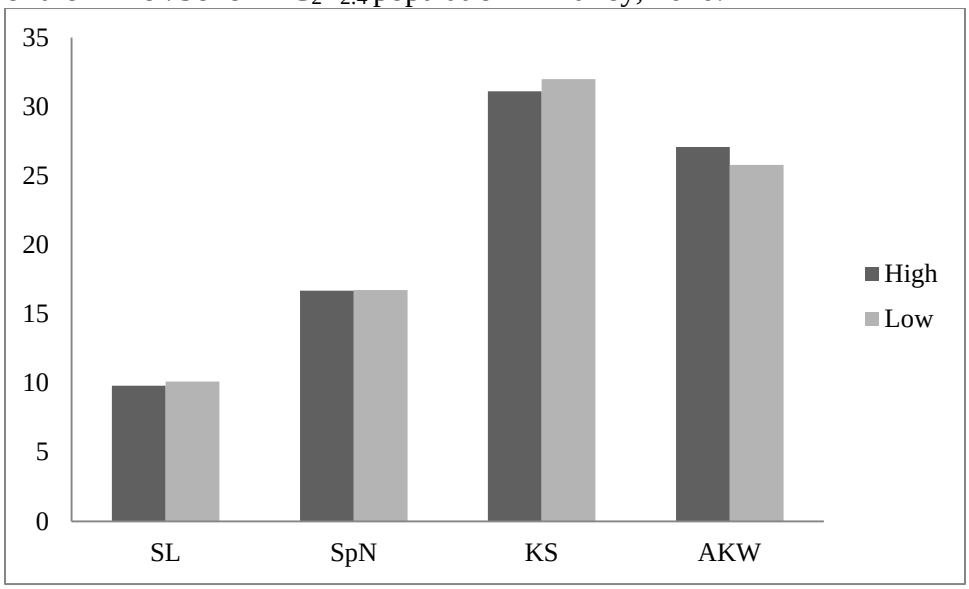
TW=test weight; AGB1=above ground biomass sample at jointing; AGB2=above ground biomass sample at booting; AGB3=above ground biomass sample at heading; AGB4=above ground biomass sample at harvest; KWB4=kernel weight of sample 4; TSN=total spike number ($x10^3$) per m^2.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.14. Means of five traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Dailey, 2010.



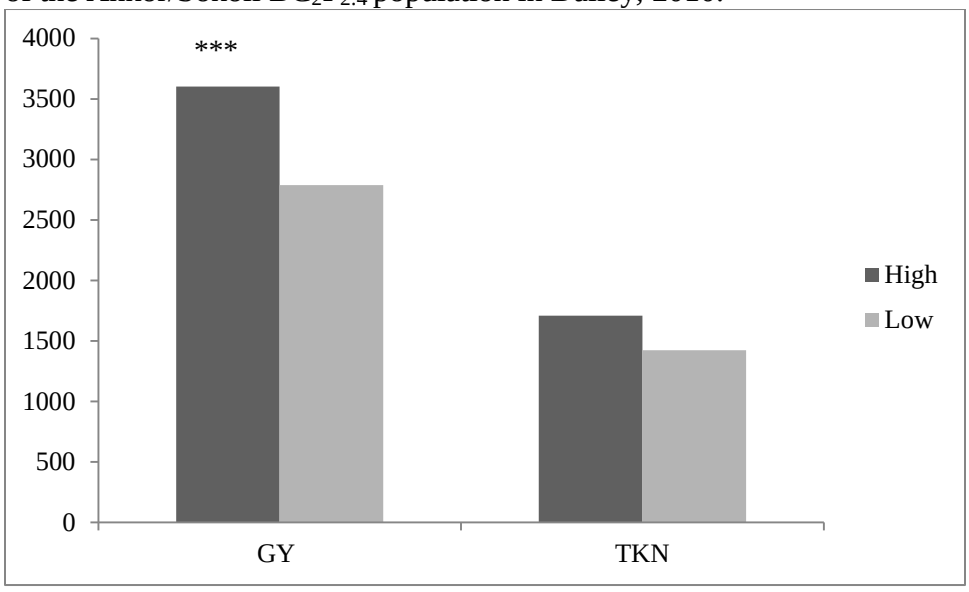
HI=harvest index; KWS= Kernel weight per spike, B2:SS=spike to stem ratio at booting; B3:SS=spike to stem ratio at heading; B4:SS=spike to stem ratio at harvest.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.15. Means of four traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Dailey, 2010.



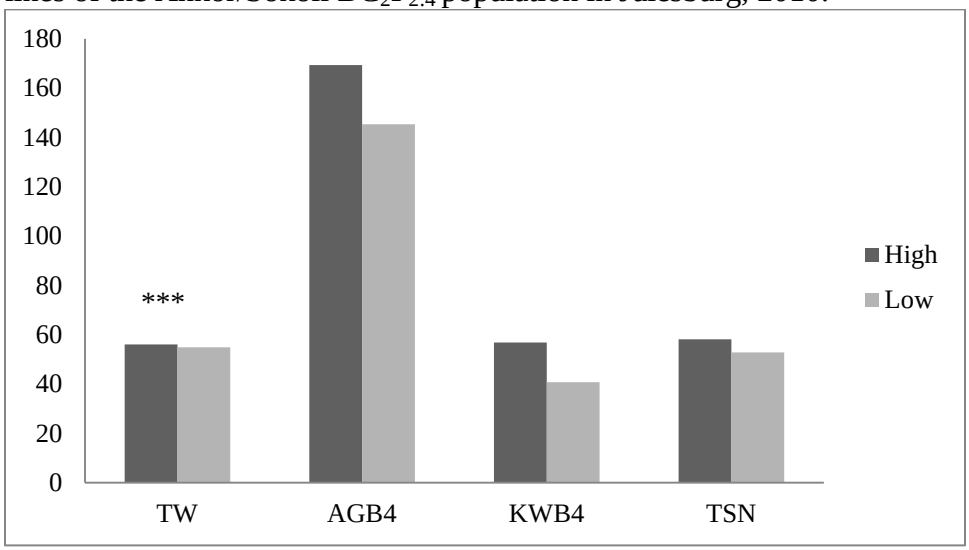
SL=spike length; SpN=spikelet number per spike; KS=kernel number per spike; AKW=average kernel weight.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.16. Means of two traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Dailey, 2010.



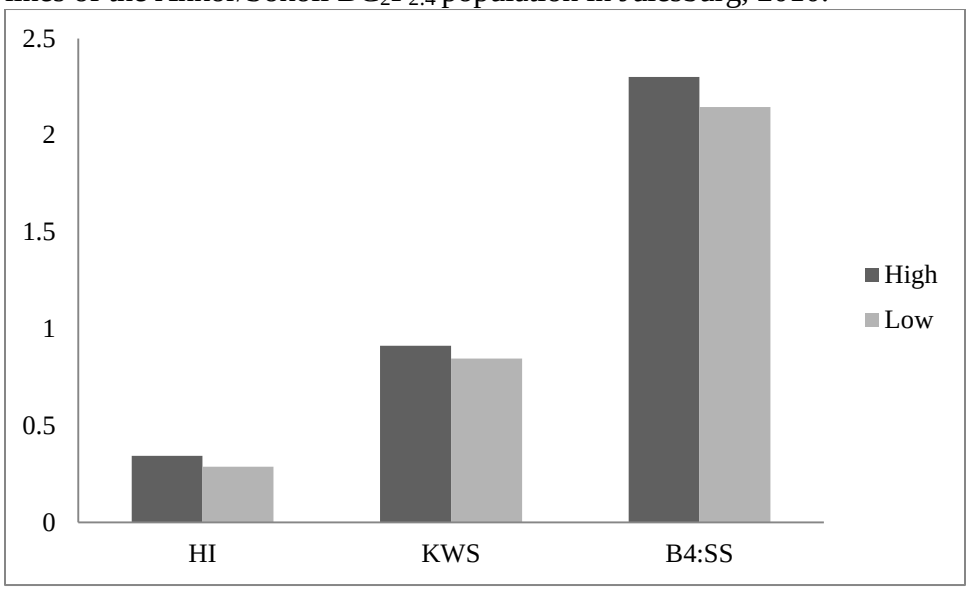
GY=grain yield; TKN=total kernel number ($x10^3$) per m^2.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.17. Means of seven traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Julesburg, 2010.



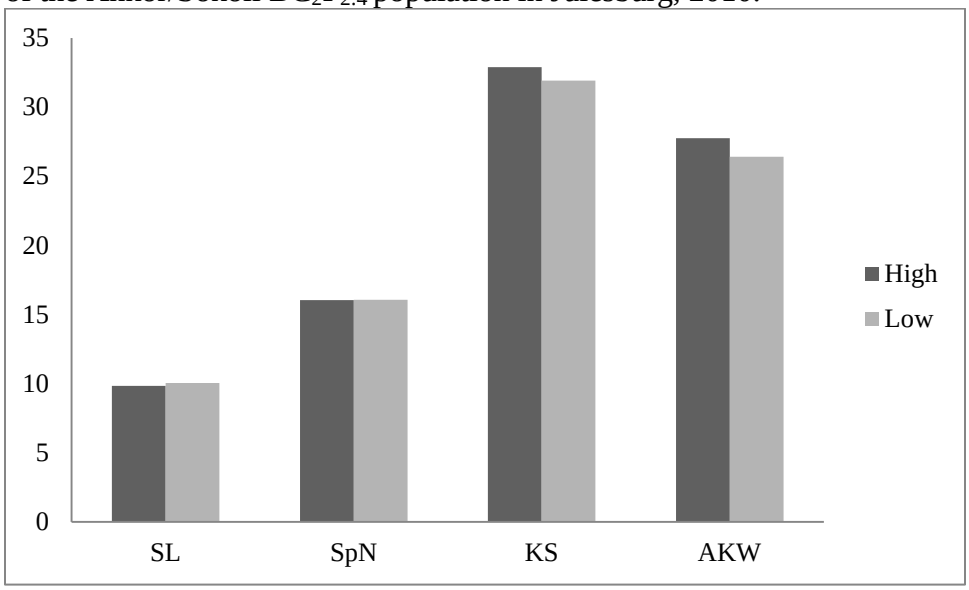
TW=test weight; AGB4= biomass sample at harvest; KWB4=kernel weight of sample 4; TSN=total spike number ($x10^3$) per m^2.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.18. Means of three traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Julesburg, 2010.



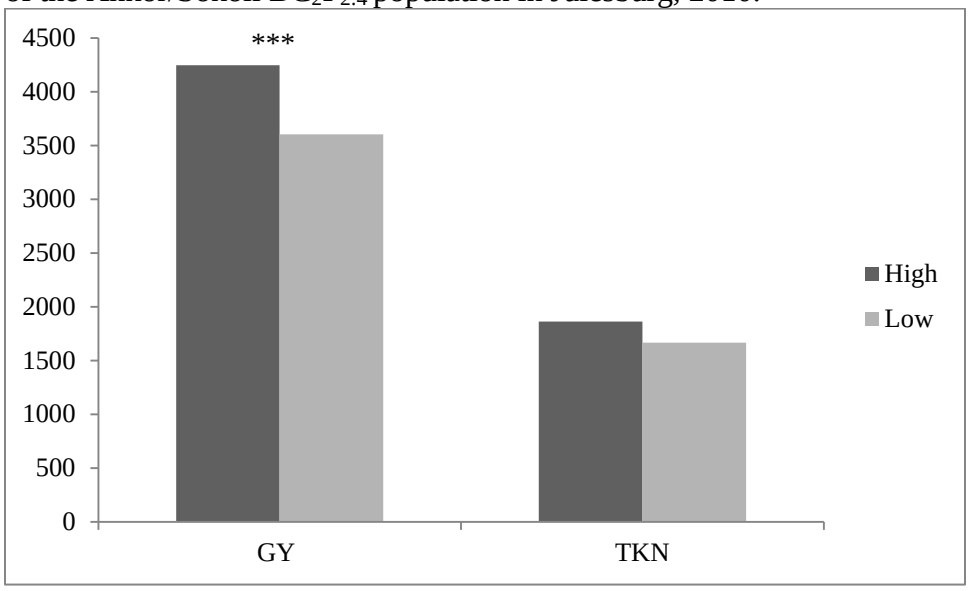
HI=harvest index; KWS= Kernel weight per spike, B4:SS=spike to stem ratio at harvest.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.19. Means of four traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Julesburg, 2010.



SL=spike length; SpN=spikelet number per spike; KS=kernel number per spike; AKW=average kernel weight.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.20. Means of two traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:4}$ population in Julesburg, 2010.

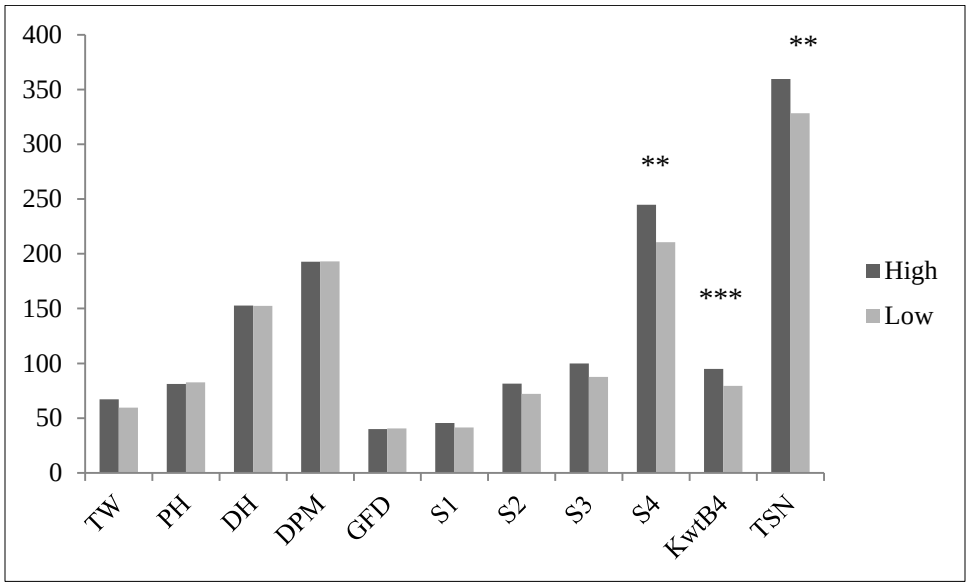


GY=grain yield; TKN=total kernel number ($x10^3$) per m^2.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Eight high and eight low means comparison, 2011

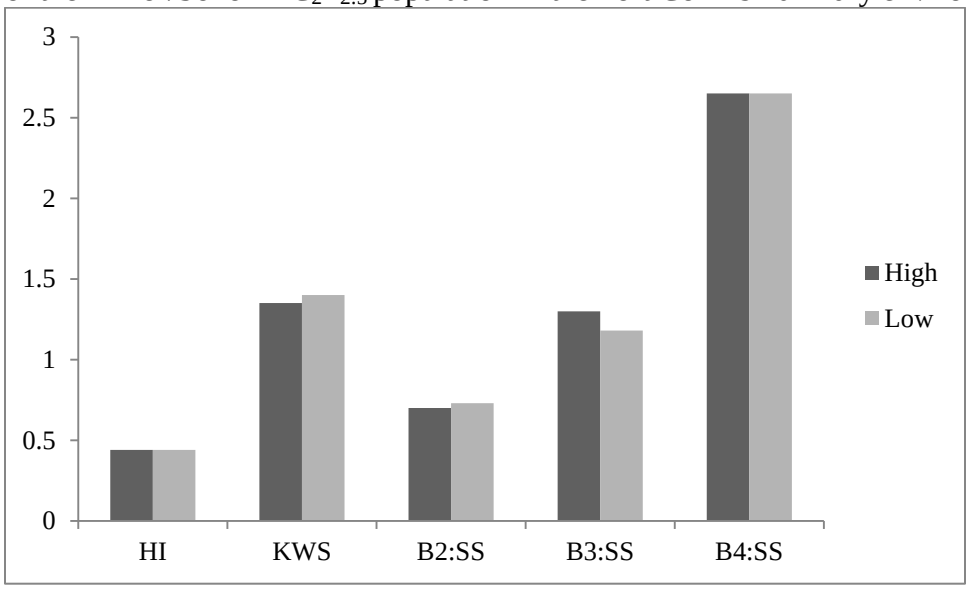
The means of the eight highest and eight lowest lines based on performance from the 2010 trials were calculated for all traits in the 2011 field trials (see Figures 3.21 to 3.28). In both the wet and dry environments, grain yield was significantly ($P \leq 0.05$) different in the high versus the low lines. In the Fort Collins 2011 dry treatment, means of the high eight lines were significantly ($P \leq 0.01$) different than means of the eight low lines for total estimated spike and kernel number. In addition, the biomass sample at harvest and the final kernel weight of the biomass sample at harvest were significantly ($P \leq 0.01$and 0.001, respectively) different between the high and low lines. In the 2011 wet treatment, the spike: stem ratio at harvest was the only trait besides grain yield that was significantly different between the eight high and eight low lines. It is again surprising that there was not a greater difference between high and low lines for biomass samples at all stages and in both treatments. However, the mean of the high lines was generally greater than or about the same as the mean of the low lines for most traits in the dry and wet treatments.

