THESIS

QUANTITATIVE TRAIT LOCUS MAPPING OF YIELD AND YIELD COMPONENTS IN CANOLA (*BRASSICA NAPUS* L.) UNDER IRRIGATED AND RAINFED TREATMENTS

Submitted by

Annie Heiliger

Department of Soil and Crop Sciences

In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2012

Master's Committee:

Advisor: Patrick Byrne

John McKay
Jerry Johnson

ABSTRACT

QUANTITATIVE TRAIT LOCUS MAPPING OF YIELD AND YIELD COMPONENTS

IN CANOLA (*BRASSICA NAPUS* L.)

UNDER IRRIGATED AND RAINFED TREATMENTS

Rapeseed (*Brassica napus* L.) is an oilseed crop that has a variety of uses, including applications in industry as well as for food, feed, and fuel. Improved *B. napus* cultivars with decreased levels of two disease-causing compounds are known commonly as canola or double-low cultivars, and are approved for human and animal consumption in the U.S., Canada, and Europe. Canola is currently grown in the northern U.S. and in several areas of Canada and Europe, but has potential to be grown in other areas of the U.S., including Colorado, either for biodiesel or to be sold in a canola commodity food oil market at the discretion of the seed producer. Additionally, the cake meal left after oil extraction has a high protein concentration and can be added as a supplement to animal feed.

In recent years, water availability for crop production in the western U.S. has declined due to competition with non-agricultural water uses, and the increasing demands for water will likely increase with global climate change. Therefore, in order to be sustainable, crops grown in Colorado must be high-yielding with limited or no irrigation inputs, and consequently canola cultivars adapted to the semi-arid climate of Colorado and the U.S. High Plains will need to be drought tolerant.

To provide information relevant to improving adaptation of canola to Colorado conditions, a study was conducted with the following objectives:

1) to evaluate yield, yield components, and days to flowering (DTF) in two doubled haploid (DH) canola mapping populations under rainfed and irrigated conditions;

2) to determine relationships among yield and yield components by analyzing trait correlations and to study trait inheritance patterns;

3) to determine areas of the *B. napus* genome that are implicated in yield and yield component traits under both rainfed and irrigated conditions by quantitative trait locus (QTL) analysis; and

4) to study the sensitivity of yield and yield component traits to drought stress by performing analysis of variance and by performing a QTL analysis on the difference in trait values from the rainfed and irrigated treatments.

Two DH canola mapping populations were grown in side-by-side irrigated and rainfed treatments near Fort Collins, Colorado: population SE1 in 2010 (n=183) and population DHYB (n=150) in 2011. DTF, seed yield, and yield-related traits were measured in order to understand relationships among these traits under different water regimes, to study trait heritabilities, and to better understand genotype, treatment, and treatment by genotype interaction effects. QTL mapping was conducted separately for each treatment in each population using R-QTL software to detect additive and epistatic effects. Yield components that were studied included siliques per main inflorescence (SMI), seeds per silique (SS), and thousand seed weight (TSW). Seed coat color was also classified for the DHYB population.

Analysis of variance revealed an influence of genotype (P<0.0001) on all traits in both populations, treatment effects on seed yield, SMI, and SS (P<0.05) in the SE1 population, and treatment effects on seed yield, SMI, TSW, and DTF in the DHYB population. Genotype by treatment interactions were significant (P<0.01) for all traits in the SE1 population and for seed yield and TSW (P<0.05) in the DHYB population.

In the 2010 study, three DTF QTL were detected that colocated with most of the other QTL, demonstrating the strong influence of flowering time on seed yield and yield components in this

population. These QTL explained 73 and 65 percent of phenotypic variance for DTF in the wet and dry treatments, respectively, in a multiple QTL model. Several novel QTL were detected in the 2011 study, including a locus on LG 17 that explained 7.54% of trait variation for seed yield in the dry treatment and a locus on LG 16 that explained 11.41% of seed yield in the wet treatment. A novel QTL for SS was detected on LG 7 in both treatments. Two QTL reported for DTF in the wet treatment on LGs 14 and 18 are novel, as are two QTL for SMI in the dry treatment.

Of the yield components studied, SS consistently had the lowest amount of genotype by treatment interaction and direct treatment effects in both populations, as well as high heritability estimates in both populations. SS also correlated positively and significantly ($P<0.05$) with seed yield in both years of the study. Additionally, the QTL detected for SS in the 2011 study were the same for the wet and dry treatments, indicating QTL stability. It is our conclusion that SS is a good candidate for direct selection in a breeding program, and that he QTL reported for SS could also be useful for marker-assisted selection for improved yields in Colorado.

TABLE OF CONTENTS

CHAPTER ONE: INTRODUCTION

Renewable energy in the U.S.

There are numerous reasons for considering alternative energy sources in the U.S.: reliance on non-renewable and finite sources of fossil fuels has reached unprecedented levels (U.S. Energy Information Administration, 2011); the world's population is predicted to rise which will increase the global demand for energy (United Nations, 2010); the U.S. imports large amounts of oil from some of the world's most politically unstable regions, which has put the security of the U.S. at risk (Naylor, 2007); and greenhouse gas emissions causing global climate changes are largely attributed to burning fossil fuels for energy (IPCC, 2001). Each of these reasons alone could be considered solid support for development of alternative and renewable energy sources, but together they demonstrate a clear need to change the way energy is produced and used in the U.S.

The U.S. government, attuned to the fact that policy will be instrumental in making changes to energy production in America, established the Renewable Fuel Standard (RFS) (EPA, 2002) and later the RFS2 under the Energy Policy Act of 2005 (EPA, 2005). These regulations dictated the amount of renewable transportation fuel to be used in the U.S. by certain dates. The first RFS goal of nine billion gallons was met and exceeded in 2009, so an update to the RFS2 followed in late 2009 which included new requirements. Specifically, an increase in renewable domestic transportation fuels to 36 billion gallons by 2022 is targeted. This governmental approach to mandate incremental renewable and sustainable energy production goals acknowledges that infrastructure and feedstock changes will need to be made, each of which will take time and financial investment.

Although a number of recent studies have recommended specific feedstocks for biofuel use in the US (e.g. Heaton et al., 2008), it is generally agreed that a single feedstock will not answer

the nation's need for renewable transportation fuel (e.g. Somerville et al., 2010). The need for sustainable bioenergy sources will constrain certain feedstocks due to regional differences in natural resources and climate. The United States Department of Agriculture (USDA) formalized this concept in their June 2010 publication entitled "A USDA Regional Roadmap to Meeting the Biofuels Goals of the Renewable Fuels Standard by 2022" (USDA, 2010). This report includes a biofuels framework with specific domestic goals for each U.S. region that take advantage of the department's agricultural and climatic knowledge. For example, in parts of the Western U.S. such as Colorado, the USDA recognizes that limited rainfall will hinder any effort to grow perennial grasses and many other dedicated bioenergy crops sustainably, due to the fact that most of these feedstocks require significant amounts of rainfall or irrigation inputs. Therefore, this region is expected to contribute only 0.3% of the total volume goal of 36 billion gallons from advanced biofuel production, mainly through conversion of woody biomass and growth of oilseed crops for biodiesel (USDA, 2010).

According to the USDA (2010) and recent research articles (e.g. Fore et al., 2010; Fore et al, 2011), oilseed production could provide energy independence to producers if used directly as straight vegetable oil (SVO) or if processed into biodiesel. Fuel price volatility is a major concern for growers, leading to a fluctuation in profits and losses from year to year. Implementation of a small-scale on-farm biodiesel operation using oilseeds could produce energetically favorable returns to growers (Fore et al., 2011), thereby decreasing the influence of unpredictable fuel prices on farmers' bottom lines. Additionally, oilseed producers could decide instead to sell their harvest in the canola commodity market for food use if the conditions are favorable to do so. Growth of oilseeds in an on-farm bioenergy capacity allows farmers much needed control over erratic petroleum fuel prices while providing an additional option for income.

The use of crops as fuel sources in the U.S. has led to skepticism and the "food versus fuel" debate, which has some people asserting that by diverting valuable croplands to bioenergy we will shrink our food supply (e.g. Tenenbaum 2008; Chakravorty et al., 2009). However, the concept of cropping intensification may resolve some issues surrounding this debate. In most of the High and Central Great Plains area of the U.S., agricultural crop rotations typically include summer fallow periods (Farahani et al., 1998). Although water is the limiting factor in these areas, this fallow period has been shown to be an inefficient use of soil water and nutrient storage (Farahani et al., 1998; Nielsen, 1998; Nielsen et al., 2005). Diversifying and adding to the crop rotation could advantageously disrupt pest and weed infestations that may arise from monoculture or fallow practices, as well as provide a more efficient use of the low and erratic precipitation that this area receives annually (Nielsen, 1998; Johnston et al, 2002). Thus, by replacing a fallow period with a different crop, such as an oilseed for biofuel production, there is no reduction to the food supply, and the debate over bioenergy sources depleting our food supply is partially circumvented.

Although other oilseed crops might fit agronomically into the majority of crop rotations used in the Great Plains, few have as many positive features as *Brassica napus* L. (canola) for use as an on-farm biofuel option (Fore et al., 2011). Not only can spring and winter-adapted varieties of canola fit into the fallow period in most of the traditional crop rotations found in the Great Plains (Farahani et al., 1998), but a market is already established for canola, which allows for sale in the canola commodity market at the discretion of the producer. Importantly, the U.S. Food and Drug Administration granted canola oil and its oil-free meal 'Generally Recognized as Safe' status, allowing its sale and distribution in the U.S., as opposed to other oilseeds such as *Camelina sativa* (false flax) which have not yet been approved for food use. Furthermore, canola seeds yield on average 40-50% oil content (Wittkop et al., 2009; Nesi et al., 2008; Kimber and

McGregor, 1995), which is an added benefit of its use when compared with other oilseeds that have lower percentages of oil such as *C. sativa* and *Glycine max* (soybean) (Fore et al., 2011). Many herbicide-resistant canola cultivars are also available, which can help combat weed growth economically and therefore increase yield. Lastly, the high-protein cake meal residue post-oil extraction is used as feed for livestock, primarily dairy cattle and poultry (Bell, 1993), which provides additional income to the producer. Canola seems to be a viable option for income, versatility and rural energy independence in Colorado.

Origin and use of rapeseed

Brassica napus L., known commonly as rapeseed, swede rape, Argentine rape, oil rape or oilseed rape, is believed to have originated hundreds of years ago in the Mediterranean region with many of its ancestral Brassicaceae family members. The plant was originally cultivated in Europe and used for its oil, and its industrial use has persisted throughout time (Shahidi, 1990). In recent history, rapeseed oil was limited to applications in cosmetics, lubrication, plastics and inks, but 20th century improvements in seed oil chemistry have broadened the uses for rapeseed to include human consumption and animal feed.

Members of the Brassica genus include cultivated vegetable and root crops such as *Brassica rapa* ssp. *Rapa* (turnip), *Brassica oleracea* ssp. *Italia* (broccoli), *Brassica oleracea ssp. gemmifera* (brussels sprouts), *Brassica oleracea ssp. Cabitata (*cabbage) and *Brassica oleracea ssp. Viridis* (kale). Six economically important oilseed Brassica species are linked genetically, a theory first described by U in 1935 and now known as the Triangle of U (Fig. 1.1). U postulated that three extant Brassica species, *Brassica oleracea* (AA), *Brassica nigra* (BB) and *Brassica rapa* (CC)

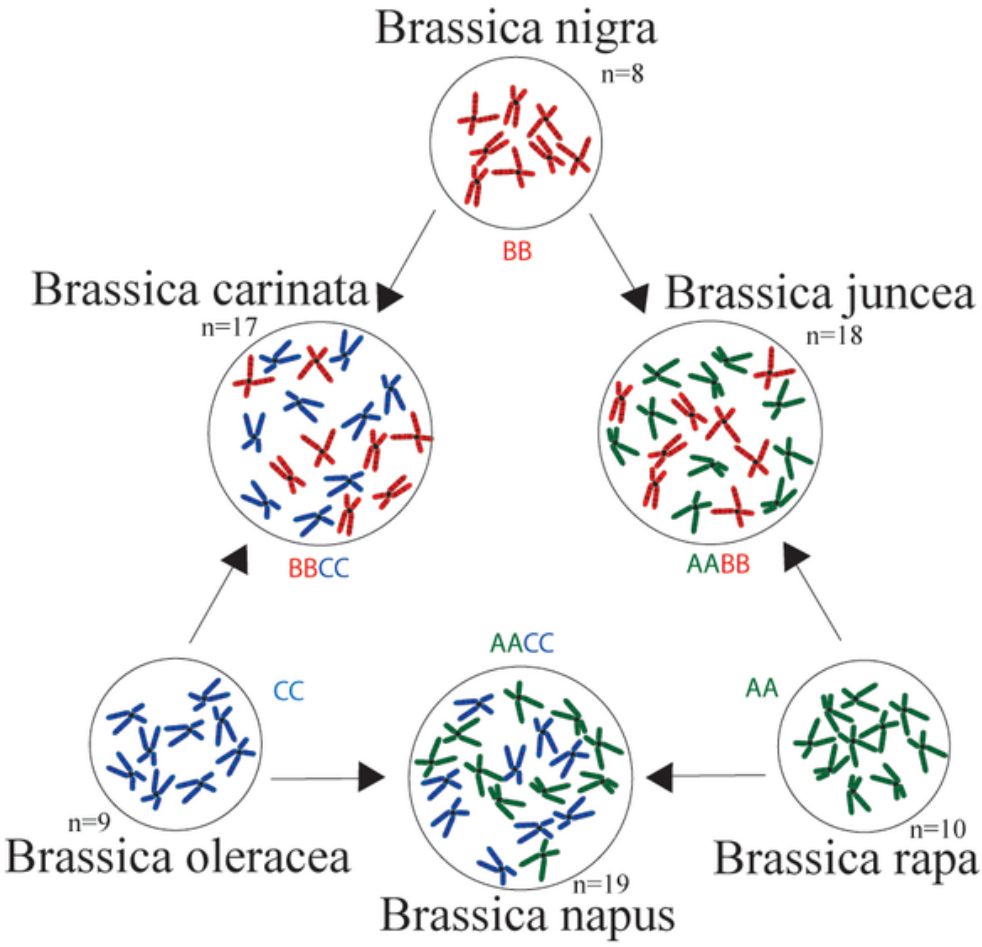


Figure 1.1 The triangle of U depicts the genomic relationship of six Brassica species. Each letter *n* indicates the number of chromosomes found in the gametes of each species. Diploid species are found in the corners, which hybridized with one another to create the respective species found in between. Adapted from the Wikimedia Commons file "Image: Triangle of U Simple.PNG" http://commons.wikimedia.org/wiki/File:Triangle_of_U_Simple.PNG

hybridized in three independent events to produce the novel allopolyploids, *Brassica juncea* (AABB), *Brassica napus* (AACC), and *Brassica carinata* (BBCC). This evolutionary relationship has had important implications in plant breeding; interspecific crosses with other species based on these close relationships have been a source of several trait improvements, including yellow seed coat color in *B. napus* and low glucosinolate levels in *B. juncea* (Branca and Cartea, 2011).

An additional member of the Brassicaceae family with tremendous biological importance is the model plant *Arabidopsis thaliana* (Arabidopsis), which has been the center of much scientific exploration in the last two decades. Its use as a model plant came about due to several factors including its small genome size (around 140 megabase pairs on five chromosomes), short life cycle, availability of genetic tools (e.g., mutant libraries) and small physical size. The full genome sequence became available for public use in 2000 (Arabidopsis Genome Initiative [AGI], 2000), which has in turn allowed for large amounts of gene and sequence annotation information to be shared with scientists worldwide. Fortunately, there is a large amount of genome synteny between Arabidopsis and many of the Brassica oilseed species (Lagercrantz, 1998; Hall et al., 2002; Lukens et al., 2003, Panjabi et al., 2008)*;* several segments of the Arabidopsis genome have been shown to exist in triplicate in *B. rapa* and *B. oleracea*, which are the progenitor species of *B. napus* (Cavell et al., 1998; Parkin et al., 2005; Kimber and McGregor; 1995; Kole, 2011). Consequently, genetic and genomic tools from Arabidopsis are ideal for gene discovery and improvement efforts of Brassica species (Hall et al., 2002).

Rapeseed seed chemistry

The composition of rapeseed grain makes it ideal for use in many applications, including animal feed and human consumption. Rapeseed typically contains at least 40% oil but can yield up to 52% oil depending on the cultivar; for comparison, *C. sativa* and soybean yield about 30%

and 20% oil, respectively. On a dry basis, 40-60% of the meal weight after oil extraction is crude protein with a well-balanced assortment of essential amino acids (Kimber and McGregor, 1995; Shahidi, 1990; Nesi et al., 2008). This high protein percentage, along with its rich vitamin and mineral profile, makes the meal residue favorable for use as an animal feed (Canada Canola Council, 2009; Nesi et al., 2008). After protein, carbohydrates make up the next largest portion of rapeseed meal, of which the fiber component is least desirable because of its negative effect on digestibility in animals. Opportunely, rapeseed has naturally low fiber content, yet ultra-low fiber cultivars have also been developed to further improve digestibility of the meal (Canola Council of Canada, 2009).

While fatty acid composition of rapeseed oil is highly variable (see Table 1.1), the specific oil profile determines the end use of the seed oil in either industrial or nutritional applications. Traditional rapeseed varieties contain 22-60 percent erucic acid (22:1 ω-9) in their oils, which are known as HEAR (high erucic acid rapeseed) varieties. Although these varieties are suitable (and, in fact, preferable) for industrial applications, concerns in the 1970s about the negative effects of erucic acid on cardiac health led the Canadian government to enforce regulations for food-grade oils which limited erucic acid amounts to less than five percent. LEAR (low erucic acid rapeseed) cultivars were introduced as a response to these health concerns (Kimber and McGregor, 1995). Later, glucosinolates and their degradation products in the meal were discovered to be associated with antinutritive and toxic effects in animals, leading to further breeding efforts to lower the levels of these plant products. Today, in some countries, cultivars are officially known as “canola” (derived from **Can**adian **o**il **l**ow **a**cid) if the oil contains “less than 2% erucic acid, and the solid component of the seed (contains) less than 30 micromoles of any one or any mixture of (specific) glucosinolates per gram of air-dry, oil-free solid.” According to the Canola Council of Canada (2009), the term “was trademarked by the Western Canadian

Table 1.1 Typical chemical composition of traditional rapeseed varieties, single-low varieties, and double-low (canola quality) varieties. Adapted from Bell and Keith, 1982.

Fatty Acid (common name)	**Traditional rapeseed (High Erucic Acid) %**	**Single-low (Low Erucic Acid) %**	**Double-low (Canola quality) %**
Saturated Fatty Acids			
16:0 (Palmitic)	1.7	3.5	4.0
18:0 (Stearic)	0.9	1.5	1.4
20:0 (Arachidic)	1.2	1.1	1.1
22:0 (Behenic)	0.9	0.3	0.3
24:0 (Lignoceric)	0.5	0.1	0.1
Monounsaturated Fatty Acids			
16:1 (Palmitoleic)	0.0	0.4	0.5
18:1 (Oleic)	12.3	58.9	56.9
20:1 (Eicosenoic)	5.8	1.5	1.5
22:1 (Erucic)	59.4	0.1	0.0
24:1 (Nervonic)	1.6	0.2	0.3
Polyunsaturated Fatty Acids			
18:2 (Linoleic)	12.7	21.8	23.8
18:3 (Linolenic)	7.6	10.5	10.1

Oilseed Crushers' Association (now the Canadian Oilseed Processors Association) to differentiate the superior low-erucic acid and low-glucosinolate varieties and their products from the older rapeseed varieties." Some countries adopt these standards, yet refer to the oil instead as "00," "double-zero" or "double-low" rather than by the name canola. Canola oil has been given GRAS (generally recognized as safe) status in Canada, Europe and the U.S., and production has risen to account for nearly 20% of food-grade vegetable oils globally (USDA, 2012).

As illustrated in Figure 2.1 and Table 1.1, the typical fatty acid profile of canola oil is exceptionally healthy compared with other cooking and food-grade oils. The low amounts of saturated fatty acids coupled with the balanced 2:1 ratio of linoleic (18:2 ω-6) to alpha-linolenic (18:3 ω-3) polyunsaturated fatty acids make the nutritional profile of canola excellent for human consumption (Wittkop et al., 2009; Coonrod, 2005). Consequently, canola ranks second in the world behind soybean for use as a food-grade vegetable oil (USDA, 2011).

Rapeseed breeding efforts

The greatest effort in rapeseed breeding has been given to improving oil quality (Wittkop et al., 2009, Nesi et al., 2008; Kimber and McGregor, 1995). As mentioned above, the largest success in rapeseed breeding was inarguably the development of canola-quality or "double-low" rapeseed cultivars, made by decreasing erucic acid in seed oil and glucosinolate levels in the oil-free meal. A separate undertaking for scientists has been to decrease amounts of linolenic acids, which are beneficial for human health, but unfortunately very unstable. This instability causes rancidity and undesirable odors from extended shelf-life or cooking, so breeders have worked to decrease levels of linolenic acids, increase levels of linoleic acids and also increase oleic (18:1 ω-

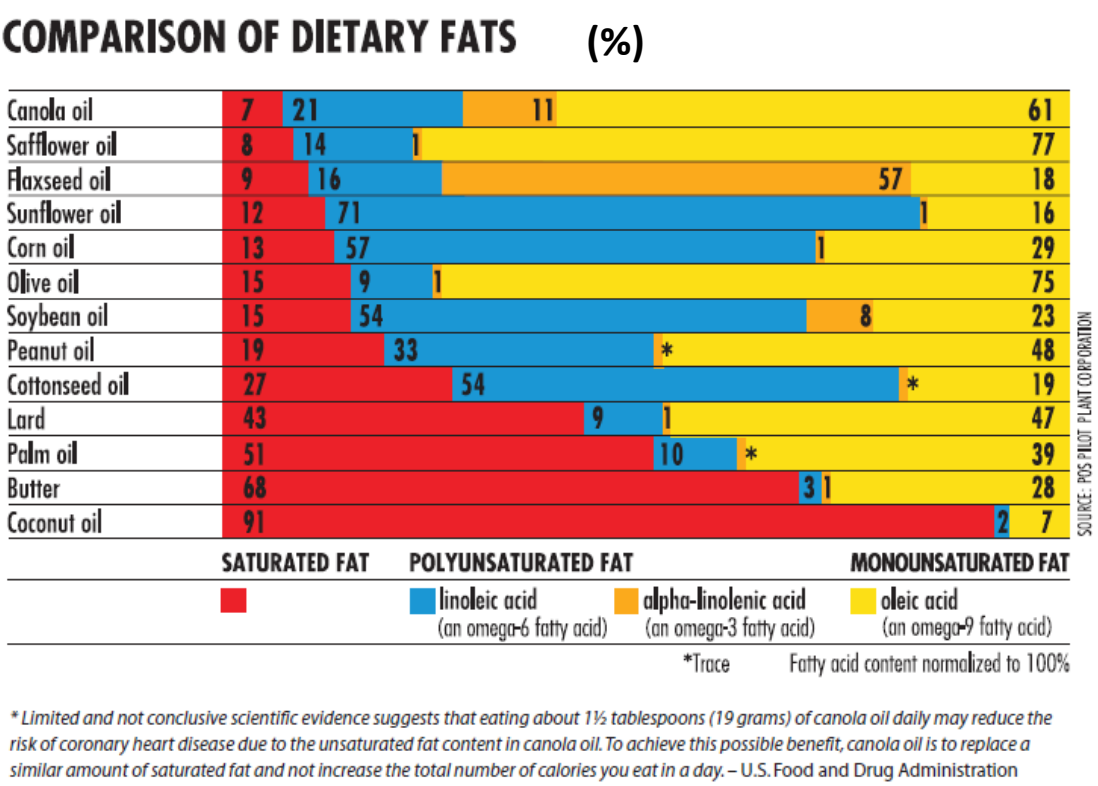


Figure 2.1 Comparison of dietary fat percentages in canola and other popular cooking oils. File used by permission from Canola Council of Canada.

9) acids for beneficial health effects and improved shelf-life (Coonrod, 2005; Scarth and Tang, 2005). These new double-low rapeseed cultivars with high oleic acid and low linolenic acid are sometimes termed 'HOLLi' cultivars (Addabi et al., 2011, Wittkop et al., 2009). A separate oil quality breeding goal which allows for a more diverse application in food products has been to increase saturated fatty acids (Scarth and Tang, 2005). This is useful to margarine and confectionary producers because introduction of saturated fats can then occur without the use of hydrogenation, which promotes formation of unhealthy *trans*-fatty acids. As a result of the many uses of rapeseed, the objective of improving its oil composition for specific applications will continue to be an ongoing breeding effort.

Breeding goals for quality in rapeseed have not been limited to the oil portion of the seed. It has been a goal of rapeseed breeders to improve the energy value of the oil-free meal which is used to supplement livestock feed, as well as to continue to decrease levels of glucosinolates, tannins and sinapate esters for improved livestock palatability (Nesi et al., 2008; Canola Council of Canada, 2009). Seed protein improvements, such as increase in essential amino acid fraction have been successful using genetic engineering approaches (e.g. Altenbach et al., 1992). Furthermore, the reduction in tannins, which are responsible for the black seed coat color in rapeseed, has recently occurred as a result of interspecific crossing strategies. The resulting yellow-seeded *B. napus* varieties also tend to have higher oil and protein content in addition to the intended consequence of decreased fiber and hull seed proportion (Wittkop et al., 2009; Nesi et al., 2008; Zhi-wen et al., 2005; Tang et al., 1997; Xiao et al., 1982). Breeding strategies to modify meal properties continue in rapeseed to increase utilization of this value-added product.

In addition to the continued attention given to oil and meal quality in canola, breeders are also concerned with increasing seed oil concentration. This has been met with some success due to the introduction of yellow-seeded canola varieties as previously discussed. These varieties

tend to have higher oil and protein content (Nesi et al., 2008), so improvement of yellow-seeded varieties is being emphasized in many current breeding programs. Another prospect for increasing oil content could include breeding for larger seed sizes, which seem to have higher oil concentration and better germination ability as well (Fan et al., 2010; Geritz et al., 1999). Finally, several quantitative trait loci (QTL) have been discovered for oil content recently (e.g. Mahmood et al., 2006, Zou et al., 2010) which could be used in marker-assisted breeding to increase oil concentration. Improvement in oil content continues to be one of the largest challenges that rapeseed breeders face today.

One other major target for rapeseed breeders has been, unquestionably, improvement of seed yield. Although rapeseed cultivar yields have increased by over 50% in the last fifty years (Nesi et al., 2008), the major objective of many crop breeding programs, including canola, is to continually increase seed yields for a rapidly increasing population. A growing demand in oilseed use for food, feed and fuel is placing added pressure on canola breeders to advance higher-yielding cultivars, which has led to the recent introduction of hybrid varieties. Hybrid production in the U.S., Europe, Australia and Canada has increased significantly in the last twenty years, but developments are still needed to maintain yield stability. A major aim of hybrid rapeseed development programs is therefore to establish distinct heterotic pools in order to take advantage of hybrid vigor and yield stability (Abbadi et al., 2011). Additionally, there are numerous opportunities for advancement of yield under low-input systems, such as under limited irrigation and low nitrogen. The increase in availability of agricultural technologies such as inexpensive, high-density genotyping as well as high-throughput phenotyping will no doubt assist in the efforts to breed for low-water or low-nutrient environments. Breeding for increased yield will continue to be a priority in rapeseed breeding programs, with specific attention given to hybrid varieties and use of advancements in molecular and field technologies.