Figure 3.21. Means of 10 traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 dry environment.



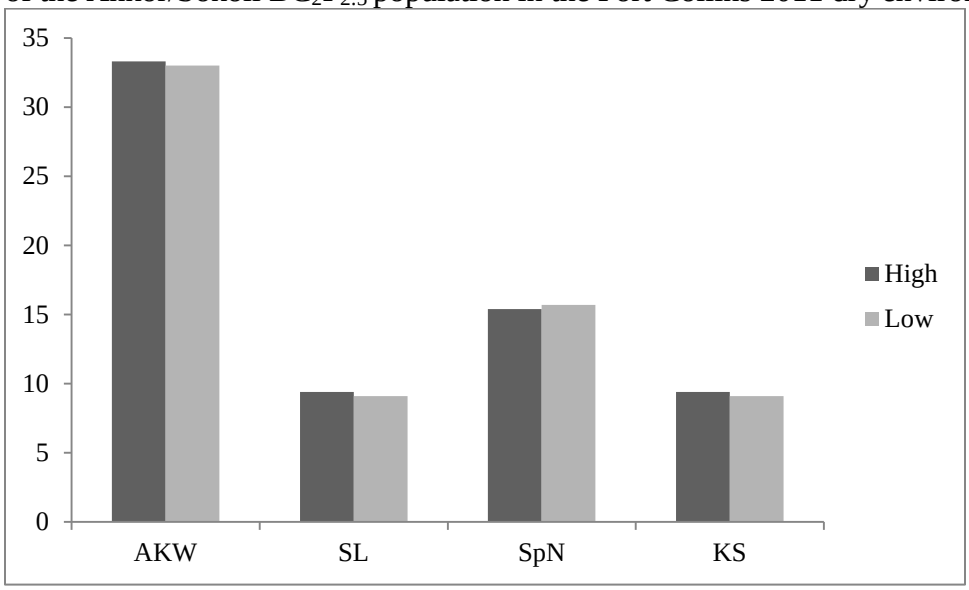
TW=test weight, PH=plant height, DH=days to heading, DPM=days to physiological maturity, GFD=grain fill duration, S1=biomass sample at jointing, S2=biomass sample at booting, S3=biomass sample at heading, S4=biomass sample at harvest, TSN=total spike number ($x10^3$) per m^2, KwtB4: kernel weight from biomass sample at harvest.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.22. Means of five traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 dry environment.



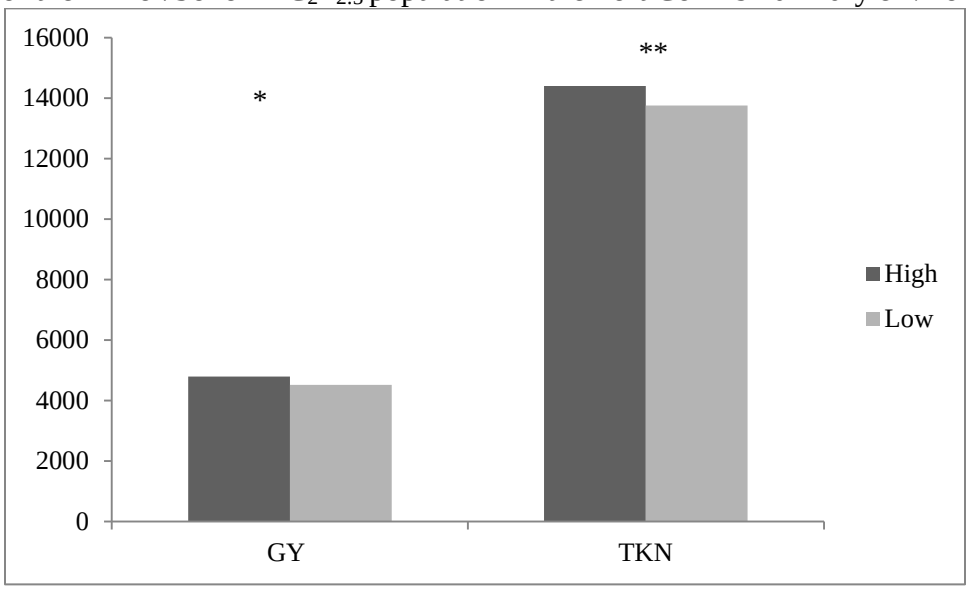
HI=harvest index, KWS=kernel weight per spike, B2:SS=spike: stem ratio at booting, B3:SS=spike: stem ratio at heading, B4:SS=spike: stem ratio at harvest.

Figure 3.23. Means of four traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 dry environment.



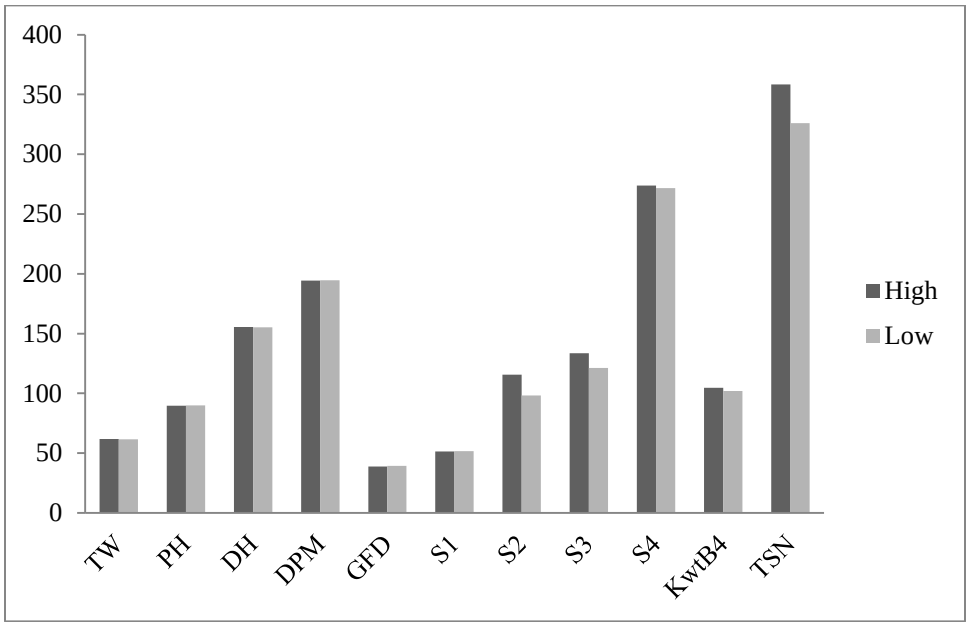
AKW=average kernel weight, SL=spike length, SpN=spikelet number per spike, KS=kernel weight per spike.

Figure 3.24. Means of two traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 dry environment.



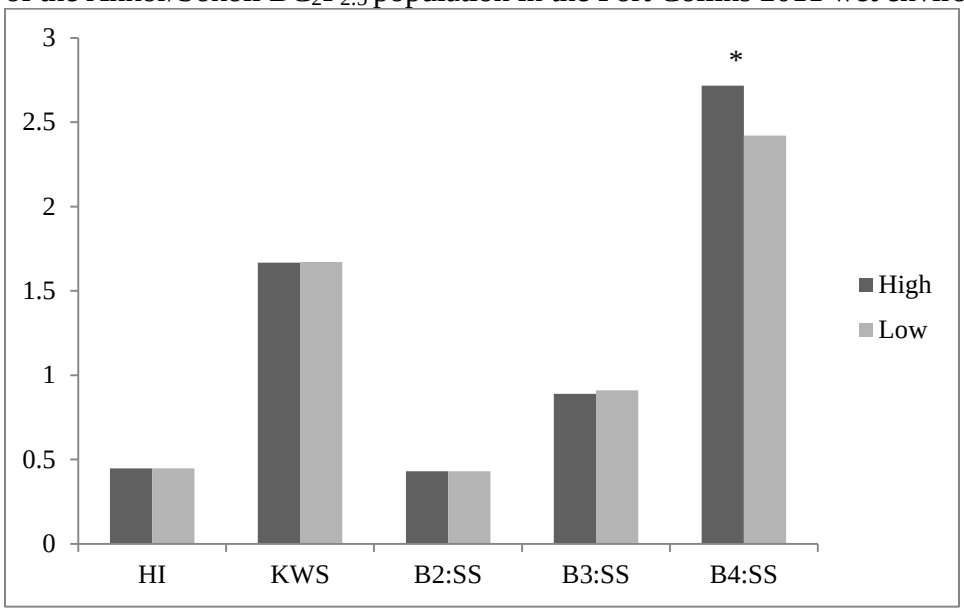
GY=grain yield, TKN=total kernel number ($x10^3$) per m^2.

Figure 3.25. Means of 10 traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 wet environment.



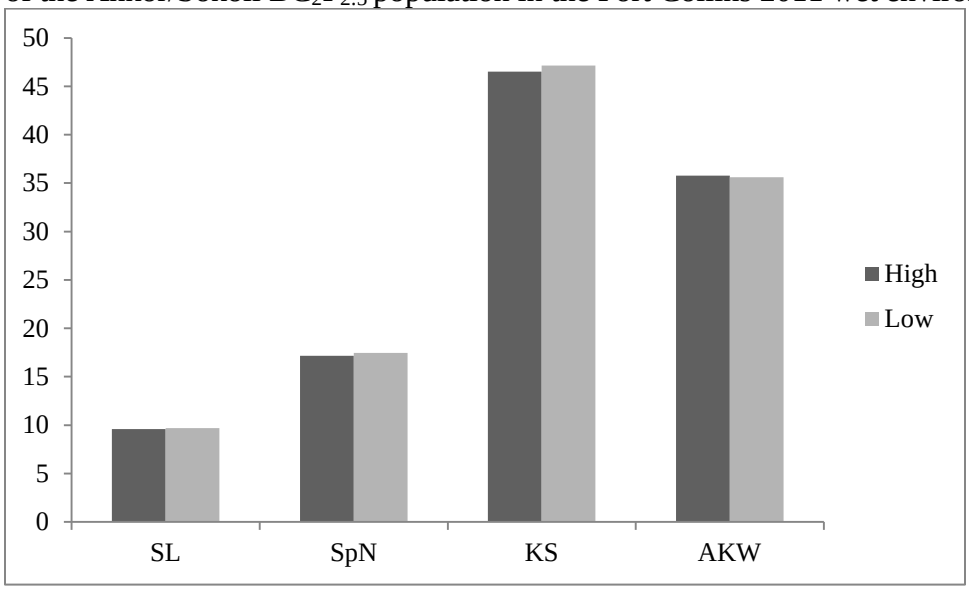
TW=test weight, PH=plant height, DH=days to heading, DPM=days to physiological maturity, GFD=grain fill duration, S1=biomass sample at jointing, S2=biomass sample at booting, S3=biomass sample at heading, S4=biomass sample at harvest, TSN=total spike number ($x10^3$) per m^2, KwtB4: kernel weight from biomass sample at harvest.
***, **, *. Significant at the 0.001, 0.01, and 0.05 levels, respectively.

Figure 3.26. Means of five traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 wet environment.



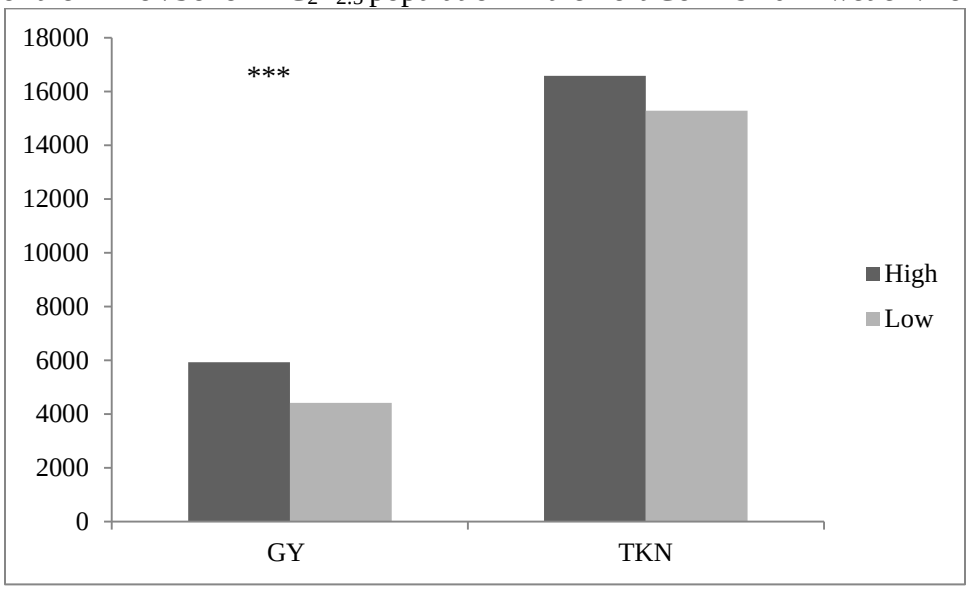
HI=harvest index, KWS=kernel weight per spike, B2:SS=spike: stem ratio at booting, B3:S:S=spike: stem ratio at heading, B4:SS=spike: stem ratio at harvest.

Figure 3.27. Means of four traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 wet environment.



KW=average kernel weight, SL=spike length, SpN=spikelet number per spike, KS=kernel weight per spike.

Figure 3.28. Means of two traits for the eight high- yielding and eight low-yielding lines of the Ankor/Sokoll $BC_2F_{2:5}$ population in the Fort Collins 2011 wet environment.