Due to the complex nature of yield, it has been suggested that indirect selection via selection of yield components may be effective (Thurling, 1974; Richards and Thurling, 1979; Mahmood et al., 2005). However, successes regarding use of yield components to increase seed yield in rapeseed have been variable. The components most predictive of yield reported in scientific literature include siliques per plant, (for which siliques per main raceme is often used as a proxy), seeds per silique, plant height, silique length, seed weight and oil content (Marjanovic-Jeromela et al., 2011; Fan et al., 2010; Zhang et al., 2010; Zhang et al., 2011; Shi et al., 2009; Mahmood et al., 2005; Thurling, 1974). Numerous studies have been done to examine correlations of these traits to yield, which have been valuable with respect to dissecting this complex trait. However, these results have been inconsistent in the literature. For example, Chay and Thurling (1989) reported a positive correlation between seeds per silique and silique length, but a negative correlation between seeds per silique and seed weight. Although this negative relationship was also reported by Zhang and colleagues (2011), Shi and colleagues (2009) reported a significant positive relationship between seed yield, seeds per silique, siliques per plant, plant height and seed weight. Also, Mahmood and colleagues (2005) reported that selection indices which included one or several yield components did not appear to be effective in increasing yield. The results from each study represent different environments, locations and years, so clearly more studies need to be completed in specific environments in order to determine usefulness of indirect selection in regional breeding programs.

Effect of genes, environment and GxE on yield and yield components, oil content and oil profile

Decline in crop productivity can occur due to environmental stresses such as limited water availability, insect damage, disease and high temperatures, among many other factors.

However, more of the US is subjected to drought stress than any of the other production-limiting factors, and drought affects yields more severely than any of these stressors combined (Boyer, 1982). Adaptations to drought stress exist in many wild species across the globe, so in theory, the genetic potential to increase crop yields under limited water availability exists. Harnessing this potential has been a large and recent goal of many crop plant breeding programs (focus on “more crop per drop”) world-wide (Pennisi, 2008).

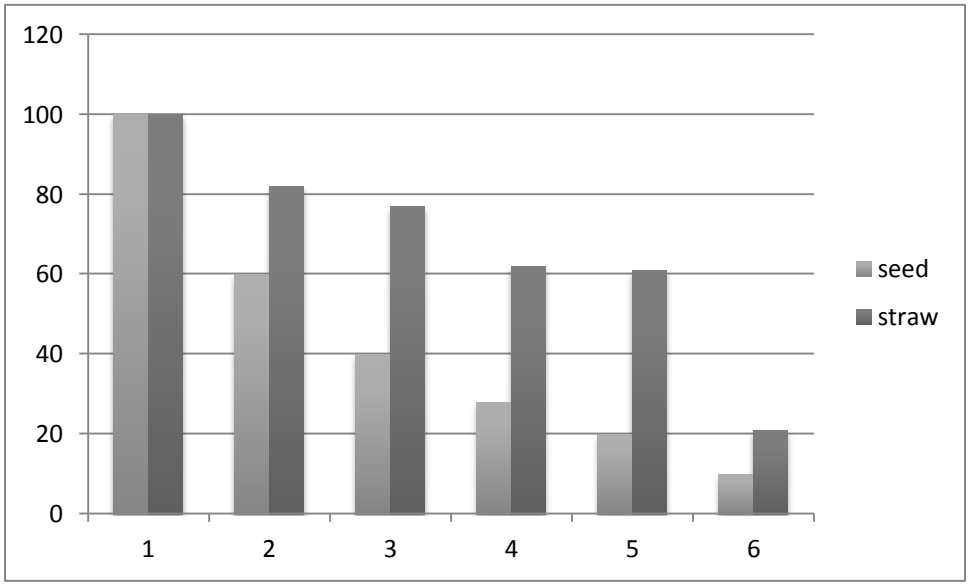
Gene by environment interaction (GEI) is a component of phenotypic variance that exists when a genetic effect is only realized under certain circumstances or environments. Existence of GEI might hinder breeding progress by adding a level of phenotypic variation that is difficult to account for. However, knowledge of the extent and/or the mode of GEI can assist breeders who may be able to exploit the interaction, thus tailoring cultivars to a specific set of environments. Although GEI can be a complicated phenomenon, researchers are beginning to better understand and take advantage of this interaction in order to induce gains in diverse and often stressful environments.

Effects of water stress on canola cultivars have been well-studied (Fig. 3). Seed yields as low as 10% of irrigated checks due to drought stress have been reported in several studies (Hergert et al., 2011; Canola Council of Canada, 2009; Si and Walton, 2004; Si et al., 2003). Extreme impacts have also been reported on yield components, oil content, oil profile and protein content due to limited water availability and heat stress. A summary of heat and drought effects as well as GEI on yield, yield components, fatty acid profile and oil content in canola are discussed below.

Seed yield in many crop plants is a complex phenotype which is affected by genotype, environment and GEI, as is oil concentration in most oilseeds (Moghaddam and Pourdad, 2011;

Table 2.1 Effects of temperature and water stress applied from the end of flowering until maturity on yield, yield components and oil content for *B. napus*. From Canola Council of Canada (http://www.canolacouncil.org) and Richards and Thurling (1978).

Temperature (high/low)	18/10 C		26/18 C	
Water treatment	Irrigated seed Yields (%)	Water stress seed yields (%)	Irrigated seed Yields (%)	Water stress seed yields (%)
Seed Yield	100	60	67	56
Pods/m2	100	72	82	65
Seeds/m2	100	84	83	72
Seed weight	100	76	81	77
Oil content	100	88	89	83



- 1=Optimum irrigated check
- 2= Dry up to floral bud appearance and irrigated thereafter
- 3=Early irrigation, dry during flowering, irrigated during pod formation
- 4=Irrigated up to flowering and dry afterwards
- 5=Dry up to full bloom and irrigated
- Dry=No irrigation after crop establishment – no rainfall

Figure 3.1 Effect of water stress during different developmental stages on Brassica plants. Chart reproduced from Canola Council of Canada (http://www.canolacouncil.org) and Richards and Thurling (1978).

Gunasekera et al., 2006; Abbadi and Leckband, 2011; Si et al., 2003; Si and Walton, 2004). Both of these traits represent the most economically-important qualities which should remain as stable as possible in order for growers to benefit from the growth of Brassica cultivars. However, both seed yield and seed oil content have been reported to show a negative response to heat and drought stress (Moghaddam and Pourdad, 2011; Gunasekera et al., 2006; Si et al., 2003; Siand Walton, 2004). Although a significant GEI for both traits has also been reported in several studies, some studies have reported insignificant GEI (Si and Walton, 2004). Fortunately, seed oil content has also been reported to have a high heritability and mostly additive gene action, indicating that GEI and environment do not contribute as much to the variation in the trait as main effects of genes. Therefore, breeding for high oil content in water-limited environments has great potential.

Seed yield has been reported to decrease under heat and drought stress (see Fig 3; Angadi et al., 2000), with more severe penalties if the stressors occur post-anthesis in addition to during the time of flowering. Yield components, including seeds per silique, siliques per plant, silique length, and seed weight have also been shown to suffer under heat and water stress (see Table 2; Champolivier and Merrier, 1996; Clarke and Simpson, 1978).

Intense breeding efforts in the 1970s rapidly changed the fatty acid profile of rapeseed cultivars, leading to the enhancement of health benefits and introduction of canola-quality cultivars. Although quite a bit about the genetic control of these traits was discovered during this time, it was also discovered that the oil profile is highly influenced by environment, especially high temperatures and low water availability. Aslam et al. (2009) reported significant relationships between amount of rainfall and fatty acid composition; this corresponds to other drought and heat stress studies on *Brassica napus* cultivars (e.g. Moghaddam et al., 2011). In this study, significant effects of genotype and environment (heat and drought stress) were

reported for all fatty acids, but GEI was insignificant at all locations. Specifically, oleic acid and saturated fats decreased with incidence of drought stress, while percentages of linolenic and linoleic fatty acids increased. These relationships are important to account for, especially since the abundance of specific fatty acids in canola cultivars affect the health profile to humans and animals that consume the oil.

Aslam et al. (2009) also reported relationships between rainfall, temperature, seed oil and seed protein content that are consistent with other reports in the literature (e.g. Wittkop et al., 2009; Nesi et al., 2008). Seed oil content and seed protein content are reliably reduced as rainfall declines and as heat stress increases. Nesi et al. (2008), Zhao et al. (2005) and Delourme et al. (2006) reported a strong environmental effect as well as incidence of additive gene action in control of seed oil content. Temperature and water availability during and after anthesis tend to predict seed oil content consistently, such that higher temperatures and lower rainfall decrease seed oil content.

Future breeding directions

Current crop breeding strategies focus on improvement of varieties that have survived a bottleneck during the domestication process, and therefore rely on a relatively small gene pool for continued improvement. *B. napus* is a crop which has had a similar fate, and therefore contains a narrow base of alleles to draw upon for continued yield and quality improvements (Abbadi et al., 2011, Kimber and McGregor, 1998). Fortunately, the species represented in the Triangle of U (U, 1935) present an opportunity to easily introgress alleles with positive adaptive and quality characteristics (Cermankova et al., 1999; Song et al., 1993) using crossing methods and tissue culture. Breeders of many crops, including *Solanum lycopersicum* (tomato), *Zea mays* (maize) and *Triticum aestivum* (bread wheat), are utilizing secondary gene pools more

frequently to improve upon modern crop cultivars, and canola breeders are following suit (McCouch, 2004).

One of the most important breeding goals of our time will be to produce cultivars which utilize resources more effectively, due to heavy competition for limited water sources from agricultural, industrial, urban and recreational use (Peterson et al., 2006) and expensive, environmentally-degrading nutrient applications. Breeding Brassica cultivars that have superior water and nutrient use efficiencies will certainly be included in this broad goal. Fortunately, the wide range of growing areas in the history of the domestication of the Brassica species has led to a variety of specific adaptations to environments and stresses. For example, *B. juncea* is considered to be the most tolerant to heat and drought of the Brassica oilseed species, *B. carinata* has better resistance to disease and shattering and *B. rapa* is earlier flowering and maturing (Kimber and McGregor, 1995). Consequently, there is considerable potential for breeding improvements in *B. napus*, which is best adapted to the cool areas of the temperate zones (daytime temperatures of 12-30˚C) with sensitivity to high temperatures and low rainfall (Angadi et al., 2000). Introgression of heat and drought tolerance traits via interspecific crossing strategies or generation of resynthesized *B. napus* varieties from *B. rapa* x *B. oleracea* crosses might allow better adaptability to hot and dry climates as typically found in the Central Great Plains.

Mapping populations, molecular genetic markers and QTL analysis

Quantitative traits are traits which are affected by several genes of large effect, small effect or a combination of both. In addition to genetic variation, environment can also influence polygenic phenotypes, due either to the environment directly or to the interaction of genes with the environment (Falconer and Mackay, 1996). These traits tend to be approximately normally

distributed in a population because the alleles that affect the trait can be of similar or conflicting effects and are disseminated differently among individuals, so therefore tend to be expressed in a continuous range. This phenotypic variation and the variability in the underlying causes of different traits make the understanding of contributory genes and environmental conditions difficult for scientists to study. Understanding the influence of genes and environment on quantitative phenotypes is a major challenge in many biological systems today.

Quantitative trait loci, or QTL, are areas of the genome that include genes or are tightly linked to genes that affect quantitative traits. QTL mapping projects focus not only on the discovery of QTL in many species and populations, but can also be used to study the influence of environment and gene by environment interaction on phenotypes. QTL mapping is being done in many species to better understand which genes affect important biological traits, from disease susceptibility or resistance in humans to yield in crop plants.

Methods used to discover or map QTLs are based on DNA molecular markers and the linkage disequilibrium that exists between these markers and the trait of interest in a specific mapping population (Falconer and Mackay, 1996). In order to study QTL adequately, a mapping population is constructed or chosen based on individuals that display phenotypic differences in the trait(s) being studied. To obtain the most accurate results, the genome of interest should be densely covered by polymorphic markers, which are essentially genomic landmarks used to determine linkage groups based on recombination frequencies. The same markers used to construct genetic linkage maps are also used to score individuals' genotypes; they must be polymorphic in the study population in order to perform both roles (Collard et al., 2005). A number of marker types are available for population characterization for genetic mapping purposes, but must be chosen carefully based on the project goals. However, a densely covered genetic linkage map is mandatory for any project in order to detect QTL effectively.

Several DNA molecular marker types are available for genotyping mapping populations. A non-comprehensive list of available and popular markers for molecular DNA analysis includes: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequences (CAPS), microsatellites or simple sequence repeats (SSRs), expressed sequence tags (ESTs), single nucleotide polymorphisms (SNPs), and diversity arrays technology (DArT) (Semagn et al., 2010). Semagn and colleagues (2010) differentiate marker types based on whether heterozygotes and homozygotes can be distinguished (dominant versus co-dominant markers), mode of transmission (e.g. maternal organelle, paternal organelle, or biparental nuclear) and method of analysis (hybridization [e.g. RFLP] or PCR-based). PCR-based markers are further broken down into arbitrarily-primed (e.g. RAPD) and site-targeted (e.g. EST and SSR) amplification methods. In the end, usefulness can be found in each of these types of markers, so every project and investigator needs to separately evaluate marker types against their utility, time requirement, and expense.