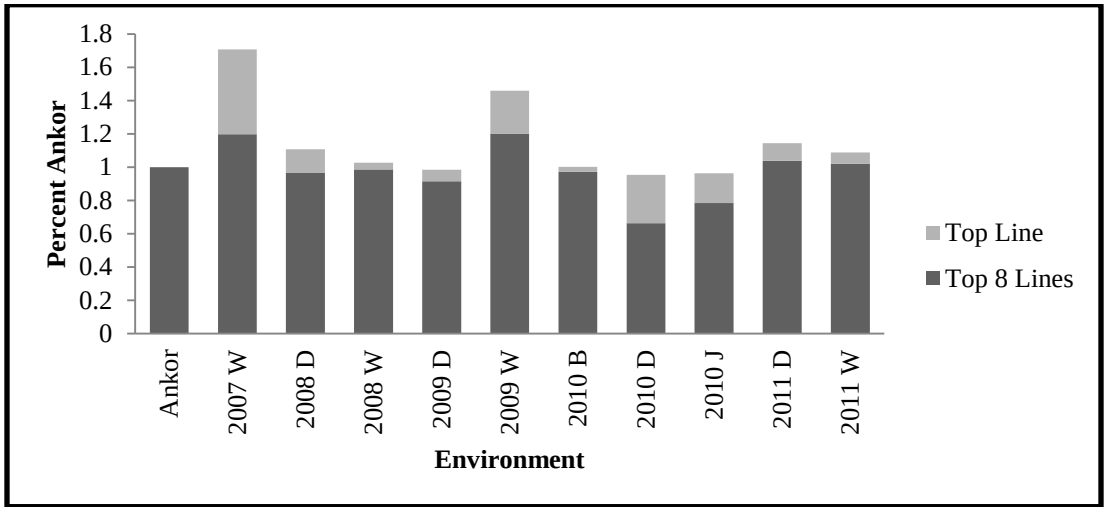


GY=grain yield, TKN=total kernel number ($x10^3$) per m^2.

Summary of yield improvement over Ankor

The percent yield value of Ankor for the top line and top eight lines in all ten environments are depicted in Figure 3.29. For the mean of the top eight lines, six environments (2008 dry and wet, 2009 dry, and 2010 Burlington, Dailey, and Julesburg) had a numerically smaller percent yield value than Ankor (percent of Ankor=97, 99, 92, 97, 66, and 78, respectively). The two environments with the greatest percent yield improvement over Ankor for the top eight lines were the 2007 and 2009 wet environments, both peaking at 120%. Examining just the highest performing line in each environment, it was discovered that the highest yielding line in the 2009 dry, 2010 Dailey, and 2010 Julesburg environments did not surpass the mean yield for Ankor. However, in the 2007 and 2009 wet environments, in particular, the best backcross line out-yielded Ankor by 46 and 71%, respectively. The top lines in both these cases also did much better than the top eight lines, out-performing the mean of the top eight lines by 26 and 51%, respectively.

Figure 3.29. Percent yield value of Ankor for the top line and top eight lines in all 10 environments.



Top Lines for Breeding

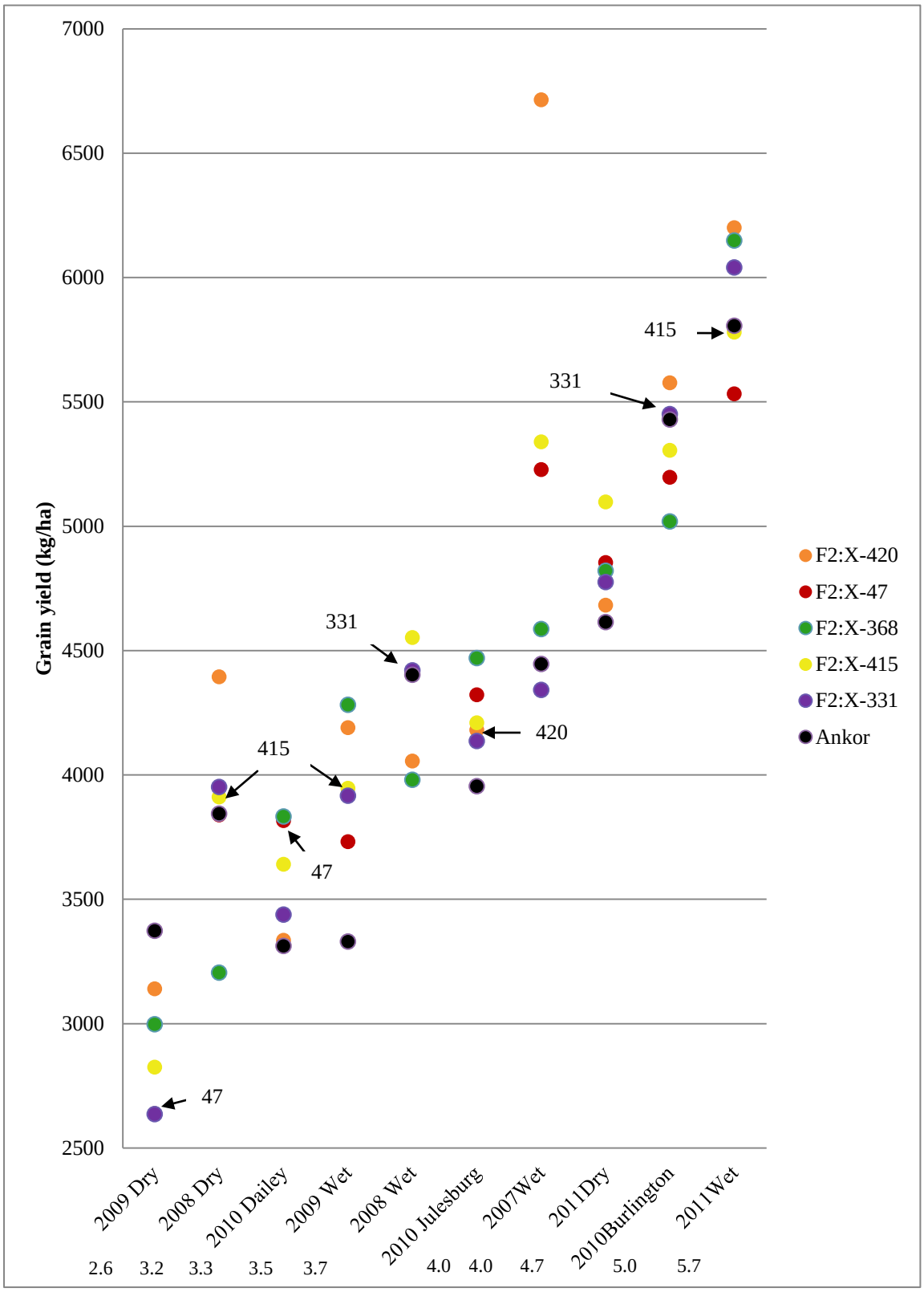
The top five lines across the five environments from 2010 to 2011 were selected based on yield for potential use as parents in a breeding program. Grain yield for the five lines and Ankor in each of the 10 environments are summarized in Figure 3.30. The environments were plotted based on the mean yield of the lines in each environment. The environments with the lower mean grain yield are on the left side of the figure, and the environments with the higher mean grain yield are toward the right side of the figure.

It is evident among the five lowest- and highest-yielding environments that there was substantial variability in line performance. In the lowest-yielding environments, Ankor yielded the highest; however, in the 2009 wet and 2010 Dailey environment, Ankor was the lowest-yielding among the lines represented in Figure 3.36. Line 368 was the highest-yielding line in the 2010 Dailey and 2009 wet environments. Line 420 performed near or at the top of the lines in three of five low-yielding environments.

Among the five high-yielding environments, the top five lines yielded more than Ankor in the 2010 Julesburg and 2011 dry environments. Line 420 out-yielded all lines in three of five environments. Line 415 was the highest-yielding line in the 2011 dry environment and ranked in the middle of the five lines for all other high-yield environments. It is evident that line 420 was the most consistent, top-performing line across the 10 environments.

The top five lines were evaluated for the number of Sokoll favorable alleles they contained for all significant markers of selected yield and yield component traits (Table 3.14). Line 47 was determined to have the most Sokoll favorable alleles (4) for grain

Figure 3.30. Grain yield of the top five lines and Ankor in each of the 10 environments. Values below the environment are the line means for each environment in tons/ha.



yield, lines 420 had the most Sokoll alleles for average kernel weight (5), and line 368 had the most for total kernel number (4). This indicates certain lines received more Sokoll alleles at specific loci than others.

Table 3.14. Number of 'H' scores at all significant ($P \leq 0.01$) markers for four traits and the top five lines in the Ankor/Sokoll BC_2F_2-derived population.

	Line				
Trait	**47**	**331**	**368**	**415**	**420**
Grain yield	4 (2)†	3 (2)	2 (2)	2(1)	1 (0)
Average kernel weight	3 (0)	2 (0)	1 (1)	1 (1)	5 (2)
Total kernel number	2 (2)	1 (1)	4 (4)	0 (0)	1 (1)
Total spike number	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)

†Number in parentheses represents the number of markers for which Sokoll had the favorable allele and for which an H score was recorded for the line.

QTL Validation, 2010-11

Grain yield: Of the 12 significant ($P \leq 0.01$) markers detected in the 2007 to 2009 environments for grain yield, 10 were significant ($P \leq 0.05$) in at least one of the five field trials from 2010 to 2011 (Table 2.15). Three markers for grain yield were validated in multiple field trials. The adjacent marker pair *Xwmc603/Xbarc108*, found on chromosome 7A, was detected in the Dailey and Julesburg dry trials, contributing 13.7% and 12.9% of the phenotypic variation in their respective environments. *Xwmc603/Xbarc108* was previously detected in the 2008 Fort Collins dry and 2009 Greeley wet trials. In addition, marker *Xgwm193*, found on chromosome 6B, was also validated in the Dailey and Julesburg dry trials. *Xgwm193* was previously detected in the 2008 Fort Collins wet field trial. Furthermore, marker *Xgwm268* (chromosome 1B), previously detected in the 2008

Table 3.15. QTL validation for agronomic and morphological traits of the Ankor/Sokoll BC_2F_2-derived population in five Colorado environments in the 2010 and 2011 growing seasons.

Trait	Environment†	Chromosome	Nearest Marker	*P*	% R^2	Effect‡	Previous Environment (s)
Grain yield	2010 Dailey	1B	*Xgwm153*	0.027	11.4	-288.5	08FW, 09GW
		1B	*Xgwm268*	0.037	10.0	-305.4	08FD, 09GW
		3A, 1D	*Xgwm155*	0.010	15.2	283.0	09GD
		3A	*Xwmc532*	0.040	9.8	231.9	08FD
		6B	*Xgwm193*	0.045	9.2	-250.0	08FW
		7A	*Xwmc603*	0.015	13.7	293.1	08FD, 09GW
	2010 Julesburg	2A, 2B, 2D, 6B	*Xgwm311*	0.040	9.6	-181.2	09GD
		6B	*Xgwm193*	0.022	11.8	-231.8	08FW
		7A	*Xwmc603*	0.018	12.9	233.9	08FD, 09GW
	2011 Dry	1B	*Xgwm268/153*	0.022	32.4	-340.5	08FW, 09GW
		3D	*Xgwm484*	0.009	41.6	-327.1	08FW
	2011 Wet	2B	*Xgwm630*	0.044	25.9	-312.0	07FW
		2D	*Xgwm539*	0.040	28.5	-374.5	09GW
Plant height	2011 Dry	2B	*Xgwm630*	0.028	30.0	1.9	08FD
Days to heading	2011 Wet	3D	*Xgwm484*	0.006	45.9	-1.4	07FW, 08FW
Days to maturity	2011 Dry	6A	*Xwmc553*	0.044	25.9	0.5	07FW
Average kernel weight	2010 Julesburg	2A, 2B	*Xwmc170*	0.048	9.2	2.3	09GD

		4A	*Xwmc219*	0.031	10.6	-2.3	08FW
Kernels per spike	2010 Dailey	2B	*Xbarc55*	0.004	46.0	-3.3	09GD, 09GW
		7B	*Xgwm213*	0.009	16.8	-1.8	07FW
	2011 Dry	2D	*Xgwm261*	0.020	32.9	-2.3	07FW, 08FW
Spike length	2010 Julesburg	1A	*Glu-A1*	0.042	9.5	-0.3	08FW
		2A, 2B	*Xgwm122*	0.048	9.6	0.4	08FW
	2011 Dailey	2A	*Xwmc522*	0.0006	58.1	-1.8	08FW, 08FD
Total kernel number	2010 Burlington	7A	*Xbarc108*	0.024	11.5	1016.5	08FD
	2010 Dailey	1B	*Xgwm153*	0.013	14.2	-1317.6	09GW
		3A, 1D	*Xgwm155*	0.001	25.4	1537.2	09GD
	2010 Julesburg	2B	*Xbarc18*	0.046	9.2	-905.3	08FW
Total spike number	2010 Dailey	1B	*Xgwm153*	0.016	13.4	-45.8	08FW, 09GW
		2D	*Xgwm539*	0.013	14.8	-48.1	09GD

†The five environments: 2010 Burlington dry, 2010 Dailey dry, 2010 Julesburg dry, 2011 Fort Collins dry, and 2011 Fort Collins wet trials.
‡Additive effect: calculated as mean value of the heterozygous genotypic class minus the mean value of the homozygous genotypic class. Positive values indicate an increasing effect of Sokoll alleles, and negative values indicate an increasing effect of Ankor alleles.

Fort Collins dry and wet and 2009 Greeley wet environments was confirmed in the Dailey 2010 and Fort Collins 2011 dry environments. This indicates a tendency for *Xgwm268* to be significant in dry environments. Of the 10 markers validated from 2010 to 2011 environments, Sokoll contributed the favorable allele for markers *Xwmc532*, *Xwmc603*, and *Xgwm155*.

Test weight: No markers were able to be validated for test weight in 2010 or 2011. It is possible this is related to the small sample size in the 2010 and 2011 environments (n=44 and 16, respectively). A smaller sample size would decrease the power of detection and not allow potentially significant QTL to be validated.

Plant height: One QTL marker out of eight detected plant height markers from 2007 to 2009 was validated for plant height in 2010 and 2011. Marker *Xgwm630*, located on chromosome 2B and previously detected for grain yield in the 2008 Fort Collins dry environment, was confirmed in the 2011 Fort Collins dry environment. *Xgwm630* contributed 30.0% of the phenotypic variation for plant height in the 2011 dry treatment.

Days to heading: Of the four QTL detected for days to heading, one QTL was validated on chromosome 3D. The maker pair *Xgwm484*/*Xgwm261* contributed 45.9% of the total phenotypic variation in the Fort Collins 2011 wet environment. *Xgwm484*/*Xgwm261* was previously detected in the 2007 wet, 2008 wet, and 2009 dry and wet environments. Ankor consistently contributed to later heading for this marker, and was detected most strongly in wet environments.

Days to maturity: Of the three QTL detected for days to physiological maturity, one was validated in the 2011 Fort Collins dry environment. Marker *Xwmc553* on chromosome

6A was found previously in the 2007 Fort Collins wet environment, and it contributed 25.9% of the total phenotypic variation in the 2011 Fort Collins dry environment.

Grain fill duration: Neither of two QTL detected previously for grain fill duration was confirmed in the 2010-11 environments. Again, this could either be due to the inaccuracy or inconsistency of evaluating the trait or the small sample size. In addition, grain fill duration was not evaluated in 2010; consequently, days to heading, days to maturity, and thus grain fill duration were only able to be validated in the 2011 dry and wet treatments where the sample size was n=16. Therefore, the power to detect and validate grain fill duration was low.

Average kernel weight: Of the 12 QTL detected previously for average kernel weight, two were validated in the 2010 Julesburg dry environment. Marker *Xwmc170* contributed 9.2% and *Xwmc219* contributed 10.6% of the total phenotypic variation. These markers were previously detected in the 2008 Fort Collins wet and 2009 Greeley dry environments, respectively. Sokoll contributed the favorable allele for average kernel weight for *Xwmc170,* and Ankor contributed the favorable allele for marker *Xwmc219*.

Kernel number per spike: Of the seven QTL found from 2007-09, three were validated and represented all four environments for which QTL were previously detected in 2007-09. Marker *Xgwm213* validated the 2007 Fort Collins wet treatment by the 2010 Dailey environment and contributed 16.8% of the phenotypic variation. Marker *Xbarc55,* found on chromosome 2B, previously detected in the 2009 wet and dry treatments, was validated in the Dailey 2010 environment. Finally, *Xgwm261* validated the 2007 and 2008 Fort Collins wet treatments through its detection in the 2011 dry treatment, where it contributed 32.9% of the phenotypic variation. Ankor contributed the favorable allele for

all three markers, and in the previous four environments, Ankor contributed the favorable allele for the majority (66.7%) of the significant markers for this trait.

Spike length: Of the 13 QTL detected for spike length, three QTL were validated. Marker *Glu-A1* contributed the favorable allele and 9.5% of the total phenotypic variation in the 2010 Julesburg environment. Marker *Xgwm122* contributed 9.6% of the total phenotypic variation in the 2010 Julesburg environment. Marker *Xgwm522* contributed 58.1% of the phenotypic variation in the 2011 dry environment on chromosome 2A.

Total kernel number: Of the 10 QTL detected for kernel number in four environments, four QTL were validated in those four environments. Marker *Xgwm155* found on chromosome 3A or 1D was validated in the Dailey 2010 environment and had the greatest phenotypic contribution (25.4%). Marker *Xgwm155* was previously detected in the 2009 dry environment. Sokoll contributed the favorable allele for markers *Xgwm155* and *Xbarc108*, and Ankor contributed the favorable allele to markers *Xgwm153* and *Xbarc18*.

Total spike number: Of the 11 QTL found for spike number, two were validated in the 2010 Dailey environment on chromosomes 1B and 2D, and Ankor contributed the favorable allele for these markers. Markers *Xgwm539* and *Xgwm153* validated QTL from the 2008 wet and 2009 dry and wet environments and contributed 7.7 and 8.1% of the phenotypic variation.

DISCUSSION

Trait Analysis, 2010 & 2011

Means and correlations

In comparison to Burlington and Julesburg, Dailey had the highest spike: stem ratio at heading and at harvest for all environments (Julesburg was not recorded at heading). At Burlington, the wheat plants experienced less moisture stress due to favorable rainfall. Therefore, plants could develop more biomass, which would in turn give more opportunities for the plant to photosynthesize and grow. However, the Dailey trial received low rainfall throughout the crop cycle, and plants were more stressed than either Burlington or Julesburg. Because the Dailey trial was more stressed, it appears more energy, resources, and photosynthates were being invested into the development and preservation of the grain, rather than biomass. This may explain the higher spike: stem ratio at later stages of development in the Dailey 2010 rainfed environment.

In addition, in the Dailey and Julesburg 2010 trials and the 2011 wet trial, the spike: stem ratio at harvest was correlated with final grain yield (r=0.41, 0.34, and 0.53, respectively, $P \leq 0.05$). Grain yield was not correlated with any spike: stem ratio at any other growth stage. One potential explanation is the low sample size (n=10 tillers) used to determine spike: stem ratio. While plots were generally uniform, there was still evidence of within-plot variability among plants. This indicates spike: stem ratio is not the best predictor of final grain yield at the current sample size. However, biomass seemed to be a better indicator of final grain yield. Grain yield was correlated with the biomass sample weights at all growth stages from booting to harvest for the Dailey 2011 (r=0.39, 0.44, 0.61, and 0.33, respectively, $P \leq 0.05$) and Fort Collins 2011 dry

environments (r=0.71, 0.76, 0.79, and 0.49, respectively, $P \leq 0.05$). Grain yield was correlated with biomass weight at all growth stages except harvest for the Fort Collins 2011 wet environment (r=0.58, 0.73, and 0.61, respectively, $P \leq 0.01$). Biomass samples were taken from 1 m strips in four reps in the Fort Collins 2011 environments, whereas they were taken mostly from 0.5 m strips in 2010. Therefore, to find strong correlations between grain yield and other traits such as biomass, it might be beneficial to take larger samples at multiple growth stages and from multiple reps. Overall, biomass was a better indicator than spike: stem ratio of final grain yield.

Ankor vs. backcross lines

Determining entries that performed significantly ($P \leq 0.05$) better than Ankor is important in validating the advanced backcross strategy, as well as identifying lines which can be advanced to further generations or used for crossing in a breeding program. In the Ankor/Sokoll population, multiple lines performed significantly better than Ankor in multiple environments. For kernels per spike, kernel weight per spike, and average kernel weight, all of which are important yield components, at least one line performed significantly better than Ankor in three environments. Biomass, another component correlated with grain yield, was also found to have lines significantly greater than Ankor at all biomass growth stages in the 2011 dry and wet environments. At least two lines yielded more than Ankor in four of five environments from 2010-11, indicating genetic improvement of Ankor and the potential for future use of these lines in a breeding program.

High vs. low comparison

In 2010, the 22 highest and 22 lowest yielding lines from the five 2007-09 environments were evaluated to determine if there were significant differences in trait performance between these two categories. In addition, in 2011, the eight highest and eight lowest yielding lines were grown and evaluated for differences. There were significant ($P \leq 0.05$) differences between spike: stem ratios at harvest for Dailey and 2011 wet environments, and total spike number was significant ($P \leq 0.05$) for Burlington, Dailey, and 2011 dry environments. Also, grain yield was found to be significantly ($P \leq 0.05$) different between high and low groups of lines in all five environments from 2010-11. This validates the selection procedure used for the five environments (2007 to 2009) based on a yield rank summation procedure.

Top lines for breeding

Five lines were selected based on yield in the five environments from 2010 to 2011. These lines were highlighted as potential germplasm for a wheat breeder to use in a crossing block and as a way to incorporate genetic diversity from a synthetic-derived line into their germplasm. Lines 47, 331, 368, 415, and 420 had fairly high levels of heterozygosity (29, 30, 23, 11, and 25%, respectively), indicating the Sokoll alleles were incorporated into the Ankor background in these lines. Line 420 was the most consistent, high-yielding line; it received the most Sokoll alleles for average kernel weight of the top five lines, and it would be the top recommended line for a breeder to use in crossing.

Conclusion

This study confirmed the hypothesis that biomass was correlated to and possibly a good predictor of yield in the Dailey 2010 and Fort Collins 2011 dry and wet

environments. Biomass sampling with multiple replications was beneficial in detecting these correlations. This study rejected the hypothesis that the spike: stem ratio was correlated to or was a good predictor of yield. The spike: stem ratio was subject to within-plot variability when taking biomass samples and to the variability within the 10-stem samples. Spike: stem ratios at booting and heading were not correlated to and thus not good predictors of final grain yield. The most significant differences between the high- and low-yielding lines tested in 2010 and 2011 were detected for grain yield, total spike number, and spike: stem ratio at harvest. Five lines were identified that could be useful for a wheat breeder in the crossing block of a breeding program. This study also confirmed 28 QTL in nine of traits on chromosomes 1A, 1B, 2B, 2D, 3A, 3D, 4A, 6A, 6B, 7A, and 7B, and 10 of these 28 QTL were confirmed for grain yield.

CHAPTER 4

SUMMARY AND CONCLUSIONS

The genetic understanding and improvement of wheat will be increasingly important as the world population continues to grow. Wheat must be better able to combat abiotic factors such as drought each year to reduce the 25% annual production loss due to these stresses. In this study, we attempted to understand the genetic basis of drought tolerance, yield, and other agronomic traits through the introgression of synthetic-derived wheat alleles into a Colorado cultivar and subsequent AB-QTL analysis. The incorporation of favorable alleles at QTL for drought tolerance into a wheat breeding program could lead to marker-assisted breeding and screening of traits of interest at earlier generations, facilitate the development of more stress-tolerant cultivars, and cut production loss.

The specific objectives of this study were 1) to collect and analyze phenotypic data from an AB-QTL population grown in five Colorado environments differing in soil moisture level, 2) to gather DNA molecular marker data on the AB-QTL population and on an F_2 population derived from a cross of the same parents, 3) to locate and characterize QTL associated with drought tolerance and other agronomic traits using the collected phenotypic and genotypic data, 4) to determine if there is a correlation between spike:

stem ratio and biomass at various growth stages and final grain yield, and 5) based on a subset of the population evaluated in 10 environments to identify promising lines for use in breeding programs.

To determine QTL affecting yield and other agronomic traits, a population of 188 backcross lines derived from a cross between the Colorado cultivar 'Ankor' and the CIMMYT synthetic-derived line 'Sokoll' was grown in replicated field trials in five Colorado environments from 2007 to 2009. In addition, the 22 highest and 22 lowest performing lines from 2007-09 were grown in replicated field trials in three environments in 2010; the eight highest and eight lowest performing lines from 2010 were grown in replicated field trials in dry and wet environments in 2011. Field trials in 2008, 2009, and 2011 were grown under moderate moisture stress and under fully irrigated conditions in adjacent parts of the same field, for a total of two environments per location per year.

Traits evaluated from 2007 to 2009 included grain yield, test weight, days to heading, days to physiological maturity, grain fill duration, kernel number per spike, average kernel weight, plant height, spike length, and total kernel and spike number per plot. An additional 11 agronomic traits comprising plant phenology, morphological traits, yield and yield components were also evaluated in the 2010 and 2011 environments.

The F_2 and BC_2F_2-derived populations were genotyped using mostly microsatellite markers distributed across the wheat genome. Forty-one linkage groups were constructed from 141 microsatellite markers and three gene-specific markers (*Ppd-D1*, *Glu-A1*, and *Glu-D1*) in the F_2 mapping population. These linkage groups spanned 1414.4 cM (about 55% of the wheat genome) and covered parts of all 21 chromosomes. Quantitative trait loci were detected and characterized by single-factor analysis of

variance for 76 markers that were genotyped in the backcross population. Our analysis detected a total of 88 QTL, from three to 13 significant ($P \leq 0.01$) QTL for each trait. Individually, the QTL explained from 3.6 to 14.6% of the phenotypic variation. Sokoll contributed the favorable allele for 34 (38.6%) QTL, while Ankor contributed the favorable allele for 54 (61.4%) QTL.

The 88 QTL from the 2007-09 environments were tested on data collected in five environments in 2010 and 2011. This process validated 25 of 88 QTL for multiple traits. Traits such as grain yield, test weight, and total spike number showed significant ($P \leq 0.05$) differences between high and low lines from 2010 and 2011 field trials.

While our results reveal many similar genomic relationships to those QTL reported in previous AB-QTL literature, this study also identified 12 novel QTL among all AB-QTL studies reviewed for test weight, days to maturity, kernel weight, and spike length on chromosomes 2A, 3A, 4A, 5B, 6B, 7A, and 7B.

One QTL of importance was linked to marker *Xgwm499* on chromosome 5B. *Xgwm499* was a very consistent marker, proving stability across six different traits and four environments. Marker *Xgwm499* contributed to grain yield, test weight, average kernel weight, and kernels per spike, the latter two of which are yield components. However, the favorable allele in all cases was from Ankor, so a novel source of variation at that locus was not detected in this study. Interestingly, *Xgwm499* was not confirmed in any environment or trait in the five environments from 2010 to 2011. The persistence of this marker across multiple traits could be due either to linkage or pleiotropic effects, but pleiotropy is suggested because of the logical relationship among the traits. Other noteworthy markers were *Xbarc108*, *Xwmc532*, *Xbarc18*, and *Xgwm118*, all of which

were consistent across traits and locations and would be beneficial if incorporated into a breeding regime using marker-assisted selection. *Xbarc108*, in particular, contributed to yield, kernels per spike, and total kernel number, three important traits for a plant breeder, and Sokoll contributed the favorable allele at that locus. Sokoll also contributed the favorable allele for markers *Xwmc532* and *Xgwm118*.

Grain yield was correlated with many traits, the most consistent being total kernel number (r=0.55 to 0.97, $P\leq$0.05) and total spike number (r=0.35 to 0.74, $P\leq$0.05). Significant ($P\leq$0.05) correlations were detected in nine and seven of 10 environments, respectively. Grain yield was correlated with test weight (r=0.19 to 0.69, $P\leq$0.05) and with average kernel weight (r=0.24 to 0.36, $P\leq$0.05) each in five of 10 environments. In the five environments from 2010 to 2011, grain yield was significantly ($P\leq$0.05) correlated with biomass in three or more growth stages (r=0.33 to 0.79, $P\leq$0.05) in three of five environments and with the spike: stem ratio at harvest (r=0.34 to 0.53, $P\leq$0.05) in three of five environments.

Five of the top lines were also identified based on yield performance. These five lines, line 420 in particular, could all be used for making crosses to other parents in a breeding program. Alternatively, two or more of these lines could be crossed to each other to combine beneficial alleles from Sokoll in the same breeding line. These crosses combined with data from this study could then be used for marker-assisted selection to pyramid several desirable loci for traits of interest. This work has developed a further understanding of the wheat genome by locating and characterizing QTL associated with yield and yield component traits.

Though many QTL were detected for various traits, more work still needs to be done on the Ankor/Sokoll population to determine a complete set of QTL. More markers need to be genotyped in the F_2 generation to make a more complete framework map, followed by additional genotyping in the BC_2F_2-derived lines. After this is completed, simple interval and composite interval mapping can be conducted. Fine mapping of the QTL region or even specific genes of interest could proceed after that to enhance marker-assisted selection.

In conclusion, this study provided a better understanding of the genetic basis of yield formation and improved germplasm for the Great Plains regions using an advanced backcross strategy. The value of alleles from CIMMYT spring wheat germplasmwhen introgressed into an adapted winter wheat cultivar was demonstrated. This study also confirmed the hypothesis that biomass is a reliable predictor of yield, but rejected the hypothesis that the spike: stem ratio is a dependable predictor of yield.