Population types in association mapping experiments are either specially-constructed from crosses between lines that differ for the trait(s) of interest, or are natural populations chosen based on these trait differences. Parental inbred lines are the most popular choice for QTL mapping; these include backcross lines, recombinant inbred lines and doubled-haploid (DH) lines (Falconer and Mackay, 1996). Utilization of inbred lines fixes alleles such that the population is “immortal” and can be grown in many replicates and environments. Additionally, because heterozygotes are nearly non-existent due to the degree of inbreeding that occurs to construct the lines, overdominance does not complicate the analysis, and only two genotypes are possible at each polymorphic locus which adds an element of simplicity.

QTL mapping is a statistical exercise that is used to reveal linkage that exists between one or more molecular markers and a locus that may be implicated in the trait of interest. Several

methods are available to perform QTL mapping, either at a single locus (single marker analysis) or multiple markers (genome-wide scan). Researchers often prefer to carry out a single-marker analysis when strong *a priori* knowledge is available about which loci might be implicated in a trait of interest (Rafalski, 2010). Genome-wide scans take all markers into account, so reveal more information, yet become more complicated statistically due to the large number of comparisons that are being performed in the analysis.

Many methods are available to perform genome-wide QTL analysis. How to treat the missing genotype data (between known markers) and how to select a model that accurately describes the phenotype are the unknowns in QTL mapping (Broman and Sen, 2009), and generally define the differences between mapping methodologies. Many QTL mapping methods have been developed which allow genome-wide scans to be performed in between markers, even though this complete genotype information is not available, thus attempting to answer the problem of the missing data. These procedures, known as interval mapping procedures, include standard and composite interval mapping, Haley-Knott regression and multiple imputation methods, among others. The multiple imputation method (MI) is different from other methods in how it treats missing genotype data. In essence, MI guesses at the unknown genotypes between markers based on the known genotypes at the two flanking markers. These guesses, or imputations, are based on the probabilities of recombination events between the markers. After the data are imputed, t-tests can be performed at each position (using each imputation), and the results are averaged from each t-test and expressed as a LOD score (Broman and Sen, 2009). The multiple imputation method is considered to be a robust application for mapping QTL. In the view of many experts, it is considered the most valuable method for exploring multiple QTL models (Sen and Churchill, 2001; Broman and Sen, 2009).

A number of published studies have reported conclusions on influence of genes, environment and GEI on seed oil content, seed yield and yield components in Brassica species. Additionally, several QTL studies on these traits have been published, yet to our knowledge no studies have reported QTL in both well-watered and rainfed conditions. In this study, MI QTL interval analysis will be performed on two separate mapping populations in *Brassica napus* L. under rainfed and irrigated conditions. Both populations contain doubled haploid lines which were developed for genetic mapping purposes. Traits to be studied include flowering time, seed yield and yield components. The specific objectives of this study were:

1) to evaluate yield, yield components, and days to flowering in two doubled haploid canola mapping populations under rainfed and irrigated conditions north of Fort Collins, Colorado during the 2010 and 2011 growing seasons;

2) to determine relationships among yield and yield components by analyzing trait correlations and to study trait inheritance patterns;

3) to determine areas of the *B. napus* genome that are implicated in yield and yield component traits under both rainfed and irrigated conditions by QTL mapping; and

4) to study the sensitivity of yield and yield component traits to drought stress by performing analysis of variance and by performing a QTL analysis on the difference in trait values from the rainfed and irrigated treatments.

Each of these objectives will provide useful information to canola breeders in the High Plains, who will require information on trait inheritance, correlations, and marker-trait associations in order to breed high-yielding, drought-tolerant cultivars of *Brassica napus* L. for food, feed, or fuel.

CHAPTER TWO: QUANTITATIVE TRAIT LOCUS MAPPING IN CANOLA (*BRASSICA NAPUS* L.) UNDER IRRIGATED AND RAINFED TREATMENTS: YIELD AND YIELD COMPONENTS

Chapter summary

Brassica napus L. is an oilseed with potential for use as an on-farm biofuel in Colorado in order to secure rural energy independence. However, as urban water consumption rises, the amount of water available for crop production declines, and therefore rotation crops in Colorado must yield well with limited or no irrigation. In this study, two doubled haploid *B. napus* genetic mapping populations (SE1, n=183; DHYB, n=150) were grown under irrigated and rainfed treatments in Fort Collins, Colorado to study the sensitivity of yield and yield-related traits to water stress and to detect quantitative trait loci (QTL) in both treatments. Days to flowering (DTF), seed yield, siliques per main inflorescence (SMI), seeds per silique (SS), and thousand seed weight (TSW) were analyzed in both populations, SE1 grown in 2010 and DHYB in 2011. Analysis of variance revealed significant treatment, genotype, and genotype by treatment interaction (GEI) for many traits in both populations. Significant correlations between days to flowering, yield and yield components are reported in each treatment and population. Additionally, seven additive (one detected in both treatments and three novel) as well as two epistatic QTL (both novel) were detected for seed yield in both populations. Two large effect DTF QTL co-located with QTL for all yield component traits in the 2010 SE1 study, and explained 73 and 65 percent of phenotypic variance for DTF in the wet and dry treatments, respectively. Many other novel QTL are reported for SS, SMI, DTF and TSW in both treatments of both studies. These results are useful for providing insights into relationships among yield components, and have potential to be used in marker-assisted selection techniques to improve canola seed yields in the semi-arid climates of the U.S.

Introduction

Reliance on non-renewable and finite sources of fossil fuels has reached unprecedented levels in the U.S. (U.S. Energy Information Administration, 2011). Additionally, the United Nations predicts that the world's population will rise to nine billion by 2050 which will increase the demand for energy (United Nations, 2010). Furthermore, greenhouse gas emissions causing global climate changes are attributed largely to burning fossil fuels for energy (IPCC, 2001). Each of these reasons alone could be considered solid support for development of alternative and renewable energy sources, but together they demonstrate a clear need to change the way energy is produced and used in the U.S.

Brassica napus L., known also as rapeseed, is considered to be a viable option for bioenergy in the Great Plains region of the U.S. Few oilseeds has as many positive features as *B. napus* for use as an on-farm biofuel option (Fore et al., 2011). Not only can spring and winter-adapted varieties of rapeseed fit into the fallow period in most of the traditional crop rotations found in the Great Plains (Farahani et al., 1998), but a market is already established for canola, which is an improved variety of rapeseed considered to be healthier for human and animal consumption. Canola (also known commonly as double-low rapeseed) varieties are approved in the U.S. and Canada for food and animal feed purposes. Consequently, canola oil could also be sold in the canola commodity food oil market at the discretion of the producer. Many herbicide-resistant canola varieties are also available, which can help combat weed growth and therefore increase seed yield. Lastly, the high-protein cake meal residue post-oil extraction is used as feed for livestock (Bell, 1993), which provides additional income to the producer. *B. napus* seems to be an attractive option for income, versatility, and energy in Colorado.

In order to increase the use of canola in sustainable crop rotations in Colorado, it is necessary that the germplasm is highly water use efficient. As the worldwide population grows,

constraints on water use for crop production become more prominent due to competition from non-agricultural uses. Additionally, the semi-arid climate in Colorado does not suit the growth of many crops well, so it is necessary to develop specific cultivars that are adapted in this area. Therefore, development of high-yielding, high quality water use efficient cultivars is a priority for oilseed crop plants in Colorado and the High Plains area of the U.S.

Seed yield is commonly the target for improvement in crop breeding programs, including *B. napus*, yet yield is difficult to select for because of the large number of genes involved and the presence of complex genotype by environment interactions. Yield components are often studied along with yield in order to decompose the complex trait of yield into its component parts and to better understand how yield is determined. The most typical yield components studied in rapeseed include number of siliques per main inflorescence or raceme (SMI), number of braches per plant, number of seeds per silique (SS), silique length, and thousand seed weight (TSW).

Many recent publications have reported relationships between yield and various yield components in *B. napus*. A negative correlation between SS and TSW has been reported in many studies (e.g. Liu, 1987; Zhang et al., 2011; Li et al., 2007), yet positive correlations between SS and TSW have also been described (Chay et al., 1989; Liu, 1987). Zhang and colleagues (2011) were the first to study these relationships using quantitative trait locus (QTL) mapping in a doubled-haploid (DH) population. Their results confirmed a significant negative relationship between SS and TSW (R=-0.62; P<0.01; n=140) but did not indicate any co-located QTL. Additionally, their study was completed in only one location and treatment, so there is little indication of whether the study results are stable or if there could be treatment or location effects.

In this experiment, two canola mapping populations were grown under side-by-side irrigated and rainfed treatments in order to study days to flowering, yield, yield components,

and the sensitivity of these traits to drought stress. QTL mapping was conducted to detect chromosomal regions that are linked to yield and yield-related traits in rainfed and irrigated treatments, and trait correlations are reported for both years in both conditions. This information will be valuable to canola breeders whose goal is to improve yield under limited or no irrigation in the High Plains area of the U.S.

Materials and Methods

Plant material, field trials and trait measurements

The SE1 population of approximately 1,000 DH *B. napus* lines was developed at Cargill Specialty Canola Oils (SCO) in Fort Collins, Colorado, using microspore culture from an F_1 cross. The parents of the cross were 'IMC106,' a spring rapeseed cultivar for the Canadian production region developed by Cargill SCO (www.inspection.gc.ca/english/plaveg/variet/pntvcne.shtml), and `Wichita,' a winter rapeseed variety with adaptation to the Great Plains region of the U.S., developed by Rife et al. (2001; Reg. no. CV-19, PI 612846). The resulting population was grown in a greenhouse and plants were selected for spring growth habit, resulting in a population of 238 lines which were generously donated for our research use.

In spring of 2010, the SE1 DH lines and parents were planted under a linear-move sprinkler irrigation system at Colorado State University's Agricultural Research Education and Demonstration Center (ARDEC) north of Fort Collins, CO (40.66˚N/105˚W; Elev. 5110'). The soil at this location is characterized as a Nunn Clay Loam. The study was arranged as a Row-Column design (created with CycDesigN 3.0, www.cycdesign.co.nz) with four replicates for each of the treatments, rainfed and irrigated. Plots consisted of two rows approximately 1 m long, with 0.23 m between rows and 0.33 m between plots. The study was irrigated approximately 2.5 cm per week via a linear-move sprinkler irrigation system for a period of one month from planting to

allow for stand establishment. After one month, the irrigation was discontinued for the rainfed treatment but continued at 2.5 cm per week for the irrigated treatment. Weeds were controlled manually. Plots were thinned to approximately 10 plants per plot, spaced evenly within rows. Details of the 2010 growing season conditions including rainfall, irrigation, and temperature are found in Figure S1.

To complement the genetic information resulting from the 2010 SE1 study, a second *B. napus* DH population named DHYB was used to study seed yield and yield-associated traits. The Plant Biotechnology Institute of the National Research Council Canada generously donated 150 DH lines for our research use. The population was derived using microspore culture of an F_1 cross between parents DH12075 (a black-seeded DH line derived from a 'Westar' × 'Cresor' cross; Agriculture and AgriFood Canada, Saskatoon Research Centre) and YN01-429 (a yellow-seeded line; Rakow and Relf-Eckstein, 2005). Both parents were spring-habit types.

In 2011, three plots of each DH line and parents were grown under both irrigated and rainfed conditions at ARDEC north of Fort Collins, CO. Each treatment was randomized independently and the study was arranged as a Row-Column design (created with CycDesigN 3.0, www.cycdesign.co.nz) with three replicates. Plots consisted of two rows approximately 2 m long, with 0.23 m between rows and 0.33 m between plots. The study was irrigated approximately 2.5 cm per week via a linear-move sprinkler irrigation system for a period of one month after planting to allow for stand establishment. After one month, the irrigation was discontinued for the rainfed treatment but continued at 2.5 cm per week for the irrigated treatment. Weeds were controlled manually. Plots were thinned to approximately 20 plants per plot, spaced evenly within rows. Details of 2011 growing season conditions including rainfall, irrigation, and temperature are found in Figure S2.

In both experiments, prior to planting, seed was treated with Prosper FX (Bayer CropScience Inc., Alberta, Canada; PCP registration number 29159) for protection against flea beetles (*Phyllotreta vittula*), *Rhizoctonia solani*, *Fusarium*, seed-borne blackleg, seed-borne *Alternaria* and *Pythium*. Sevin (Bayer CropScience Inc., Alberta, Canada; PCP registration number 264349) was sprayed on all plots to prevent accumulation of flea beetles during the seedling stage of growth.

Days to flowering was recorded for each plot as the interval from sowing date to the date on which 50% of the plants in the plot had initiated flowering. Yield components included number of siliques per main inflorescence (SMI), number of seeds per silique (SS) and thousand seed weight (TSW). SMI data were recorded from two random plants per plot and averaged. Ten siliques were collected, two apiece from the distal end of five randomly chosen main plant inflorescences per plot to measure SS and TSW. The siliques were allowed to dry at ambient room temperature for at least one month, and then threshed by hand in order to estimate number of seeds per silique. Seeds were weighed in order to extrapolate TSW (g/1000 seed weight). All plants in each field plot were swathed manually at maturity when 60-70% of the seeds appeared black upon inspection and allowed to dry on the ground for 5-7 days. A Wintersteiger combine harvester was used for threshing, and seed was weighed immediately to determine yield per plot in grams. Stand counts were taken on each plot in order to adjust yield for stand. In the 2011 DHYB population, seed coat color was qualitatively scored as either black or yellow, even though several different shades of each are apparent in the population.