APPENDICES

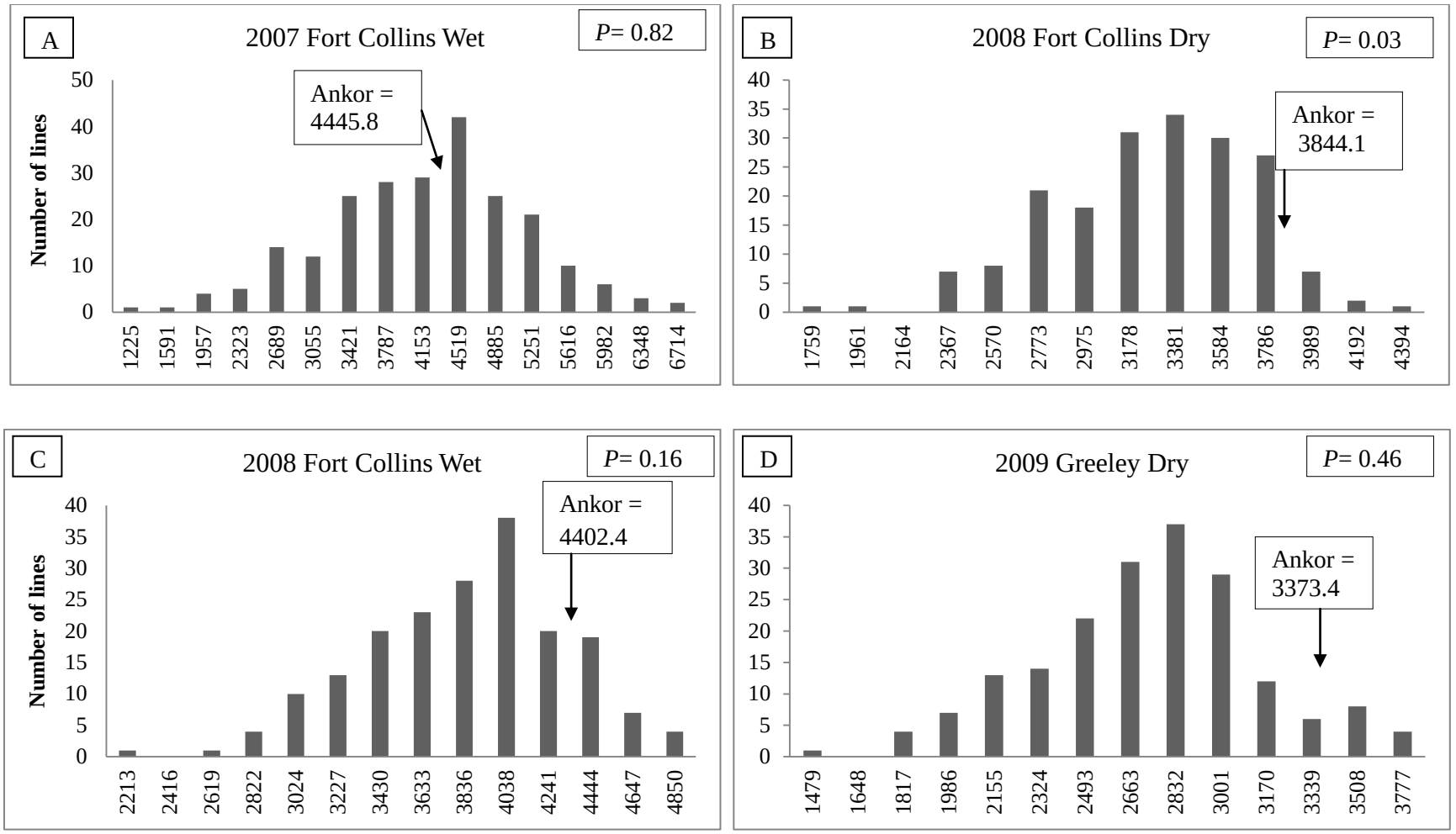


Fig. A-D. Frequency distributions for grain yield (kg/ha) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

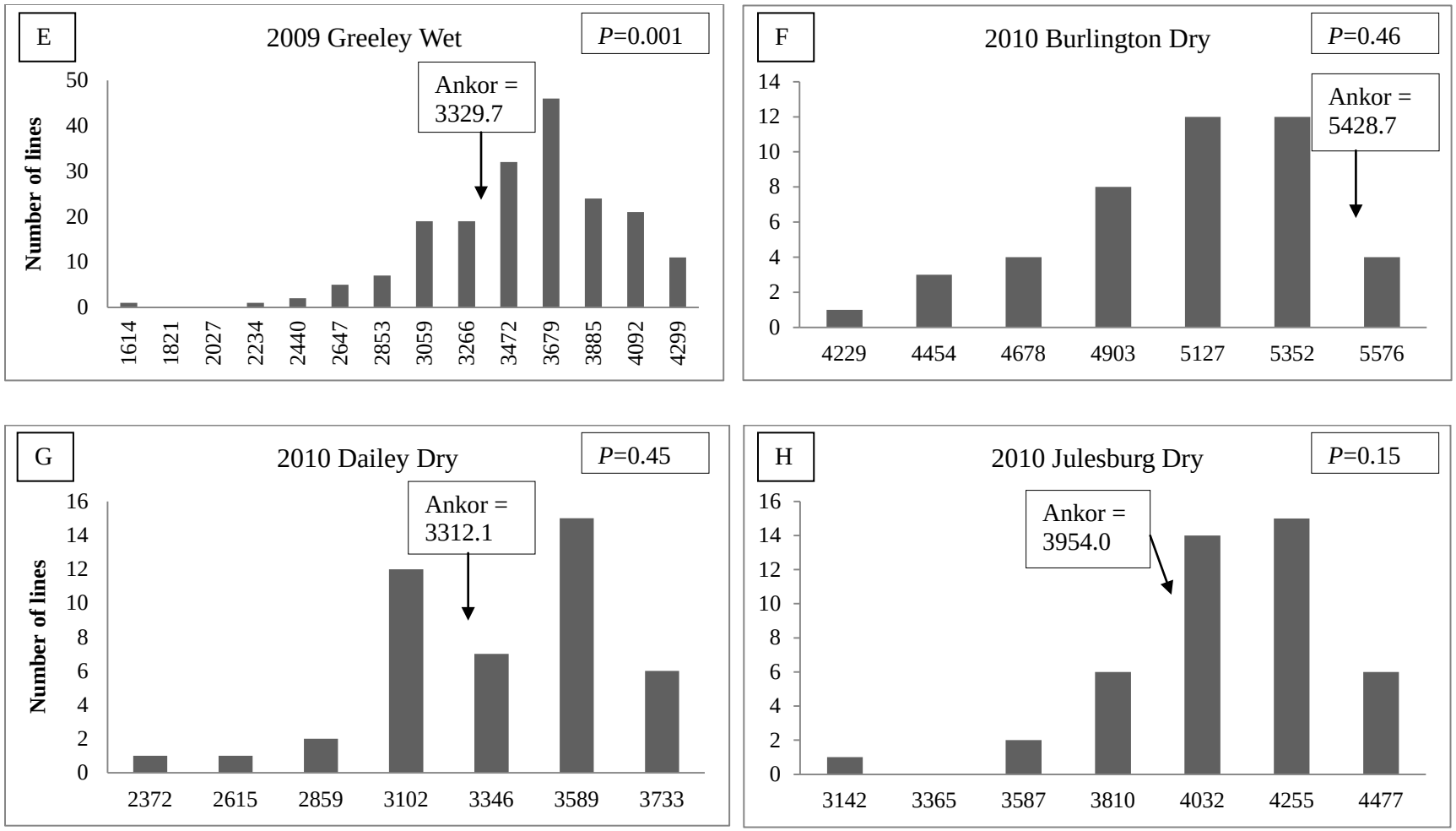
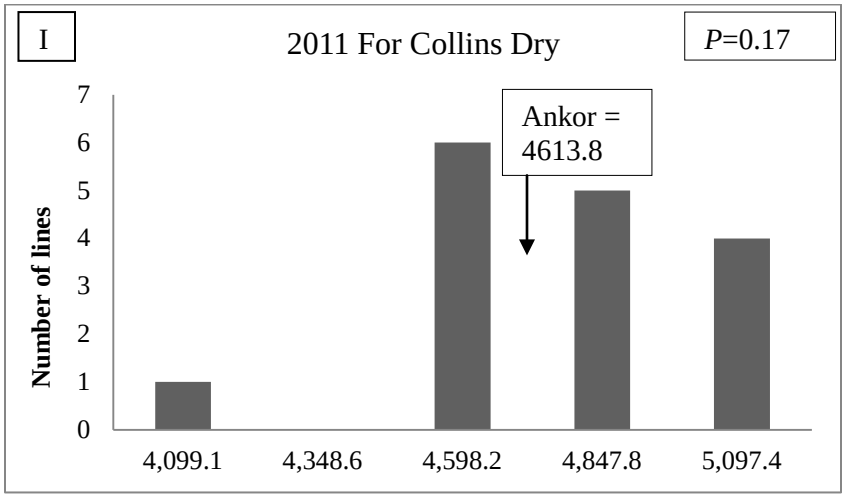


Fig. E-H. Frequency distributions for grain yield (kg/ha) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.



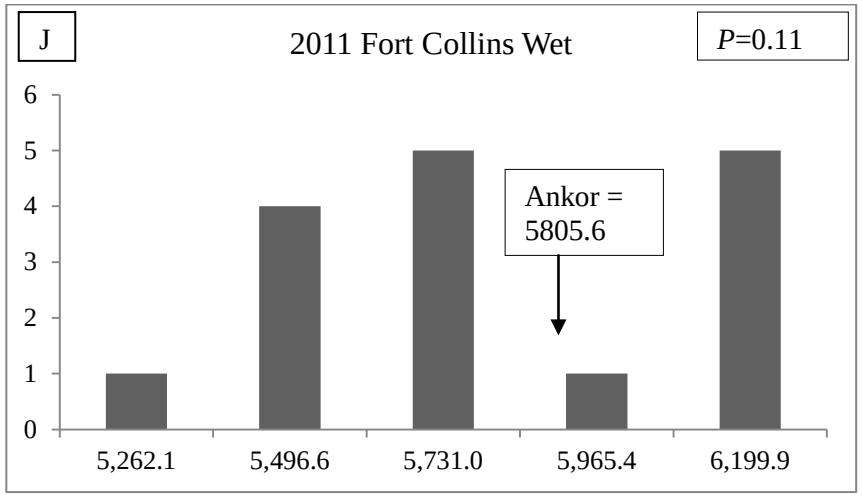


Fig. I-J. Frequency distributions for grain yield (kg/ha) in the Ankor/Sokoll BC_2F_2-derived population in two environments. P-value is from the Shapiro-Wilk test of normality.

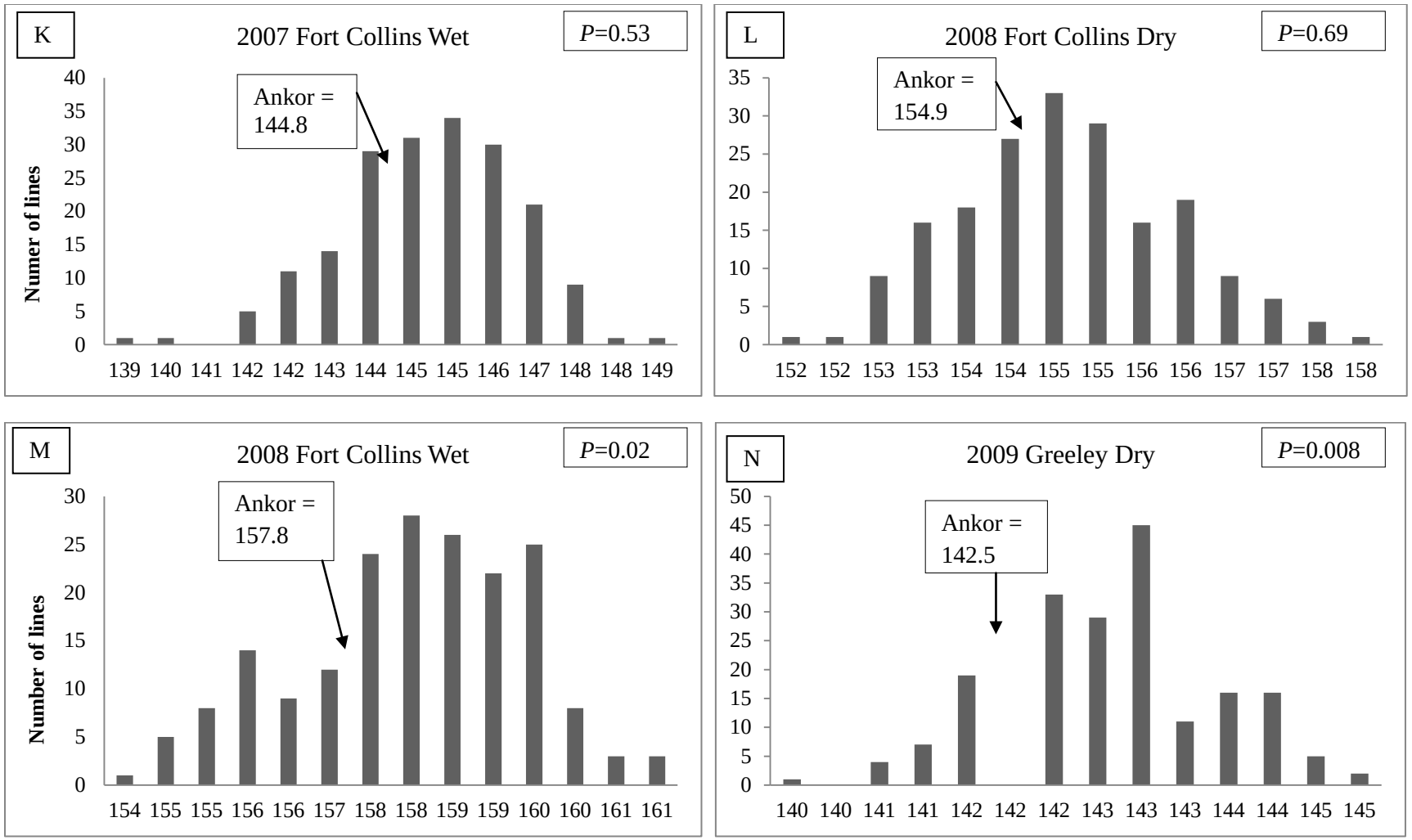


Fig. K-N. Frequency distributions for days to heading (Julian days) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

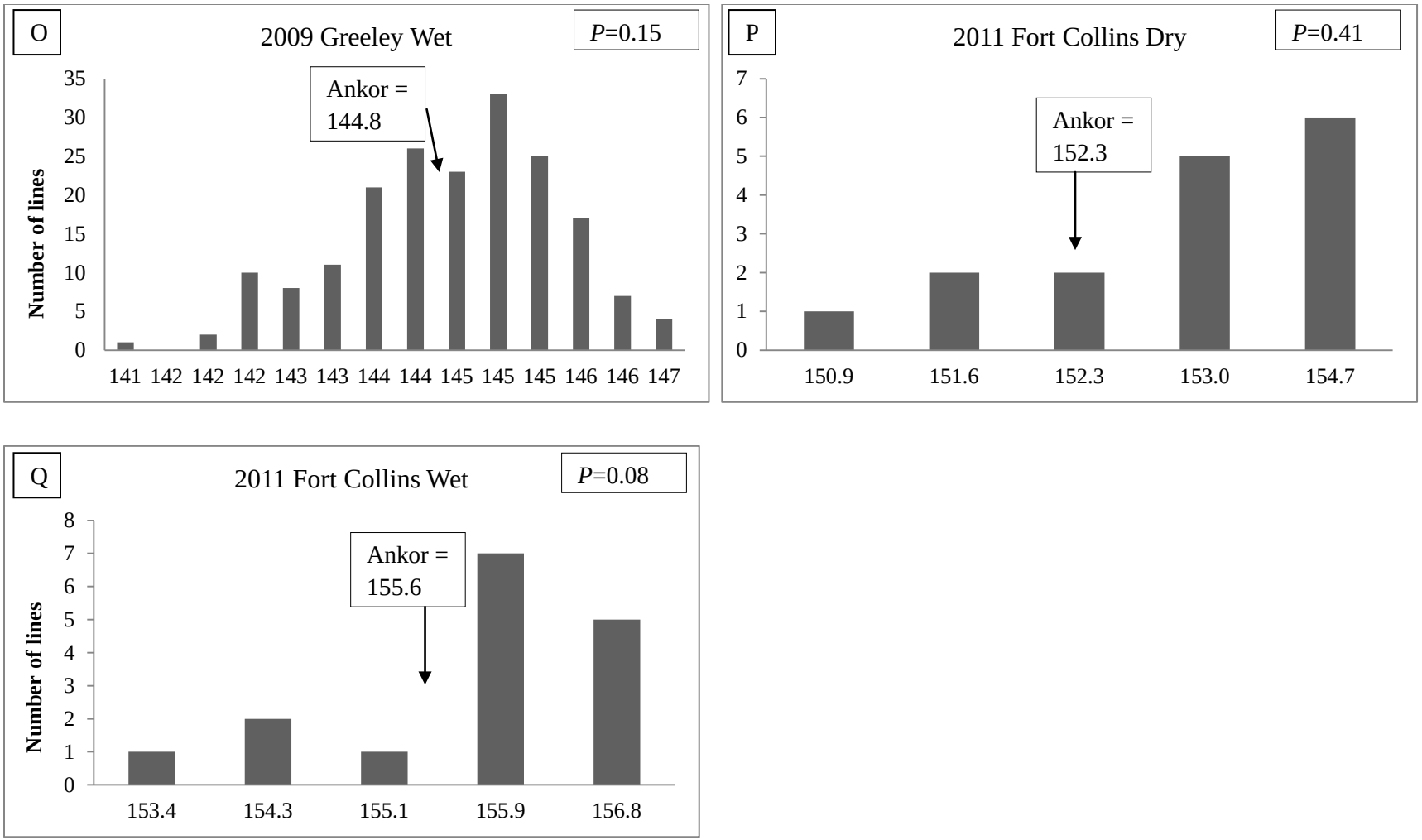


Fig. O-Q. Frequency distributions for days to heading (Julian days) in the Ankor/Sokoll BC_2F_2-derived population in three environments. P-value is from the Shapiro-Wilk test of normality.

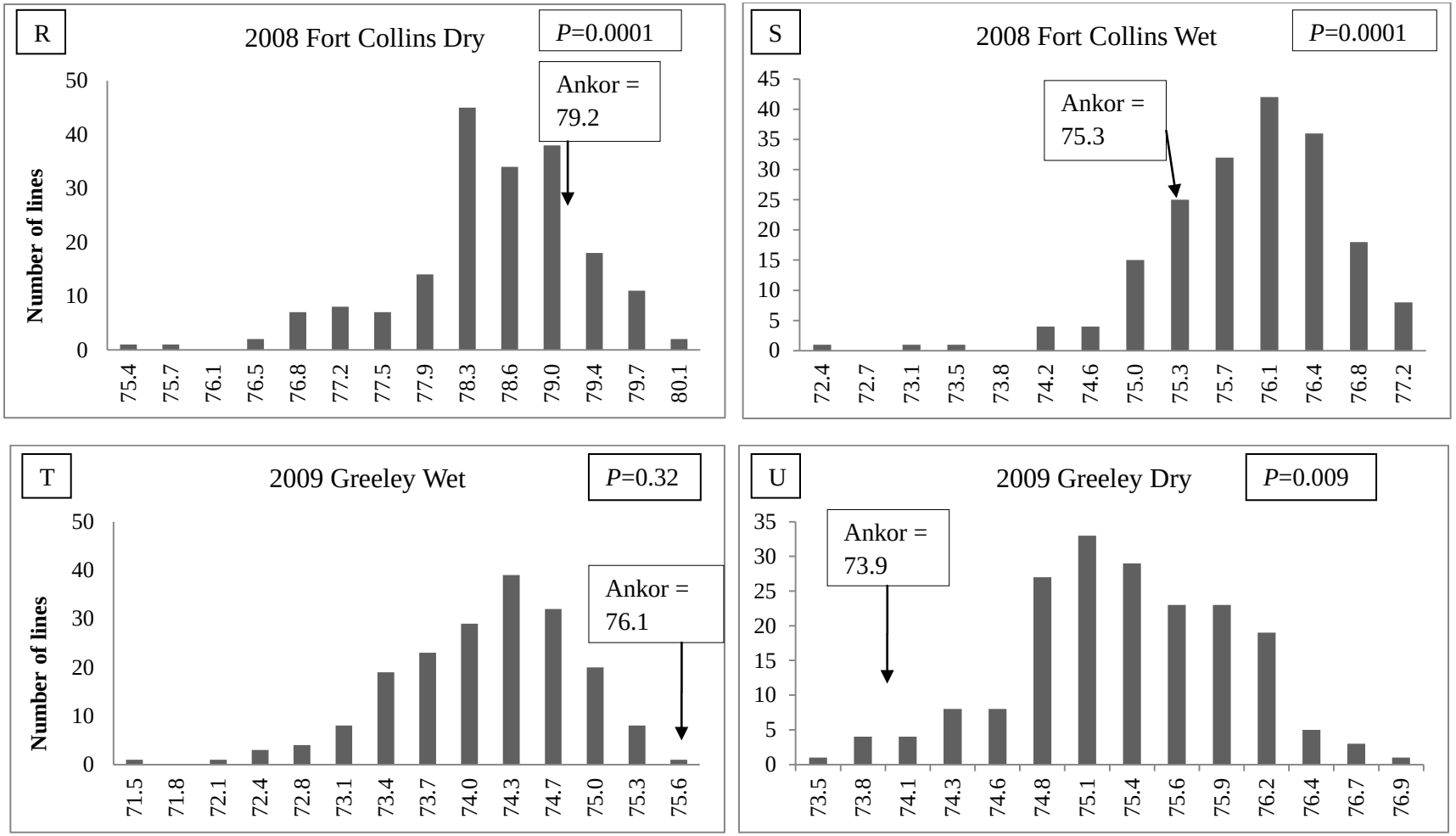


Fig. R-U. Frequency distributions for test weight (kg/hL) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

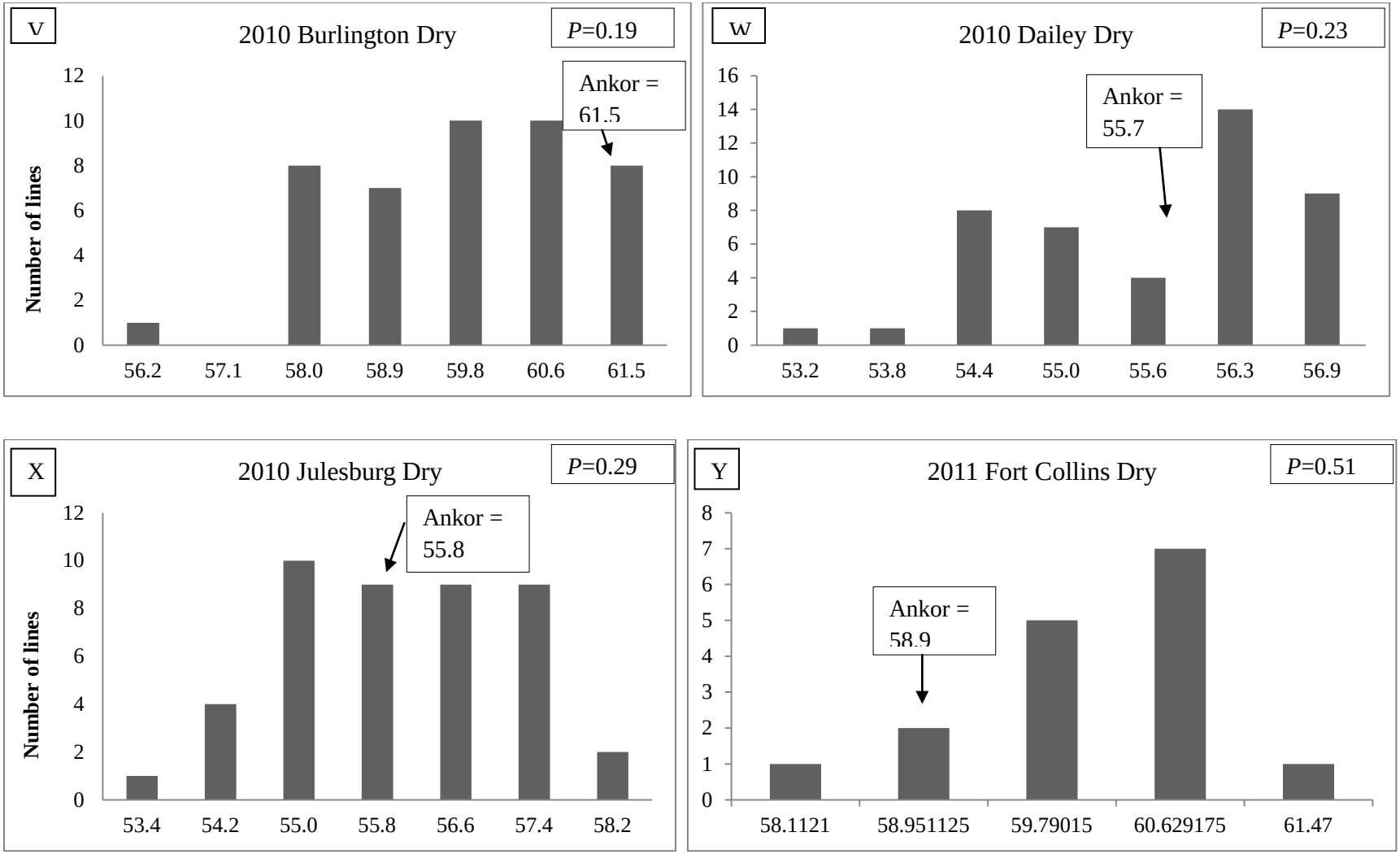


Fig. V-Y. Frequency distributions for test weight (kg/hL) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

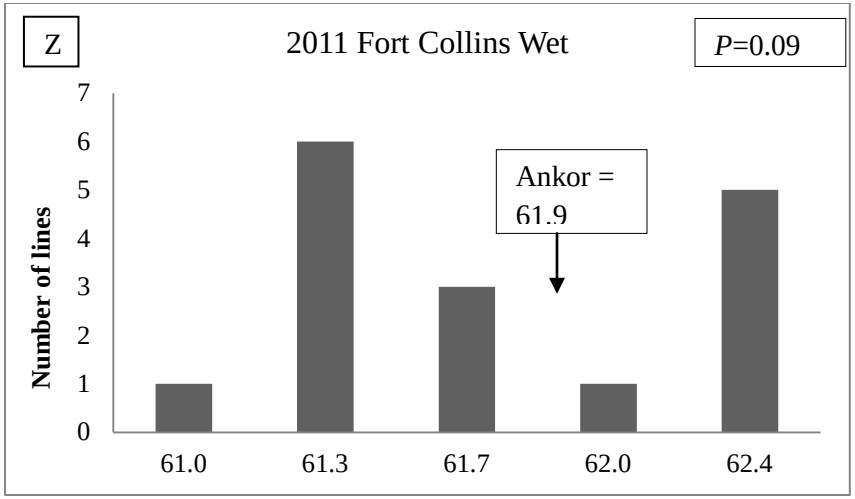


Fig. Z. Fig. Frequency distributions for test weight (kg/hL) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

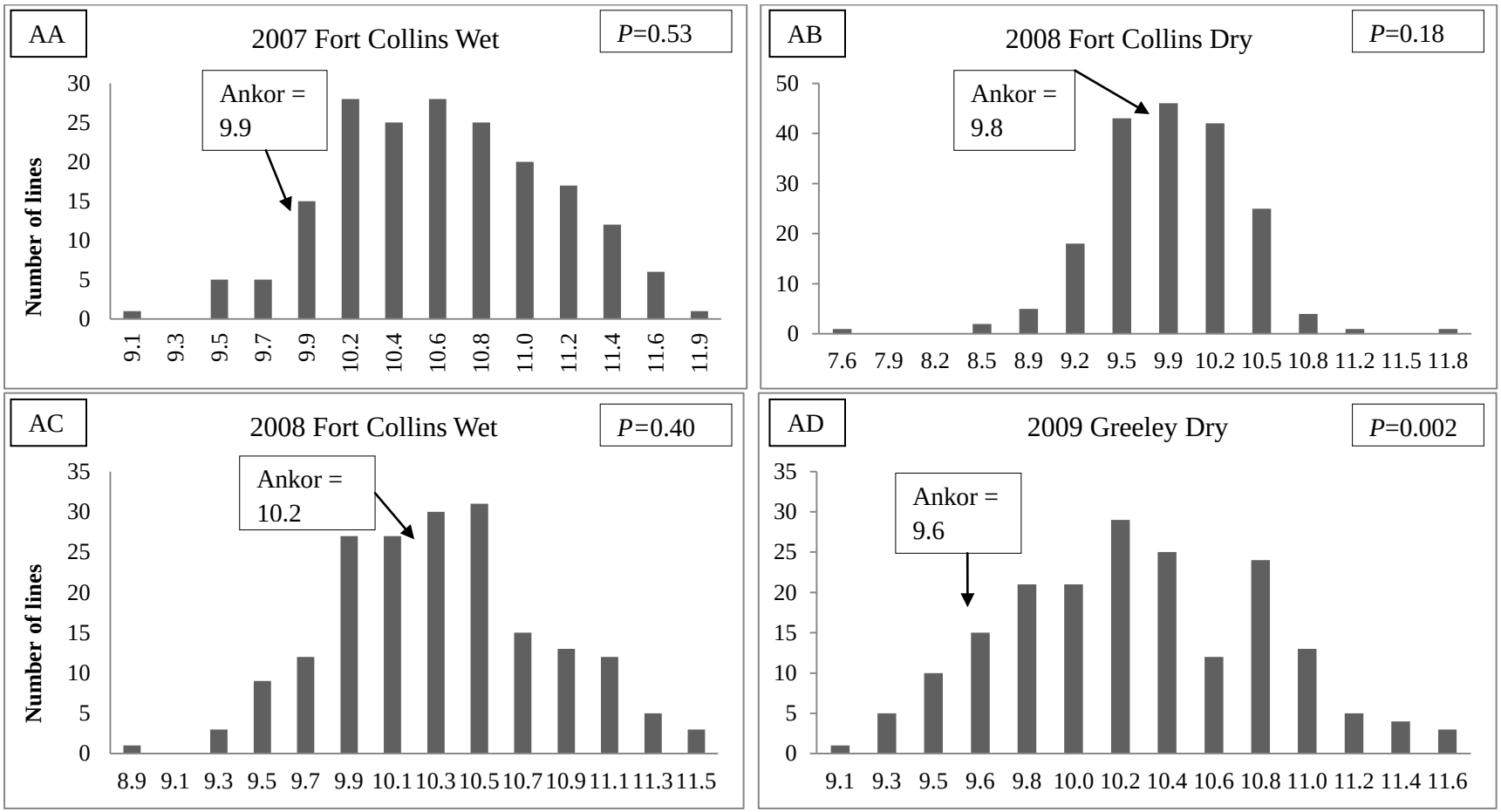


Fig. AA-AD. Frequency distributions for spike length (cm) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