Soil samples were taken at the end of the 2011 field season from approximately the top 15 cm of soil in each treatment. Samples were weighed immediately, then dried in a drying oven set to approximately 65˚C for at least one week and weighed again to determine the gravimetric water content (GWC) of the soil in both treatments. GWC was calculated as the fresh weight of

the soil samples minus the final dry weight of the soil samples, divided by the final dry weight. A WP4 dewpoint potentiometer (Decagon Devices, Pullman, WA) was used to determine a water potential curve (Figure S3) for the field soil samples in 2011, which was then used to determine water potential for both treatments in the field (Figure S4).

Construction of the linkage maps

Approximately 500 markers were used to genotype the SE1 population. They included expressed sequence tag (EST)-derived single nucleotide polymorphism (SNP) markers and simple sequence repeat (SSR) markers with known map locations. The linkage map for the SE1 population was generated with the regression mapping algorithm in JoinMap 4.0 (http://www.kyazma.nl/index.php/mc.JoinMap; Van Ooijen et al., 2001). The threshold recombination frequency was set to <0.25 and logarithm of the odds ratio (LOD) scores >2.0. A minimum LOD score of 10 was used to group loci into linkage groups. Genetic distances were calculated using the Kosambi function (Kosambi, 1944). Genetic maps and marker names are proprietary to Cargill Specialty Canola Oils and are therefore not presented in the results.

A total of 261 SSR markers was used to create the DHYB linkage map using the regression mapping algorithm in JoinMap 4.0 (http://www.kyazma.nl/index.php/mc.JoinMap; Van Ooijen et al., 2001). The threshold recombination frequency was set to <0.25 and logarithm of the odds ratio (LOD) scores >2.0. A minimum LOD score of 10 was used to group loci into linkage groups. Genetic distances were calculated using the Kosambi function (Kosambi, 1944). Linkage map illustrations were made using Map Chart 2.2 (Voorrips, 2002; http://www.biometris.wur.nl/uk/Software/MapChart).

Statistical analysis and QTL mapping

For each population, analysis of variance was conducted separately for each treatment and combined over treatments using the MIXED procedure of the statistical software SAS

version 9.3 (SAS institute; Cary, NC). Row and/or column were treated as random effects nested within treatments, while genotype and genotype x treatment interactions were treated as fixed effects. Least squares means (LS means) were also calculated in SAS using a mixed model approach (proc MIXED). LS means were determined within treatment for each DH line for all traits with row and/or column treated as random effects and genotype treated as a fixed effect.

Pearson's phenotypic correlation coefficients were computed for all pairs of traits as well as same traits between treatments using LS means and the CORR procedure in SAS. For all traits, broad sense heritability (BS h^2) was calculated from variance components from the GLM procedure in SAS as:

$$h^2 = \frac{{\sigma^2}_G}{{\sigma^2}_G + \frac{\sigma^2_{GT}}{n} + \frac{\sigma^2}{rn}}$$

where σ^2_G is the amount of genetic variance, σ^2_{GT} is the genotype by treatment variance (included if found to be significant), σ^2 is the error variance, r is the number of replicates and n is the number of treatments. To calculate heritability estimates within treatments, σ^2_{GT} was removed from the equation.

R/qtl (Broman et al. 2003; www.rqtl.org), an add-on program for the statistical package *R* (R Development Core Team, 2011), was used for QTL interval mapping of all traits in both treatments using LS means. Significance thresholds were derived from 1,000 permutations according to a genome-wide type I error rate of 5% (Churchill and Doerge, 1994). Multiple imputation (MI), Haley-Knott regression (HK) and standard interval (EM) mapping strategies were compared to determine the most robust method for interval mapping in each mapping population. Standard interval mapping uses maximum likelihood estimation under a mixed model to predict genotype data between markers, whereas the HK method provides a faster approximation to determine genotype data. The MI method differs in that it guesses, or

imputes, the genotypes between markers for every individual given the marker locations, marker scores and probability of recombination events between markers given the population type. In this study the imputations are done many times and the LOD score averaged for all imputations to provide LOD scores at and in between markers (Broman and Sen, 2009). For the MI method, scans were computed using 512, 256, 128 and 64 draws at 0, 0.5, 1.0, 2.0 and 4.0 cM step intervals. For HK and EM methods, the error probability rate was set to 0.0001 and the steps set to 1.0. The most robust method for the DHYB and SE1 populations was determined to be HK with step interval 1.0 based on visual inspection of the one-dimensional LOD profile plots. One-dimensional scans were completed to determine putative locations of additive QTL. Two-dimensional scans determined positions for additional additive and epistatic loci. Stepwise model selection was done using one and two-dimensional scan results for additive and epistatic loci as inputs. Penalties were assessed from 1,000 permutations to adjust LOD scores and R^2 estimates for additional model marker and/or interaction terms. Proportion of phenotypic variance explained by a detected QTL (R^2) and Bayes confidence intervals for QTL positions were also determined from *R/qtl*. *R/qtl* was also used to study trait sensitivity to treatment, which was done by QTL mapping using the difference of the LS means in the dry treatment and wet treatment for each trait.

Results – 2010 SE1

Trial Performance

Overall, the growing conditions for the trial were favorable. Insect infestations, including cabbage aphids (*Brevicoryne brassicae*) and false chinch bugs (*Nysius raphanus*) were attended to promptly with chemical sprays. Chemical seed treatment prevented damage from flea beetles (*Phyllotreta vittula*) and common Brassica diseases. There was no evidence of disease in any of the lines. Climatic details are shown in Appendix Table S1. Damage from house finches

(C*arpodacus mexicanus*) was observed during the 2010 field season, potentially because of trial location, which was close to hedges where finches perched. Trees and a water reservoir were also close to the study, which caused an increase in the number of birds at the study location.

Descriptive Statistics and Analysis of Variance

All traits were distributed approximately normally in both irrigated and rainfed treatments, indicating the quantitative genetic control of yield, yield components, and days to flowering (Figure S7). Many of the lines in each population outperformed the parents for all traits, suggesting transgressive segregation.

The parent and overall population means, standard errors, maximum and minimum values, as well as F and *p* values for the differences in the trait means, are listed in Table 2.1. All SE1 lines yielded higher in the wet treatment than the dry treatment (Figure 2.1). Yield components differed between parents in both treatments as well. Mean seed yield decreased 85% from irrigated (wet) to rainfed (dry) treatment. Number of seeds per silique (SS) decreased 13.76%, thousand seed weight (TSW) decreased 5%, and siliques per main inflorescence (SMI) decreased 47.32% from the wet to the dry treatment.

Average number of days to flowering (DTF) was significantly different between treatments in this study ($P<0.001$); the dry treatment in the study finished flowering sooner than the wet treatment. In addition, in both treatments there was a 46 day range from the line that first reached 50% flowering to the line that last reached 50% flowering between both treatments. This was likely due to the nature of the population, which resulted from a cross between a winter-habit cultivar and a spring-habit cultivar. Even though the population had been selected for spring habit in a greenhouse, there were apparently winter and spring alleles still segregating in this population, which caused a wide range of flowering times. Initial heritability estimates for

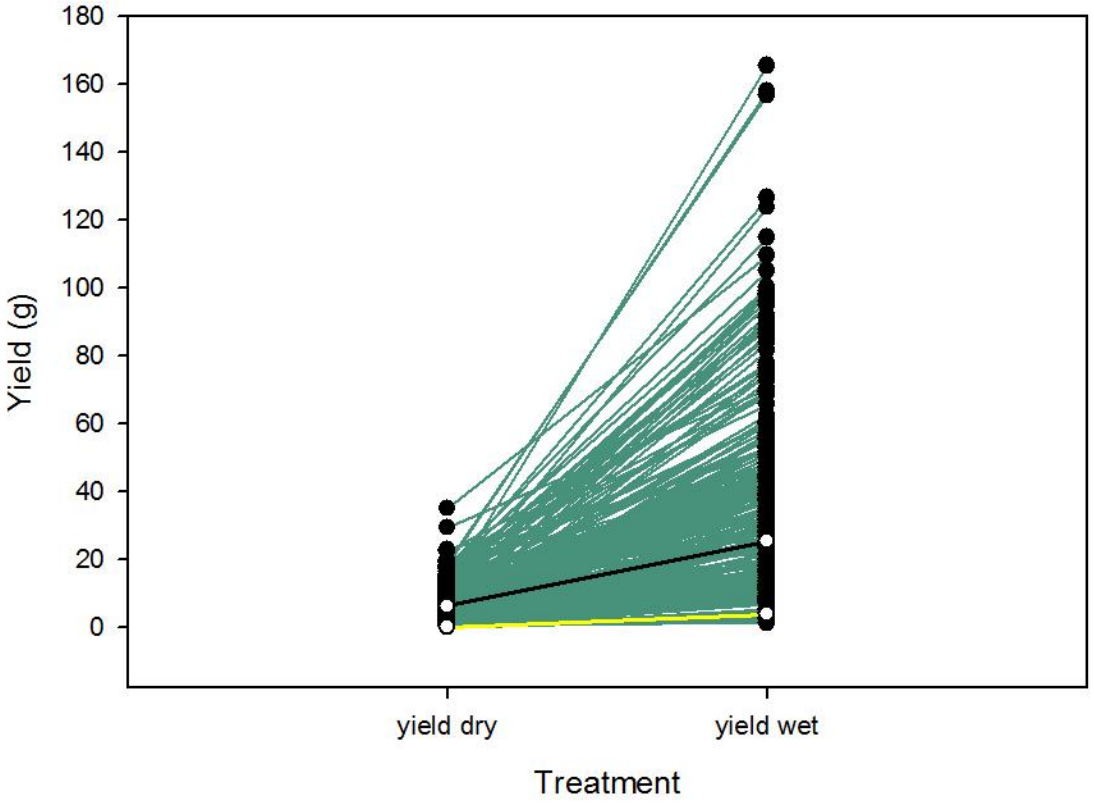


Figure 2.1. Reaction norm seed yield for 2010 SE1 field study (n=183). Green lines (filled circles) represent lines which had higher yield in the irrigated treatment, while the yellow and black lines (open circles) represent reaction norms for parent lines Wichita and IMC-106RR, respectively. All lines yielded higher in the irrigated treatment than the rainfed treatment.

Table 2.1. Descriptive statistics for the 2010 (SE1) study for seeds per silique (SS), days to flowering (DTF), siliques per main inflorescence (SMI), and thousand seed weight (TSW).

	Yield (g)		SS		DTF		SMI		TSW (g)	
	wet	**dry**	**wet**	**dry**	**wet**	**dry**	**wet**	**dry**	**wet**	**dry**
N	902	949	452	603	911	759	833	904	451	604
Mean	39.73	5.95	22.92	19.77	77.43	75.34	45.12	23.77	2.56	2.43
Std error	1.51	0.28	0.27	0.30	0.32	0.29	0.42	0.52	0.05	0.04
Max value	305	55	39.6	46.0	105	101	97	69	4.60	5.26
Min value	0	0	2.8	0.1	59	59	0	0	0.35	0.17
IMC-106	30.24	6.94	27.36	24.37	63.8	63.8	41.48	34.63	2.8	2.4
Wichita†	-	-	-	-	-	-	-	-	-	-
F value for wet & dry diff	601.12***		75.26***		24.21***		1293.92***		13.78***	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Parent 'Wichita' did not flower in any of the field replicates

yield-related traits in this population were high due to a lack of flowering, and consequently zero yield, in a portion of the population in both treatments. Because of this confounding effect, lines that did not flower (n=55) were discarded, resulting in 183 lines which were used in all analyses.

Broad sense heritability estimates (h^2) are summarized in Table 2.2 along with a summary of treatment, genotype and genotype by environment interaction effects. Heritability estimates ranged from 0.18 for TSW in the dry treatment to 0.95 for DTF in the wet treatment. Significant treatment, genotype and genotype by environment interaction effects (P<0.05) were detected for yield, SMI, and SS. There was not a significant treatment effect on TSW or DTF. Genotype by environment and genotype effects were highly significant (P<0.0001) for all traits.

Correlation analysis

Pearson's correlation coefficients are presented in Table 2.3. Significant phenotypic correlations between yield and all yield-related traits were observed in the dry treatment of the study (P<0.01). Significant correlation coefficients were also observed between seed yield and SS, TSW, and DTF in the wet treatment. The highest magnitude of correlation observed among yield and yield-related traits was -0.69 (P<0.0001) between yield and DTF in the dry treatment. Between treatments, DTF had a highly significant correlation (r=0.89, P<0.0001). Values in the two treatments were also correlated for SS (r=0.46, P<0.0001), SMI (r=0.29, P<0.05) and seed yield (r=0.45, P<0.0001). TSW correlated negatively with SS in both the dry treatment and the wet treatment (r=-0.59, P<0.001).

Linkage map construction and QTL analysis

The assembled SE1 genetic map revealed a total of 21 linkage groups. The resulting map had dense marker coverage, with an average interval between markers of 2.6 cM. A χ^2 goodness of fit test exposed 50 markers (10.1%) with segregation distortion at the p<0.001 level. These were

Table 2.2. Analysis of variance results and heritability estimates for the SE1 population (n=183) grown in Fort Collins, CO in 2010.

Trait	Genotype F value	Treatment F value	Genotype x Environment F value	h^2 wet treatment	h^2 dry treatment	h^2 overall
Yield†	3.68***	64.15***	2.04***	0.54	0.78	0.39
SMI	3.90***	181.56***	2.06***	0.58	0.89	0.21
SS	4.79***	13.57*	1.33**	0.70	0.77	0.72
TSW	2.45***	1.25	1.72***	0.69	0.18	0.23
DTF	31.40***	1.17	1.46***	0.95	0.94	0.96

† Yield was adjusted for stand in the dry treatment.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 2.3. Pearson correlation coefficients among days to flowering (DTF), seed yield, thousand seed weight (TSW), siliques per main raceme (SMI), seeds per silique (SS) in irrigated (wet) and rainfed (dry) treatments for the 2010 SE1 population (n=183).