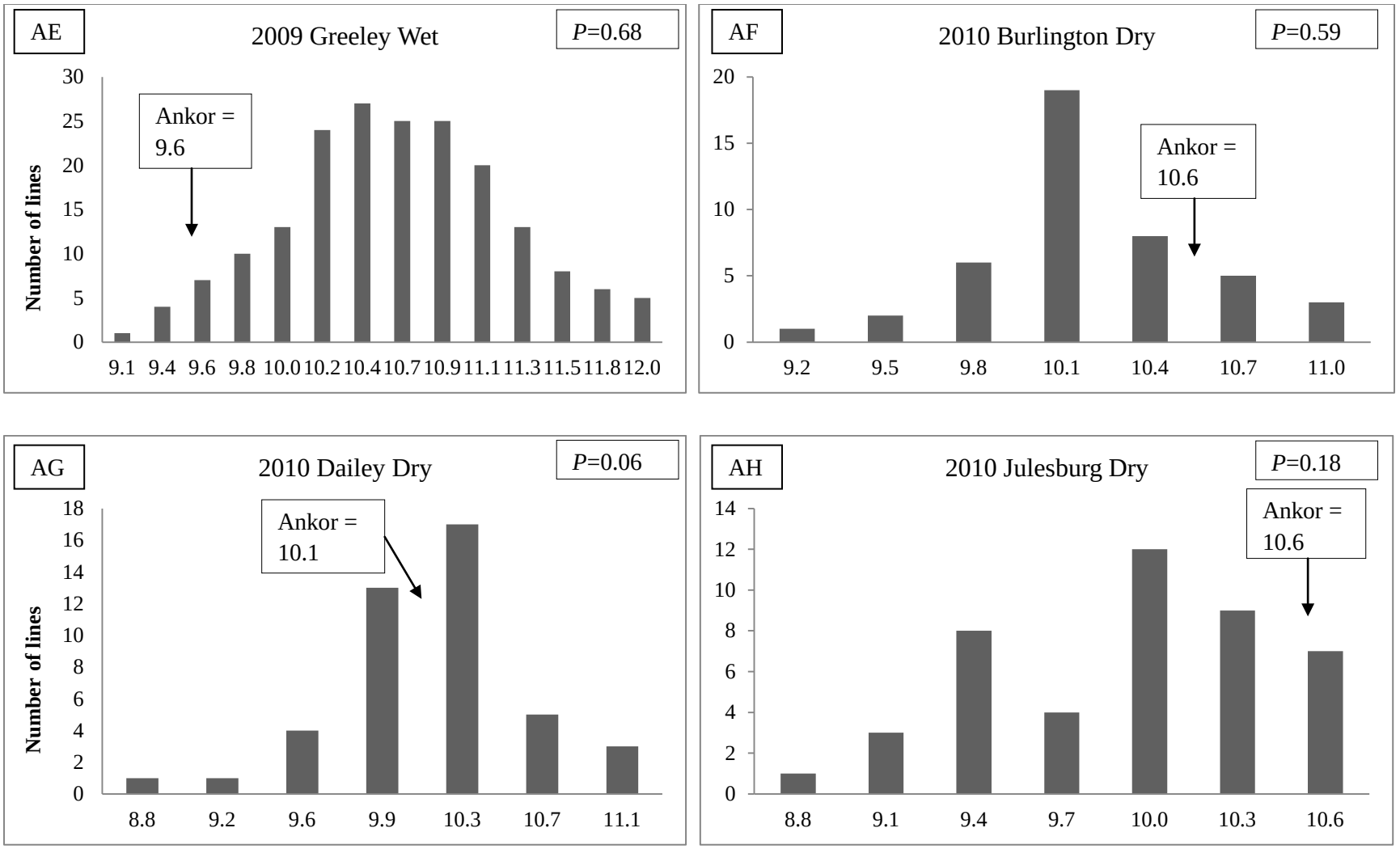


Fig. AE-AH. Frequency distributions for spike length (cm) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

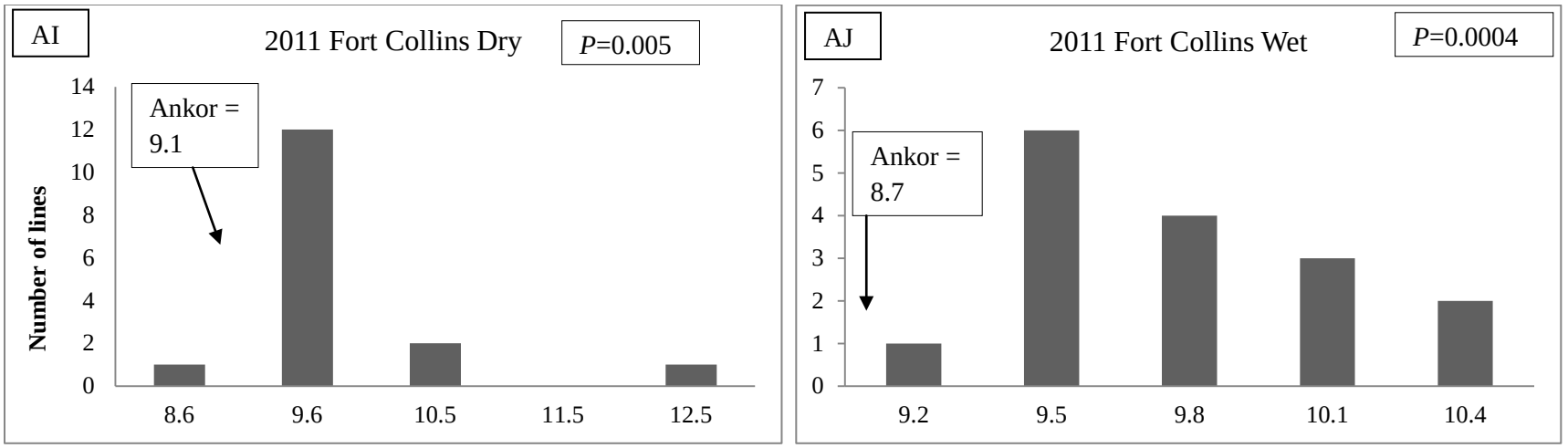


Fig. AI-AJ. Frequency distributions for spike length (cm) in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

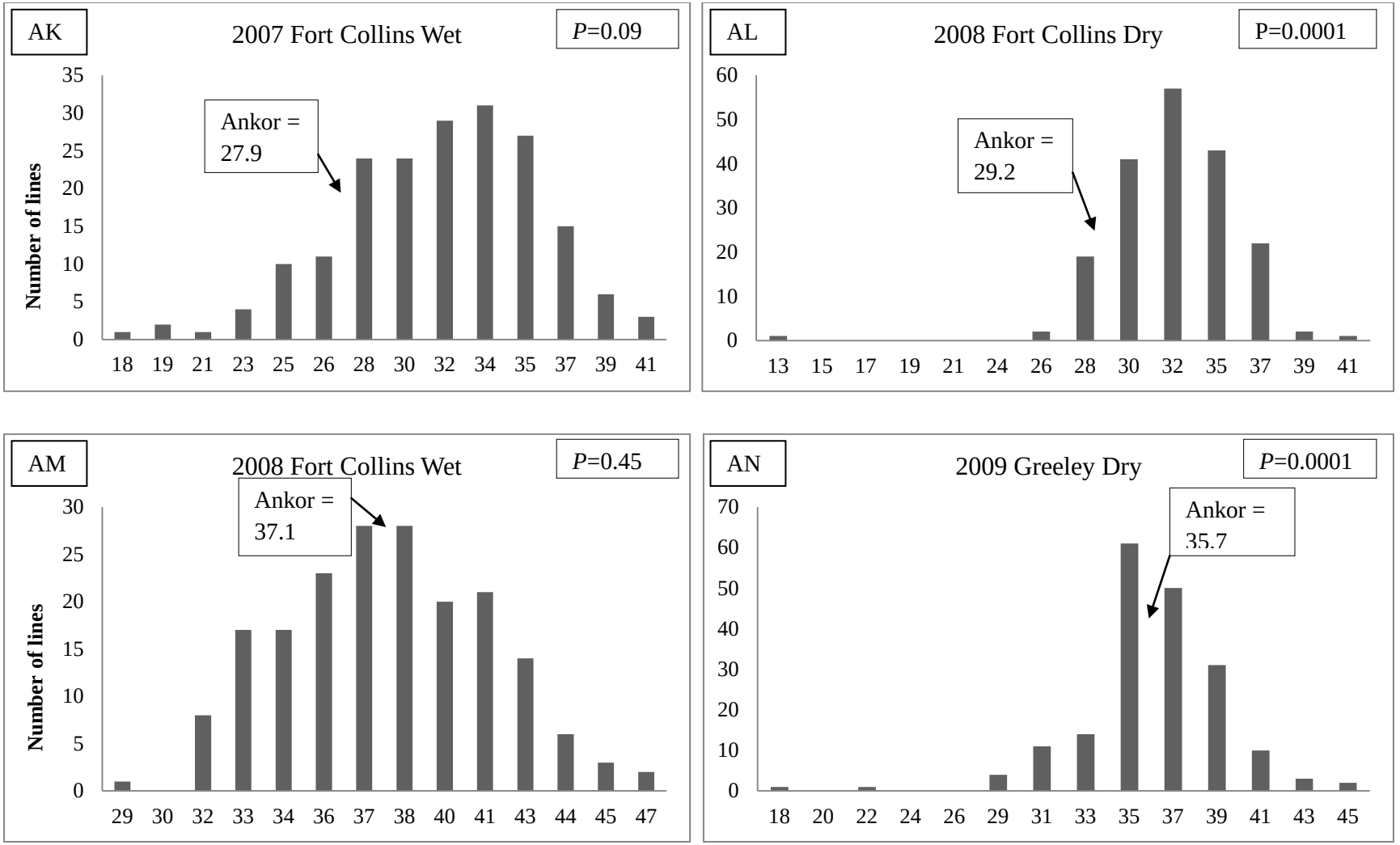


Fig. AK-AN. Frequency distributions for kernel number per spike in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

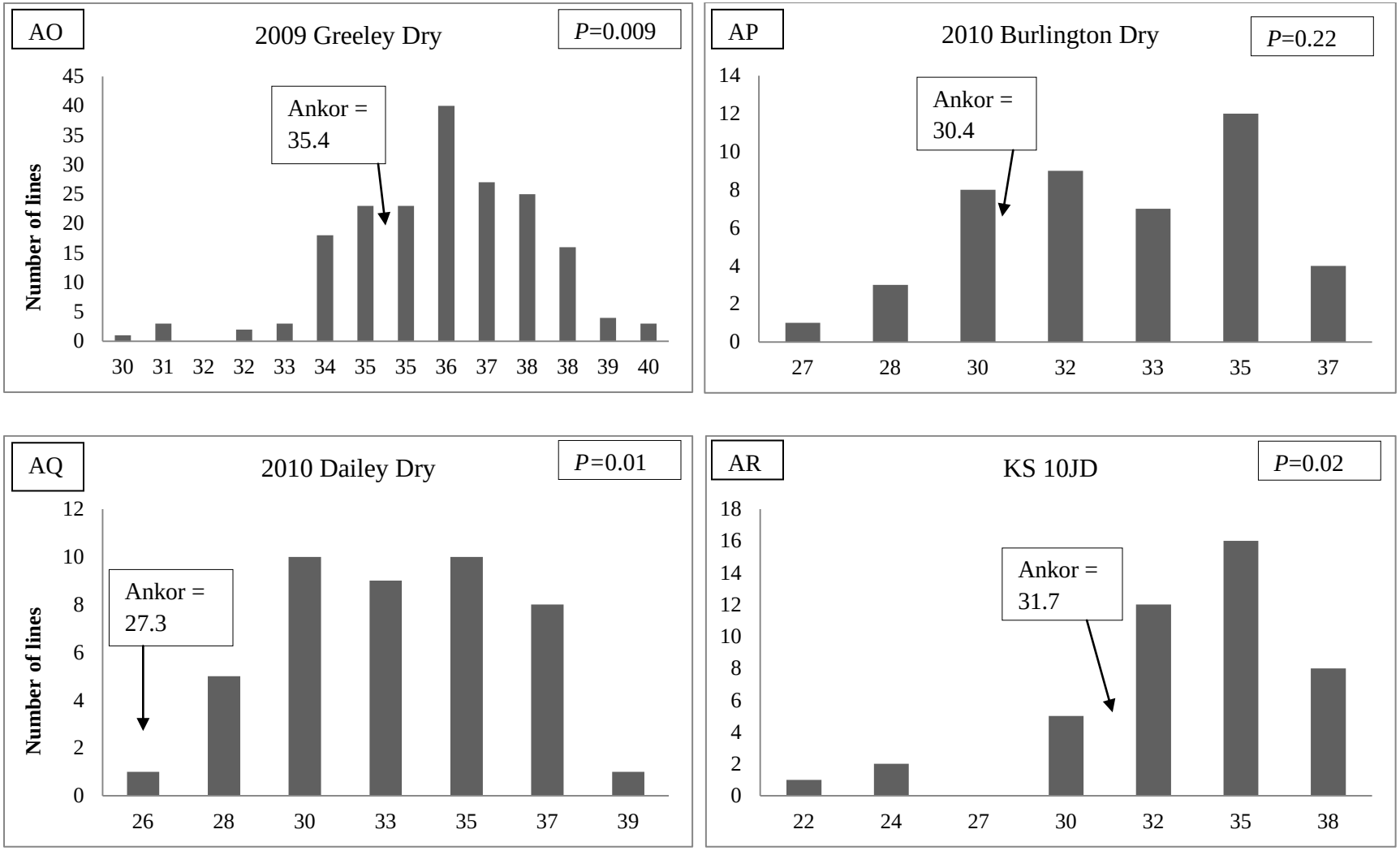


Fig. AO-AR. Frequency distributions for kernel number per spike in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

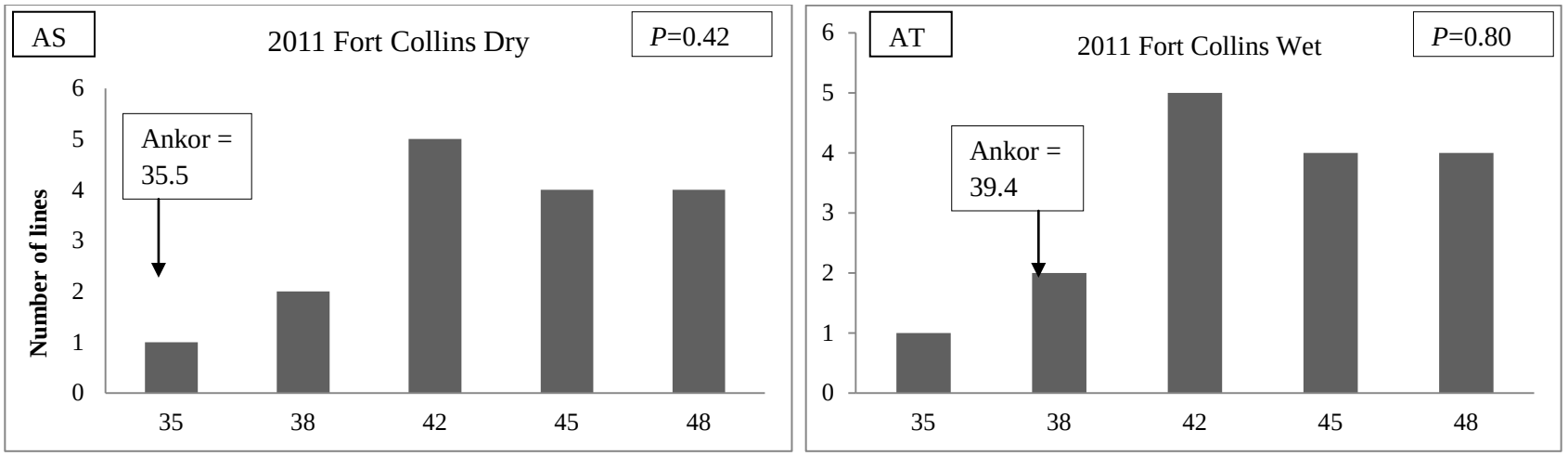


Fig. AS-AT. Frequency distributions for kernel number per spike in the Ankor/Sokoll BC_2F_2-derived population in four environments. P-value is from the Shapiro-Wilk test of normality.

Table A1. Mean check values for the Burlington 2010 environment.

Trait	Ankor	Hatcher	Ripper	RonL	Snowmass	TAM111
Grain Yield (kg/ha)	5428.7 ± 202.4	5745.8 ± 202.9	5457.6 ± 198.9	5149.2 ± 203.6	5560.5 ± 203.5	5358.5 ± 206.2
Test Weight (kg/hL)	61.5 ± 7.0	61.7 ± 1.1	59.6 ± 1.1	59.0 ± 1.1	61.7 ± 1.1	61.6 ± 1.1
Biomass Sample 1 (g)	58.1 ± 7.0	76.4 ± 7.0	56.6 ± 7.0	56.1 ± 7.0	71.7 ± 7.0	43.76 ± 7.0
Biomass Sample 2 (g)	174.2 ± 16.8	210.2 ± 17.0	193.7 ± 16.4	184.2 ± 17.0	183.94 ± 16.9	153.33 ± 17.0
Biomass Sample 3 (g)	208.2 ± 21.2	214.0 ± 21.3	226.6 ± 20.9	225.9 ± 21.2	226.1 ± 21.3	175.8 ± 21.3
Biomass Sample 4 (g)	240.5 ± 22.1	327.9 ± 22.1	241.7 ± 22.0	275.2 ± 22.1	293.7 ± 22.1	221.1 ± 22.1
KwtS4 (g)	76.0 ± 7.5	80.1 ± 7.6	89.8 ± 7.5	73.5 ± 7.6	95.6 ± 7.6	68.5 ± 7.6
Harvest Index	0.31 ± 0.03	0.34 ± 0.03	0.37 ± 0.03	0.27 ± 0.03	0.34 ± 0.03	0.31 ± 0.03
Spike Length (cm)	10.6 ± 0.3	8.9 ± 0.3	8.5 ± 0.3	7.8 ± 0.3	7.5 ± 0.3	8.1 ± 0.3
Spikelet Number	15.5 ± 0.5	14.4 ± 0.5	13.4 ± 0.5	14.0 ± 0.5	13.9 ± 0.5	13.4 ± 0.5
Kernel Number/Spike	30.4 ± 2.9	26.9 ± 2.9	28.6 ± 2.9	26.7 ± 2.9	31.2 ± 2.9	23.6 ± 2.9
Kernel Weight/ Spike (g)	0.74 ± 0.09	0.87 ± 0.09	0.94 ± 0.08	0.85 ± 0.09	1.05 ± 0.09	0.70 ± 0.09
Avg. Kernel Weight (mg)	25.2 ± 1.5	31.7 ± 1.5	31.4 ± 1.5	31.7 ± 1.5	33.4 ± 1.5	30.7 ± 1.5
B2Head:Stem (g)	0.08 ± 0.02	0.09 ± 0.02	0.07 ± 0.02	0.13 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
B3Head:Stem (g)	0.54 ± 0.06	0.53 ± 0.06	0.60 ± 0.05	0.48 ± 0.06	0.54 ± 0.06	0.57 ± 0.06
B4Head:Stem (g)	2.05 ± 0.14	2.06 ± 0.14	2.36 ± 0.14	2.10 ± 0.14	1.89 ± 0.14	1.98 ± 0.14
Total Kernel Number	3172.0 ± 177.9	2811.7 ± 176.9	2720.4 ± 171.4	2341.2 ± 171.6	2679.0 ± 176.2	2498.2 ± 174.6
Total Spike Number	105.4 ± 8.1	102.0 ± 7.8	89.5 ± 7.5	93.8 ± 7.8	83.5 ± 7.8	112.2 ± 7.9

Table A2. Mean check values for the Dailey 2010 environment.

Trait	Ankor	Hatcher	Ripper	RonL	Snowmass	TAM111
Grain Yield (kg/ha)	3312.1 ± 137.4	3843.1 ± 138.2	3226.2 ± 137.6	3971.7 ± 137.0	3733.1 ± 135.8	3525.5 ± 136.4
Test Weight (kg/hL)	55.7 ± 0.4	57.1 ± 0.4	52.8 ± 0.4	58.5 ± 0.4	59.3 ± 0.4	58.2 ± 0.4
Biomass Sample 1 (g)	254.5 ± 43.5	215.1 ± 42.9	314.2 ± 42.3	302.5 ± 42.1	345.1 ± 43.5	153.2 ± 42.2
Biomass Sample 2 (g)	36.5 ± 8.4	57.0 ± 8.5	60.1 ± 8.5	47.9 ± 8.5	35.7 ± 8.4	27.0 ± 8.5
Biomass Sample 3 (g)	108.7 ± 17.5	125.5 ± 17.5	141.5 ± 17.5	129.4 ± 17.4	115.7 ± 17.5	78.1 ± 17.4
Biomass Sample 4 (g)	157.2 ± 17.8	168.4 ± 18.1	148.1 ± 18.1	143.8 ± 18.0	183.7 ± 17.8	173.0 ± 18.1
KwtS4 (g)	52.7 ± 5.3	51.2 ± 5.4	40.7 ± 5.4	52.4 ± 5.3	57.0 ± 5.2	52.4 ± 5.3
Harvest Index	0.34 ± 0.03	0.29 ± 0.04	0.27 ± 0.04	0.39 ± 0.04	0.33 ± 0.03	0.45 ± 0.04
Spike Length (cm)	10.0 ± 0.3	7.9 ± 0.3	8.4 ± 0.3	7.7 ± 0.3	7.8 ± 0.3	8.8 ± 0.3
Spikelet Number	17.0 ± 0.4	14.9 ± 0.4	13.9 ± 0.4	14.4 ± 0.4	14.5 ± 0.4	16.0 ± 0.4
Kernel Number/Spike	27.3 ± 3.3	31.1 ± 3.3	26.5 ± 3.3	30.9 ± 3.3	36.6 ± 3.3	31.3 ± 3.3
Kernel Weight/ Spike (g)	0.74 ± 0.07	0.87 ± 0.07	0.69 ± 0.07	0.91 ± 0.07	1.01 ± 0.07	0.89 ± 0.07
Avg. Kernel Weight (mg)	26.8 ± 1.5	27.9 ± 1.5	26.9 ± 1.5	30.3 ± 1.5	27.8 ± 1.5	27.5 ± 1.5
B2Head:Stem (g)	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
B3Head:Stem (g)	0.55 ± 0.04	0.63 ± 0.04	0.77 ± 0.04	0.79 ± 0.04	0.65 ± 0.05	0.62 ± 0.04
B4Head:Stem (g)	2.95 ± 0.11	2.70 ± 0.11	2.28 ± 0.11	2.95 ± 0.11	2.57 ± 0.11	2.75 ± 0.11
Total Kernel Number	1920.0 ± 188.8	2040.5 ± 189.3	1854.8 ± 188.8	2044.3 ± 189.8	2007.9 ± 188.8	1923.7 ± 189.4
Total Spike Number	71.9 ± 6.6	64.3 ± 6.6	71.7 ± 6.6	65.4 ± 6.6	54.3 ± 6.6	60.5 ± 6.6

Table A3. Mean check values for the Julesburg 2010 environment.

Trait	Ankor	Hatcher	Ripper	RonL	Snowmass	TAM111
Grain Yield (kg/ha)	3954.0 ± 100.4	4822.7 ± 107.6	4232.9 ± 103.5	4941.4 ± 103.8	5092.2 ± 100.8	4572.7 ± 104.0
Test Weight (kg/hL)	55.8 ± 0.5	58.3 ± 0.5	54.9 ± 0.4	58.6 ± 0.4	59.5 ± 0.4	59.2 ± 0.5
Biomass Sample 4 (g)	175.1 ± 15.8	161.7 ± 16.4	136.6 ± 16.1	163.2 ± 16.0	197.4 ± 15.7	144.0 ± 16.1
KwtS4 (g)	56.7 ± 6.1	60.14 ± 6.3	44.0 ± 6.2	48.8 ± 6.22	73.4 ± 6.1	52.7 ± 6.2
Harvest Index	0.32 ± 0.02	0.37 ± 0.02	0.32 ± 0.02	0.31 ± 0.02	0.37 ± 0.02	0.36 ± 0.02
Spike Length (cm)	10.6 ± 0.4	9.5 ± 0.4	10.3 ± 0.4	9.6 ± 0.4	9.5 ± 0.4	9.7 ± 0.4
Spikelet Number	17.2 ± 0.6	14.9 ± 0.6	16.1 ± 0.6	15.7 ± 0.6	15.6 ± 0.6	16.1 ± 0.6
Kernel Number/Spike	31.7 ± 3.4	30.9 ± 3.4	32.8 ± 3.4	27.4 ± 3.4	37.4 ± 3.4	29.24 ± 3.4
Kernel Weight/ Spike (g)	0.86 ± 0.09	0.88 ± 0.09	0.73 ± 0.09	0.83 ± 0.09	1.04 ± 0.09	0.98 ± 0.09
Avg. Kernel Weight (mg)	27.1 ± 1.4	30.9 ± 1.4	24.0 ± 1.4	30.4 ± 1.4	27.7 ± 1.4	33.9 ± 1.4
B4Head:Stem (g)	1.88 ± 0.20	2.44 ± 0.21	2.58 ± 0.21	2.46 ± 0.21	2.14 ± 0.20	2.29 ± 0.21
Total Kernel Number	2098.0 ± 155.2	2320.5 ± 156.6	2609.0 ± 157.1	2449.4 ± 156.3	2709.9 ± 155.3	1986.1 ± 156.5
Total Spike Number	66.4 ± 9.8	79.8 ± 9.9	80.7 ± 9.9	91.3 ± 9.9	73.9 ± 9.8	69.1 ± 9.9

Table A4. Mean check values for the Fort Collins 2011 dry environment.

	Ankor	**Ripper**	**RonL**	**TAM111**
Trait	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Grain Yield (kg/ha)	4613.8 ± 200.4	5584.6 ± 200.1	4754.9 ± 201.5	4956.5 ± 201.4
Test Weight (kg/hL)	58.9 ± 1.0	60.4 ± 1.0	60.2 ± 1.0	62.4 ± 1.0
Days to Heading (Julian days)	152.3 ± 0.4	151.7 ± 0.4	152.5 ± 0.4	152.4 ± 0.4
Days to Maturity (Julian days)	191.8 ± 0.50	192.5 ± 0.50	192.6 ± 0.5	191.9 ± 0.5
Grain Fill Duration (Julian days)	39.4 ± 0.6	40.9 ± 0.6	40.1 ± 0.6	39.6 ± 0.6
Biomass Sample 1 (g)	43.9 ± 7.6	76.9 ± 7.8	61.8 ± 7.8	64.0 ± 7.8
Biomass Sample 2 (g)	75.9 ± 18.8	147.3 ± 18.8	87. 1 ± 18.8	112.6 ± 18.8
Biomass Sample 3 (g)	93.1 ± 14.7	173.7 ± 14.7	106.3 ± 14.7	117.2 ± 14.7
Biomass Sample 4 (g)	216.6 ± 23.4	275.3 ± 23.4	226.6 ± 23.4	213.5 ± 23.4
KwtS4 (g)	82.1 ± 9.5	104.4 ± 9.5	81.4 ± 9.5	76.8 ± 9.5
Harvest Index	0.43 ± 0.02	0.43 ± 0.02	0.41 ± 0.02	0.41 ± 0.02
Spike Length (cm)	9.1 ± 0.8	8.7 ± 0.8	6.7 ± 0.7	6.7 ± 0.6
Spikelet Number	15.8 ± 0.6	14.2 ± 0.6	12.8 ± 0.5	13.2 ± 0.5
Kernel Number/Spike	35.5 ± 2.5	36.6 ± 2.5	34.0 ± 2.5	34.2 ± 2.5
Kernel Weight/ Spike (g)	1.15 ± 0.09	1.41 ± 0.09	1.19 ± 0.09	1.18 ± 0.09
Average Kernel Weight (mg)	32.9 ± 1.2	38.7 ± 1.2	35.1 ± 1.2	35.1 ± 1.2
B2Head:Stem (g)	0.79 ± 0.33	0.60 ± 0.33	0.78 ± 0.33	0.31 ± 0.33
B3Head:Stem (g)	1.34 ± 0.10	0.89 ± 0.11	1.14 ± 0.10	1.1 ± 0.10
B4Head:Stem (g)	2.31 ± 0.17	2.41 ± 0.17	0.53 ± 0.17	2.04 ± 0.17
Plant Height (cm)	80.3 ± 1.0	73.8 ± 1.0	71.5 ± 1.0	82.0 ± 1.0
Total Kernel Number	2549.0 ± 278.9	2633.8 ± 277.8	2303.3 ± 278.5	2127.5 ± 278.5
Total Spike Number	65.6 ± 7.5	72.5 ± 7.5	68.8 ± 7.5	62.9 ± 7.5

Table A5. Mean check values for the Fort Collins 2011 wet environment.

	Ankor	**Ripper**	**RonL**	**TAM111**
Trait	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**	**Mean ± SE**
Grain Yield (kg/ha)	5805.6 ± 131.9	6690.1 ± 131.4	6112.4 ± 132.7	6091.4 ± 134.8
Test Weight (kg/hL)	61.9 ± 0.3	62.1 ± 0.3	63.4 ± 0.3	62.8 ± 0.3
Days to Heading (Julian days)	155.6 ± 0.39	153.6 ± 0.4	154.8 ± 0.4	155.2 ± 0.4
Days to Maturity (Julian days)	193.9 ± 0.4	193.0 ± 0.4	194.3 ± 0.4	193.6 ± 0.4
Grain Fill Duration (Julian days)	38.2 ± 0.5	39.5 ± 0.5	39.3 ± 0.5	38.2 ± 0.5
Biomass Sample 1 (g)	59.7 ± 8.1	94.6 ± 8.0	63.0 ± 8.1	79.0 ± 8.2
Biomass Sample 2 (g)	112.7 ± 16.2	176.7 ± 16.2	99.9 ± 16.2	140.7 ± 16.2
Biomass Sample 3 (g)	151.7 ± 17.7	179.3 ± 17.6	153.1 ± 17.8	142.5 ± 18.0
Biomass Sample 4 (g)	218.3 ± 30.8	258.9 ± 30.6	265.8 ± 31.1	254.2 ± 31.7
KwtS4 (g)	83.2 ± 11.2	101.6 ± 11.2	106.9 ± 11.3	96.5 ± 11.5
Harvest Index	0.46 ± 0.02	0.45 ± 0.02	0.46 ± 0.02	0.46 ± 0.02
Spike Length (cm)	8.7 ± 0.2	8.5 ± 0.2	6.8 ± 0.2	7.0 ± 0.2
Spikelet Number	15.2 ± 1.3	15.2 ± 0.3	18.1 ± 1.3	16.1 ± 0.3
Kernel Number/Spike	39.4 ± 2.3	38.1 ± 2.3	36.4 ± 2.3	46.5 ± 2.3
Kernel Weight/ Spike (g)	0.46 ± 0.02	0.45 ± 0.02	0.46 ± 0.02	0.46 ± 0.02
Average Kernel Weight (mg)	35.0 ± 0.9	10.4 ± 0.9	38.6 ± 0.9	36.8 ± 0.9
B2Head:Stem (g)	0.31 ± 0.19	0.56 ± 0.19	0.41 ± 0.22	0.30 ± 0.19
B3Head:Stem (g)	0.83 ± 0.04	1.02 ± 0.04	0.96 ± 0.04	1.21 ± 0.05
B4Head:Stem (g)	2.61 ± 0.27	2.29 ± 0.27	2.75 ± 0.27	2.32 ± 0.27
Plant Height (cm)	88.4 ± 1.6	83.2 ± 1.6	78.1 ± 1.6	88.4 ± 1.6
Total Kernel Number	2338.2 ± 323.0	2507.5 ± 321.9	2761.3 ± 324.9	2620.6 ± 328.6
Total Spike Number	57.8 ± 7.3	66.5 ± 7.3	74.9 ± 7.3	58.5 ± 7.4

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