	SMI dry	SS dry	TSW dry	DTF dry	Yield wet	SMI wet	SS wet	TSW wet	DTF wet
Yield dry	0.51**	0.58**	0.17*	-0.65**	0.45**	ns	0.44**	0.56**	-0.63**
SMI dry		0.42**	ns	-0.69**	0.39**	0.29*	ns	0.33**	-0.58**
SS dry			-0.16*	-0.65**	0.39**	ns	0.46**	0.41**	-0.64**
TSW dry				ns	ns	ns	0.25**	ns	ns
DTF dry					-0.60**	ns	-0.44**	0.89**	0.89**
Yield wet						ns	0.46**	0.54**	-0.68**
SMI wet							ns	ns	ns
SS wet								-0.59**	-0.66**
TSW wet									-0.69**

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

discarded, leaving 446 markers for QTL analysis. Of the 446 markers, 115 (25.8%) still revealed significant segregation distortion at $P<0.01$ but were included in the analysis.

Several additive and one epistatic QTL were detected in this study for DTF, yield and yield components, and are summarized in Table 2.4. Percent of explained phenotypic variation for single QTL effects varied among all traits from 4.28% for seed yield in the wet treatment to 38.63% for DTF in the dry treatment. Models of all loci, including epistatic and additive effects, revealed percentages of explained variation ranging from 17.71% for SS in the dry treatment to 68.21% for DTF in the wet treatment.

Significant epistasis was revealed for SMI in the wet treatment between a locus on linkage group (LG) 4 and a locus on LG 7. Both loci had additive effects on SMI as well. The source of the favorable allele at each locus differed, such that parent IMC-106 donated the favorable allele on LG 4 and parent Wichita donated the favorable allele on LG7. Several additive QTL were also detected for SMI in the wet treatment. A total of six additive loci (including the QTL on LGs 4 and 7) and the epistatic loci explained a total of 38.14% of the phenotypic variance in the study.

Two large effect QTL (each explaining over 25% phenotypic variance) on LGs 10 and 12 were detected in both treatments for DTF. These explained 28.01% and 38.63% of the trait variation in the dry treatment, respectively. The same two QTL were detected in the wet treatment, where they explained 31.24% and 33.52% of the trait variation, respectively. One additional QTL was detected on LG 3 for DTF in the wet treatment that was not detected in the dry treatment.

Under the full model, the additive QTL explained 55.11% and 68.21% of the trait variation in the dry and wet treatments, respectively. QTL detected for the remaining traits (seed yield, SS, and TSW) all co-located with the DTF QTL on LGs 3, 10 and 12 and explained trait variance percentages from 3.49% (LG 3 QTL detected for SS in the wet treatment) to 20.96% (LG 12 QTL detected for seed yield in the wet treatment).

Table 2.4. Summary of QTL detected for the SE1 population. Seeds per silique (SS), days to flowering (DTF), siliques per main inflorescence (SMI) and thousand seed weight (TSW) models for wet and dry treatments include all significant QTL detected for each trait as determined by stepwise model selection.

	Linkage Group†	Position (cM)	R^2 (%) QTL‡	F value§	R^2 (%) model¶	LOD model#	Additive effect††
Yield dry	10	51	11.26	22.02***	18.69	7.28	1.98 ± 0.51
	12	64.7	10.13	19.80***			1.92 ± 0.53
Yield wet	3	73.4	4.28	16.56***	42.125	27.07	6.96 ± 2.07
	10	70	19.34	74.85***			12.88 ±
	12	68	20.96	81.11***			13.76 ±
SS dry	10	51	7.77	16.91***	17.71	7.71	1.22 ± 0.38
	12	64.7	12.36	26.88***			1.70 ± 0.38
SS wet	3	20.9	3.49	10.28**	35.88	18.62	1.51 ± 0.41
	10	70.5	13.09	38.57***			2.03 ± 0.39
	12	64	18.76	55.29***			2.38 ± 0.38
DTF dry	10	70.5	28.01	124.8***	55.11	35.31	-3.24 ± 0.51
	12	69	38.63	172.1***			-4.17 ± 0.48
DTF wet	3	132	3.15	22.6***	68.21	57.98	-2.08 ± 0.63
	10	73	31.24	224.83***			-4.77 ± 0.51
	12	61.8	33.52	241.44***			-5.29 ± 0.48
TSW wet	10	70.5	19.39	57.81***	36.59	18.99	0.40 ± 0.07
	12	64	20.46	60.98***			0.42 ± 0.07
SMI dry	10	70.5	14.57	32.50***	20.20	8.87	2.74 ± 0.70
	12	69	12.24	27.31***			2.39 ± 0.73
SMI wet	1	51.3	4.55	15.87***	38.14	23.36	-2.21 ± 0.61
	2B	9	7.98	27.86***			2.62 ± 0.58
	7	8.3	10.81	18.86***			-1.48 ± 0.58
	10	4.8	7.13	24.91***			-2.04 ± 0.58
	15	37	6.57	22.93***			2.68 ± 0.59
	4	40	7.48	13.05***			1.32 ± 0.61
	4@40: 7@8.3		6.11	21.32***			-

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Notation for epistasis is LG1@pos1:LG2@pos2

‡ R^2 QTL (%) is the percent of phenotypic variance explained by the QTL in the full model.

§ F value for each term (QTL) in the full model.

¶ R^2 Model (%) is the percent of phenotypic variance explained by the full model.

LOD model is the logarithm of the odds value given to the full model.

†† Additive effect ± standard error for the parent 'IMC106' allele.

Results – 2011 DHYB

Trial Performance

Overall, the growing conditions for the 2011 trial were favorable. Chemical seed treatment prevented damage from flea beetles (*Phyllotreta vittula*) and common Brassica diseases. Insect infestations, including cabbage aphids (*Brevicoryne brassicae*) and false chinch bugs (*Nysius raphanus*) were attended to promptly with chemical sprays. There was no evidence of disease throughout the growing season. Alfalfa and thistle appeared as common weeds throughout the season and were controlled manually.

The 2011 growing season tended to be a rainier season and temperatures were cooler on average compared with 2010. Bird deterrents such as reflective tape, predator decoys, and a propane-fueled detonation cannon (Scare-Away M8 Multi-Bang Cannon, www.wildlifecontrolsupplies.com) acted as successful preventative measures to combat damage from birds and other non-insect pests. A hail storm on June 10, 2011 resulted in a minor amount of damage that had little apparent effect.

Field soil samples were taken in August 2011 and were used to measure gravimetric water content and water potential at the end of the field season. Water potential for the rainfed treatment was approximately -2.5 MPa, while the irrigated treatment was approximately -1.0 MPa (Figure S4), which suggests that the lines in the rainfed treatment were subjected to water stress.

Descriptive Statistics and Analysis of Variance

All traits were distributed approximately normally in both irrigated and rainfed treatments, suggesting the quantitative genetic control of yield, yield components, and days to flowering (Figure S8). Many of the lines in each population outperformed the parents for all traits, suggesting transgressive segregation.

The parent and overall population means, standard errors, maximum and minimum values as well as F and *p* values for the treatment to the dry treatment are presented in Table 2.5. Comparing the wet to the dry treatment, seed yield decreased 58%, number of seeds per silique (SS) decreased 2.4%, thousand seed weight (TSW) decreased 13.5%, and siliques per main inflorescence (SMI) decreased 9.2%.

Average number of days to flowering (DTF) was not significantly different between treatments in the 2011 DHYB study. Additionally, the 30 day range of flowering times (62 to 92) was improved from the 46 day flowering range of the 2010 SE1 population. This range decrease results from the DHYB population being a cross between two spring-habit parents.

Seed yield was higher in the wet treatment than the dry treatment for both parents as well as most of the lines (Figure 2.2). Four genotypes from the DHYB population (highlighted with red lines and open circles in Figure 2.2) had higher mean seed yield in the dry treatment, yet only Line 118 had significantly higher seed yield ($P<0.01$). Line 118 also had more siliques per main inflorescence and larger thousand seed weight, yet fewer seeds per silique in the dry treatment compared with the wet treatment.

Broad sense heritability estimates (h^2) are listed in Table 2.6 within treatments as well as overall. Treatment, genotype and genotype by treatment interaction effects are summarized in Table 2.6 as well. Heritability estimates ranged from 0.35 for SMI in the wet treatment to 0.90 for DTF (overall). Significant treatment, genotype and genotype by treatment effects ($P<0.0001$) were observed for seed yield and TSW. Genotype effects were significant ($P<0.0001$) for all traits, yet treatment was only significant for seed yield, SMI, TSW and STF. Genotype by treatment and effects were absent for SMI, SS and DTF.

Yield components differed significantly between parents and lines in both treatments as

Table 2.5. Descriptive statistics for the 2011 DHYB field study for seeds per silique (SS), days to flowering (DTF), siliques per main inflorescence (SMI), and thousand seed weight (TSW).

	Yield		SS		DTF		SMI		TSW	
	wet	**dry**	**wet**	**dry**	**wet**	**dry**	**wet**	**dry**	**wet**	**dry**
N	481	489	411	438	445	439	443	461	410	438
Mean	117.51	49.01	19.31	18.84	73.9	74.2	41.47	37.67	4.06	3.51
Std error	3.08	1.59	0.24	0.25	0.20	0.21	0.46	0.46	0.03	0.03
Max value	321	211	32	31	92	87	72.0	77.5	6.42	5.43
Min value	1	0.5	4	0	63	62	16.5	10.5	0.50	0.49
YN01-429	162	18	19.5	1.6	73.3	72.7	32	36	5	3
DH12075	141	69	16.6	18.2	72.3	71.3	54	35	5	4
F value for wet & dry diff	506.36***		23.61***		1.94		22.98***		250.30***	

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Table 2.6. Analysis of variance results and heritability estimates for the 2011 DHYB population

Trait	Genotype F value	Treatment F value	Genotype x Environment F value	h^2 wet treatment	h^2 dry treatment	h^2 overall
Yield†	2.73***	64.86***	1.26*	0.45	0.61	0.41
SMI	2.21***	5.99*	0.78	0.35	0.40	0.65
SS	6.50***	0.27	0.93	0.65	0.65	0.81
TSW	4.85***	90.76***	1.37**	0.70	0.50	0.65
DTF	5.68***	4.97***	0.90	0.80	0.84	0.90

† Yield was adjusted for stand in the dry treatment.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

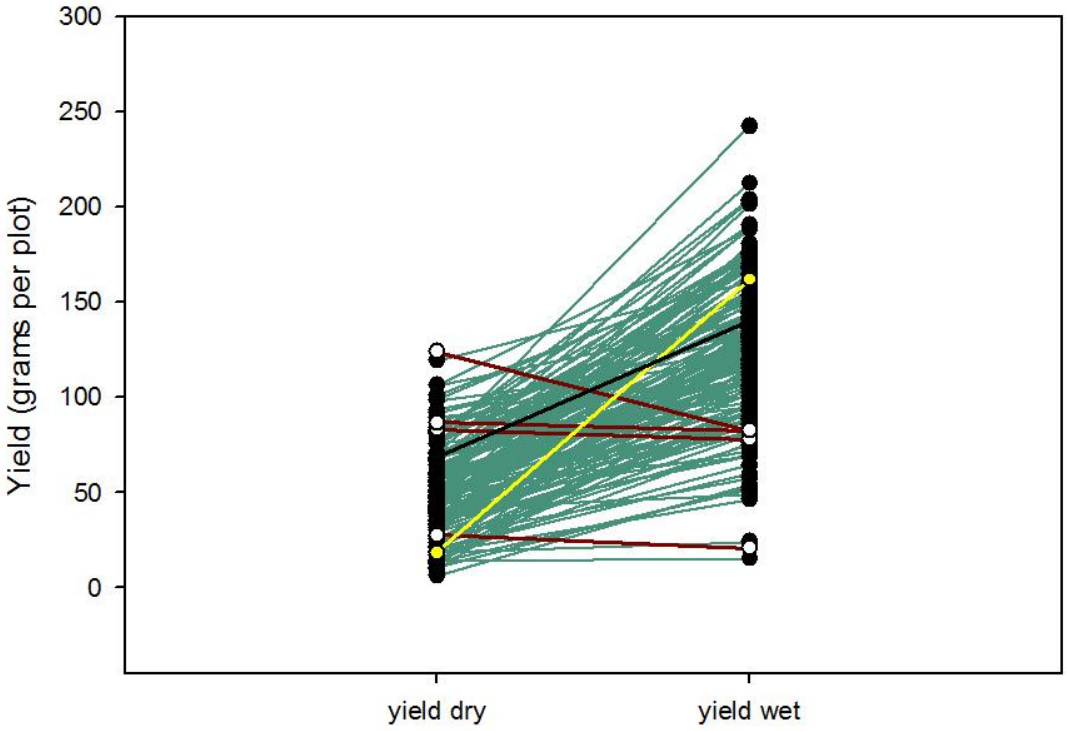


Figure 2.2. Reaction norm for seed yield2011 DHYB field study. Filled circles (green lines) represent genotypes which had higher seed yield in the irrigated (wet) treatment. Open circles (red lines) represent genotypes which had lower seed yield in the irrigated (wet) treatment. Yellow and black lines represent reaction norm for parent lines YN01-429 and DH12075, respectively.

well (*P*<0.0001; Table 2.6). Parent YN01-429 had fewer SMI in the wet treatment compared with the dry, whereas parent DH12075 had almost 20 more SMI on average when comparing wet to dry treatments. In a similar manner, DH12075 had fewer SS in the wet treatment compared with the dry treatment, yet parent YN01-429 had fewer SS in the dry treatment. While TSW did not differ significantly between the parents in the study, there was a significant difference between treatments for the lines (*P*<0.0001). DTF did not differ significantly between treatments (*P*=0.17).

Correlation analysis

Pearson's correlation coefficients are presented in Table 2.7. Significant correlation coefficients between yield and SMI, SS and DTF were observed in the dry treatment of the study (*P*<0.01). Significant correlations were also observed between yield and SS as well as yield and DTF in the wet treatment (*P*<0.001). The highest correlation coefficient observed among yield and yield-related traits was 0.52 (*P*<0.001) between yield and SS in the wet treatment. When analyzing the same trait in the two treatments, DTF had a highly significant correlation (*r*=0.77, *P*<0.001), as did SS (*r*=0.76, *P*<0.001). SMI (*r*=0.50, *P*<0.001), seed yield (*r*=0.45, *P*<0.001), and TSW (*r*=0.44, *P*<0.001) also had significant but lower magnitude correlations. DTF had a significant negative correlation with seed yield and SS in both treatments, yet had a significant positive correlation with SMI in the wet treatment (*r*=0.20, *P*<0.05). TSW correlated negatively with SMI in the dry treatment (*r*=-0.19, *P*<0.05) and SS in the wet treatment (*r*=-0.20, *P*<0.05), suggesting resource competition during the silique development phase.

Linkage map construction and QTL analysis

The assembled DHYB genetic map covered 21 linkage groups with an average interval between markers of 4 cM. A χ^2goodness of fit test for segregation distortion revealed 60 markers (18.6%) distorted at the *p*<0.001 level; these were discarded before linkage map

Table 2.7. Pearson correlation coefficients among days to flowering (DTF), seed yield, thousand seed weight (TSW), siliques per main raceme (SMI), seeds per silique (SS) in irrigated (wet) and rainfed (dry) treatments for the 2011 DHYB population (n=144)

	SMI dry	SS dry	TSW dry	DTF dry	Yield wet	SMI wet	SS wet	TSW wet	DTF wet
Yield dry	0.15*	0.45**	ns	-0.33**	0.45**	ns	0.38*	ns	-0.30**
SMI dry		ns	-0.19*	ns	ns	0.50**	ns	ns	ns
SS dry			ns	-0.24**	0.45**	ns	0.76**	-0.23*	-0.26**
TSW dry				ns	-0.16*	-0.20*	-0.20*	0.44**	ns
DTF dry					-0.28**	0.26*	-0.22**	ns	0.77**
Yield wet						ns	0.52**	ns	-0.46**
SMI wet							-0.15*	ns	0.20*
SS wet								-0.20*	-0.35**
TSW wet									ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

construction. A total of 261 markers were used to create the linkage map and were used in the subsequent QTL analysis.

Several additive and epistatic QTL were detected for DTF, yield and yield components, and are summarized in Table 2.8. Percent of explained phenotypic variation for single QTL effects varied among all traits from 2.32% to 16.21%. Models of all loci, including epistatic and additive effects, revealed percentages of explained variation ranging from 15.08% SMI in the dry treatment to 38.48% for DTF in the wet treatment.

Significant epistasis was revealed between a locus at 90 cM of LG 14 and a locus at 55 cM of LG17 for seed yield in the dry treatment (Figure 4A), and between a locus at 82 cM of LG 7 and locus at 83 cM of LG 16 for seed yield in the wet treatment (Figure 4B). All four loci also showed additive effects for seed yield. One purely additive QTL on LG 12T was discovered for seed yield in both wet and dry treatments, suggesting stability of the QTL across environments, although the locus explained about twice as much of the variation in the wet treatment. Explained phenotypic variation for the model was smaller in the dry treatment (22.03%) than the wet treatment (30.52%).

All four QTL discovered for SS were at similar positions for both wet and dry treatments, and the percentage of explained phenotypic variation for the models differed only slightly between treatments. The source of the favorable allele and nearest marker remained constant for all four QTL in both treatments. Approximately 30% of the variation was explained by the four additive loci in both treatment models. The sum of additive effects for the favorable QTL alleles at these loci totaled approximately five seeds per silique.

One QTL on LG 2 detected in both treatments for DTF explained 16.2% and 11.8% of the trait variation in wet and dry treatments, respectively. Another additive DTF QTL co-located

Table 2.8. Summary of QTL detected from the 2011 DHYB population. Seeds per silique (SS), days to flowering (DTF), siliques per main inflorescence (SMI), thousand seed weight (TSW), and seed coat color models for wet and dry treatments include al significant QTL detected for each trait as determined by stepwise model selection

	Linkage Group[†]	Position (cM)	Nearest marker(s)	R^2 (%) QTL[‡]	F value[§]	R^2 (%) model[¶]	LOD model[#]	Additive Effect of Y allele[††]
Yield dry	12T	7	R12095Ib	5.65	10.00**	22.03	7.78	-7.48 ± 2.03
	14	90	sN0539x	8.62	7.63***			6.46 ± 2.17
	17	55	sN11863a	7.54	6.68**			No add. effect
	14@90.0 : 17@55.0		sN0539x & sN11863a	7.49	13.26***			-
Yield wet	7	82	sN12480a	11.72	11.64***	30.52	11.39	12.64 ± 3.37
	16	83	sN12056c	11.41	11.33***			5.84 ± 3.85
	12T	0	R12095Ib	9.32	18.50***			-14.69 ± 3.34
	7@82.0: 16@83.0		sR12173b & sN12056c	11.31	22.46***			-
SS dry	7	82	sN12480a	5.34	10.65**	29.93	11.12	12.16 ± 3.43
	10	22	sR12317	7.71	15.29***			14.18 ± 3.43
	12T	17	R12095Ib	6.46	12.81***			-11.19 ± 3.58
	19	17	sR9251Jb	5.43	10.77**			13.00 ± 3.35
SS wet	7	77	sNRA59	4.35	8.37**	28.38	10.37	11.65 ± 3.28
	10	28	sS2066a	5.57	10.72**			12.92 ± 3.38
	12T	13	R12095Ib	5.36	10.32**			-10.82 ± 3.73
	19	16	sR9251Jb	7.48	14.41***			14.25 ± 3.30
DTF dry	2	6.4	sR9864	16.21	33.12***	32.62	12.34	1.32 ± 0.28
	12T	25	sNRE85x	10.14	20.76***			1.07 ± 0.28
	11	0	sORC20	3.81	7.80**			-0.54 ± 0.30
	2	116	sR9548	2.48	5.07*			0.30 ± 0.28
	7	1	sR4047	2.32	4.76*			0.74 ± 0.28
DTF wet	2	6	sR9864	11.84	26.70***	38.48	15.19	1.21 ± 0.29
	12T	33	sR13062x	8.78	19.83***			1.04 ± 0.27
	14	116	sN3685Ra	7.25	16.38***			-1.10 ± 0.27
	18	68	sN12646	7.07	15.99***			1.04 ± 0.28

	Linkage Group†	Position (cM)	Nearest marker(s)	R^2 (%) QTL‡	F value§	R^2 (%) model¶	LOD model#	Additive Effect of Y allele††
TSW dry	1	1	sNRA51	12.62	13.55***	36.18	14.04	-0.137 ± 0.03
	7	90	sN0818Fa	2.79	6.00*			-0.118 ± 0.04
	11	82	sN2440a	4.33	9.29**			0.117 ± 0.03
	11	2	sORC20	8.71	9.35***			0.112 ± 0.04
	19	47	sORB37a	4.70	10.09**			-0.115 ± 0.04
	1@1.0: 11@2.0		sNRA51 & sORC20	3.50	7.51**			-
TSW wet	9	106.6	sN10704a	9.60	17.80***	25.59	9.18	0.217 ± 0.04
	14	86	sN10326a	3.82	7.07**			-0.156 ± 0.05
	19	9	sS2277x	4.65	8.63**			-0.145 ± 0.04
	13	84	sN12947a	3.12	5.79*			0.092 ± 0.04
SMI dry	11	6	sN0248Ia	10.15	16.85***	15.08	5.11	-1.97 ± 0.52
	16	94	sN0818Fd	5.76	9.57**			1.50 ± 0.58
SMI wet	2	21	sR12775	4.14	7.80**	26.68	9.64	1.80 ± 0.56
	7	29	sN11682	5.30	9.98**			1.59 ± 0.51
	11	3	sN0248Ia	9.77	18.39***			-2.05 ± 0.54
	17	69.2	N12058IIIa	4.12	7.75**			-1.75 ± 0.51
Color	9	106	sN10704a	40.02	32.11***	40.02	16.01	-

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Notation for epistasis is LG1@pos1:LG2@pos2

‡ R^2 QTL (%) is the percent of phenotypic variance explained by the QTL in the full model.

§ F value for each term (QTL) in the full model.

¶ R^2 Model (%) is the percent of phenotypic variance explained by the full model.

LOD model is the logarithm of the odds value given to the full model.

†† Additive effect ± standard error for the parent 'YN01-429' allele.

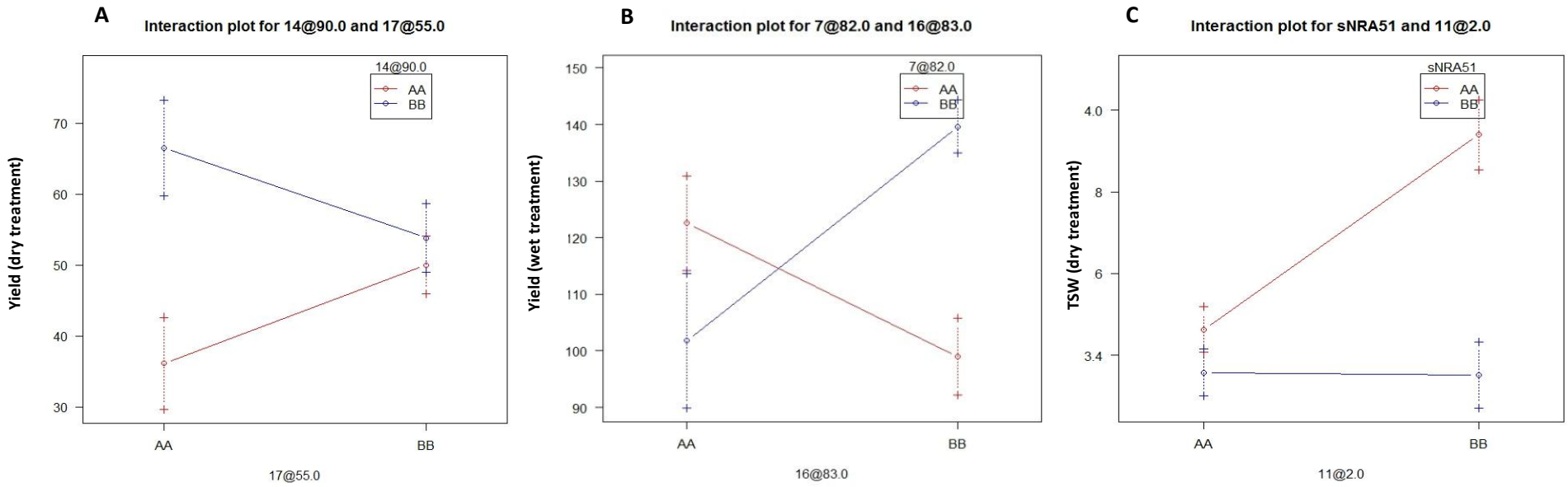
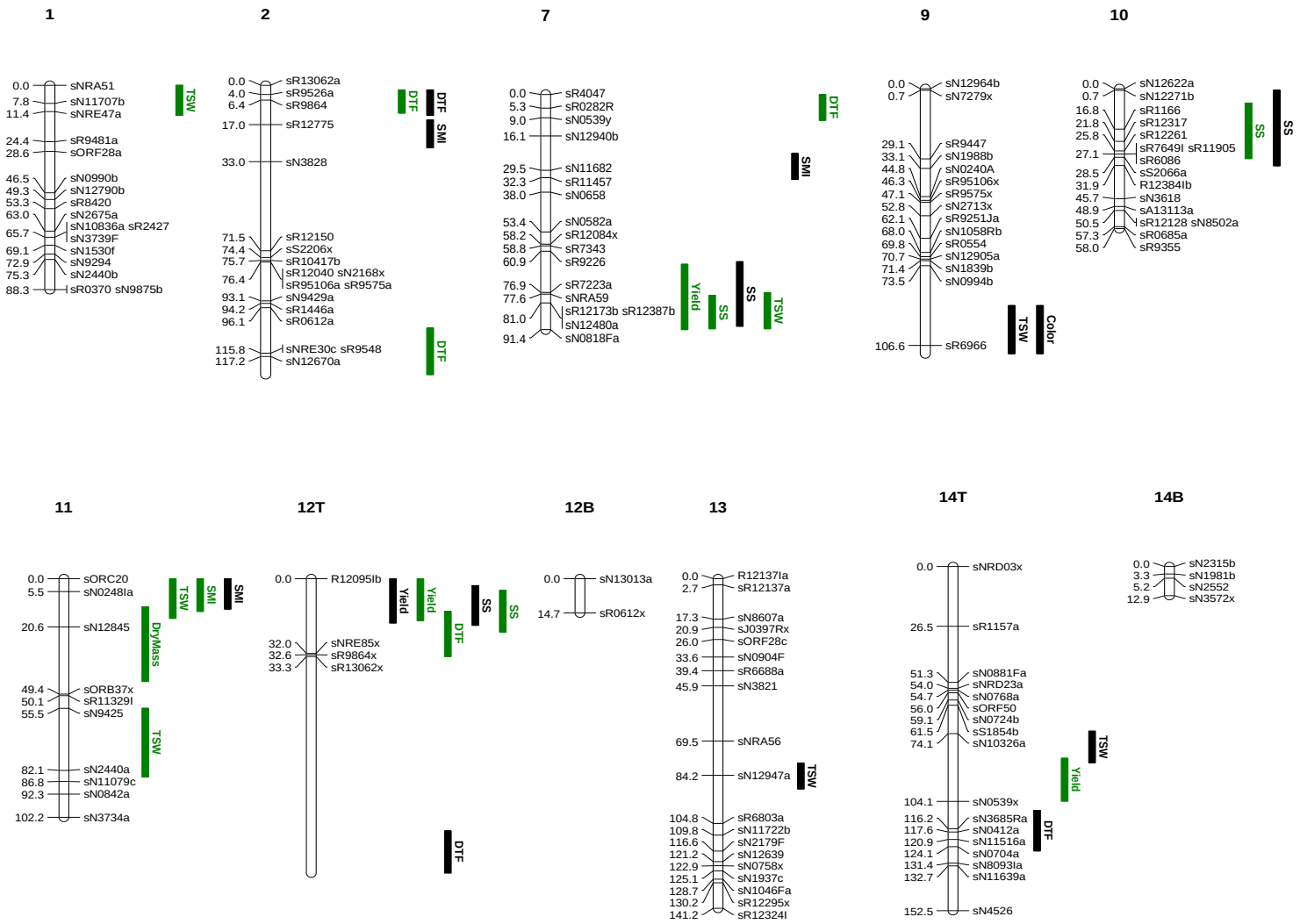


Figure 2.3. Effect plots demonstrating epistasis in the 2011 DHYB population (A) between a locus at 90 cM of linkage group 14 and a locus at 55 cM of linkage group 17 for seed yield in the dry treatment, (B) between a locus at 82 cM of linkage group 7 and locus at 83 cM of linkage group 16 for seed yield in the wet treatment, and (C) between marker sNRA51 and a locus at 2 cM of linkage group 11 for TSW in the dry treatment.


1
0.0 sNRA51
7.8 sN11707b
11.4 sNRE47a
24.4 sR9481a
28.6 sORF28a
46.5 sN0990b
49.3 sN12790b
53.3 sR8420
63.0 sN2675a
65.7 sN10836a sR2427
sN3739F
69.1 sN1530f
72.9 sN9294
75.3 sN2440b
88.3 sR0370 sN9875b
TSW
2
0.0 sR13062a
4.0 sR9526a
6.4 sR9864
17.0 sR12775
33.0 sN3828
71.5 sR12150
74.4 sS2206x
75.7 sR10417b
sR12040 sN2168x
76.4 sR95106a sR9575a
93.1 sN9429a
94.2 sR1446a
96.1 sR0612a
115.8 sNRE30c sR9548
117.2 sN12670a
DTF
DTF
SMI
DTF
7
0.0 sR4047
5.3 sR0282R
9.0 sN0539y
16.1 sN12940b
29.5 sN11682
32.3 sR11457
38.0 sN0658
53.4 sN0582a
58.2 sR12084x
58.8 sR7343
60.9 sR9226
76.9 sR7223a
77.6 sNRA59
sR12173b sR12387b
81.0 sN12480a
91.4 sN0818Fa
DTF
SMI
Yield
SS
SS
TSW
9
0.0 sN12964b
0.7 sN7279x
29.1 sR9447
33.1 sN1988b
44.8 sN0240A
46.3 sR95106x
47.1 sR9575x
52.8 sN2713x
62.1 sR9251Ja
68.0 sN1058Rb
69.8 sR0554
70.7 sN12905a
71.4 sN1839b
73.5 sN0994b
106.6 sR6966
TSW
Color
10
0.0 sN12622a
0.7 sN12271b
16.8 sR1166
21.8 sR12317
25.8 sR12261
27.1 sR7649I sR11905
sR6086
28.5 sS2066a
31.9 R12384Ib
45.7 sN3618
48.9 sA13113a
50.5 sR12128 sN8502a
57.3 sR0685a
58.0 sR9355
SS
SS
11
0.0 sORC20
5.5 sN0248Ia
20.6 sN12845
49.4 sORB37x
50.1 sR11329I
55.5 sN9425
82.1 sN2440a
86.8 sN11079c
92.3 sN0842a
102.2 sN3734a
TSW
SMI
SMI
DryMass
TSW
12T
0.0 R12095Ib
32.0 sNRE85x
32.6 sR9864x
33.3 sR13062x
Yield
Yield
SS
SS
DTF
DTF
12B
0.0 sN13013a
14.7 sR0612x
13
0.0 R12137Ia
2.7 sR12137a
17.3 sN8607a
20.9 sJ0397Rx
26.0 sORF28c
33.6 sN0904F
39.4 sR6688a
45.9 sN3821
69.5 sNRA56
84.2 sN12947a
104.8 sR6803a
109.8 sN11722b
116.6 sN2179F
121.2 sN12639
122.9 sN0758x
125.1 sN1937c
128.7 sN1046Fa
130.2 sR12295x
141.2 sR12324I
TSW
14T
0.0 sNRD03x
26.5 sR1157a
51.3 sN0881Fa
54.0 sNRD23a
54.7 sN0768a
56.0 sORF50
59.1 sN0724b
61.5 sS1854b
74.1 sN10326a
104.1 sN0539x
116.2 sN3685Ra
117.6 sN0412a
120.9 sN11516a
124.1 sN0704a
131.4 sN8093Ia
132.7 sN11639a
152.5 sN4526
TSW
Yield
DTF
14B
0.0 sN2315b
3.3 sN1981b
5.2 sN2552
12.9 sN3572x

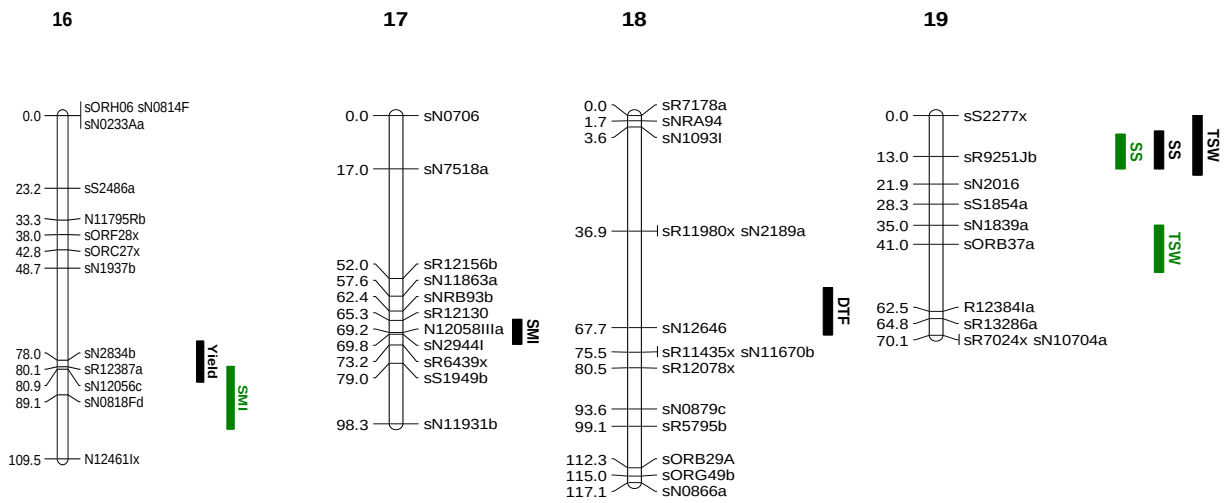


Figure 4. Schematic overview of QTL detected in the 2011 DHYB population. Green and black bars indicate QTL detected for the wet and dry treatments, respectively. Length of bars indicate QTL location +/- the Bayes confidence interval.

in wet and dry treatments and explained approximately 10% of the phenotypic variation for both. Three additional minor (explaining less than 10% phenotypic variance) additive QTL were discovered in the dry treatment, and two additional small effect QTL were discovered in the wet treatment. Under the full model, the additive QTL explained 32.6% and 38.5% of the trait variation in the dry and wet treatments, respectively.

Several QTL were detected for TSW in each treatment, however none co-located between treatments. One epistatic interaction was uncovered between marker sNRA51 and a locus at 2 cM of LG 11 for TSW in the dry treatment (Figure 2.4C), as well as an additional three loci of purely additive effect, which together explained 36.18% of the variation in this trait. A total of four additive loci were detected in the wet treatment for TSW which explained 25.59% of the trait variation. A major seed coat color QTL on LG 9 at position 106 that explained approximately 40% of the phenotypic variance co-located with a TSW QTL that was detected in the wet treatment.

SMI measured in the dry treatment revealed the fewest number of QTL of all yield-related traits. Two QTL explained 15.08% of the phenotypic trait variation. Four purely additive QTL were detected in the wet treatment, explaining 26.68% of the trait variation. One QTL on LG 11 explained about 10% of the phenotypic variance in both treatments.

In an area covering 33 cM on LG 12T, QTL for three traits (DTF, SS, and seed yield) co-located in both treatments. Additionally, the source of the favorable allele is from parent DH12075 for all six trait-treatment combinations. Other areas of co-location between traits include one QTL on LG 7 that co-located between treatments for SS and was also detected in the wet treatment for seed yield.

Discussion

In this study, two DH genetic mapping populations of *B. napus* were used to detect QTL and study drought sensitivity effects of yield and yield-related traits in Fort Collins, Colorado. Genetic linkage maps were constructed using EST-derived SNP and SSR markers. In each population, at least 19 genetic linkage groups were identified and labeled according to a reference map of *B. napus*. Each population was grown in a different season (SE1 in 2010 and DHYB in 2011) under both rainfed (dry) and irrigated (wet) treatments to better understand the sensitivity to drought of yield and yield-related traits and to detect QTL.

Correlation, ANOVA and heritability analyses – 2010 SE1

Correlation analyses revealed a number of significant relationships between yield and yield components in the irrigated treatment, most of which correspond to other studies of yield components in rapeseed (e.g. Fan et al., 2010; Zhang et al., 2011, Li et al., 2007). Heritability estimates from the irrigated treatment are also consistent with prior studies. However, few studies have reported correlation and heritability analyses of yield and yield-related traits of *B. napus* under a water stress treatment, so a number of new relationships were revealed in this study.

The correlation coefficient of seed yield between treatments (r=0.45, P<0.01) was significant, which suggests a strong genetic influence on this trait. However, this estimate is still somewhat low, which indicates that there is a genotype by treatment influence on this trait or error in the way seed yield was measured. Analysis of variance results for genotype by treatment effect on seed yield support this conclusion (P<0.0001). Broad sense heritability estimates for seed yield in the irrigated treatment are similar to those reported in other studies (e.g. Shi et al., 2009; Chen et al., 2011). However, the h^2 estimates were higher in the dry treatment than the wet treatment (0.45 and 0.61, respectively). This could be a reflection of the

influence of bird damage on yields, which appeared to be more severe in the wet treatment based on observation.

TSW analyses provided the most variable results of the yield component data in this study. A low correlation coefficient (r=0.17, P<0.05) was found between TSW and seed yield in the dry treatment of the study. Additionally, a non-significant correlation was observed between treatments for TSW. This adds further evidence to the observation that there is high amount of non-genetic variability inherent in this trait or perhaps variability that is difficult to account for using our methods, especially in the rainfed treatment. However, TSW and seed yield were found to correlate strongly (r=0.54, P<.0001) in the wet treatment, which corresponds with other studies on seed weight (Fan et al., 2010; Li et al., 2007). TSW also correlated negatively with SS in both treatments, suggesting competition for assimilates during the seed and/or silique development stage. This negative correlation has been previously reported in other studies (e.g. Fan et al., 2010; Zhang et al., 2011).

TSW has previously been reported by several researchers to have a heritability ranging from 0.49 to 0.85 under well-watered conditions (Zhang et al., 2011; Fan et al., 2010; Shi et al., 2009; Udall et al., 2006), which corresponds to the data from the wet treatment in this study (h^2=0.69). In the dry treatment, however, the heritability estimate dropped to 0.18. The low heritability and lack of correlation of TSW with seed yield in the rainfed treatment suggests that breeding for TSW in order to increase seed yield in rainfed conditions could produce variable results. However, TSW has previously been reported to be a reliable component of yield under irrigated conditions, and in fact many QTL have been published for this trait (e.g. Fan et al., 2010; Shi et al., 2009) that have explained high proportions of phenotypic variance. Therefore, more studies need to be done to confirm the relationship between TSW and seed yield under water stress.

SMI was significantly correlated with seed yield only in the dry treatment of the study (r=0.51, P<0.001). Conversely, heritability estimates for SMI were high in both treatments (h^2=0.89 [dry]; h^2=0.58 [wet]). The results are consistent with previous studies, most of which reported high heritability estimates and high correlation with seed yield (Li et al., 2007; Chen et al., 2007; Shi et al., 2009).

Of the yield components studied, seeds per silique (SS) showed the most stability between treatments. Broad sense heritability estimates were similarly high in both wet (h^2=0.70) and dry (h^2=0.77) treatments. Correlation coefficients between SS and seed yield were also moderatley high in both treatments (r=0.58, P<0.001 [dry]; r=0.46, P<0.001 [wet]). Furthermore, the correlation coefficient between SS in wet and dry treatments was relatively high (r=0.46, P<0.001), which suggests a large genetic effect on this trait. Genotype effects, as well as genotype by treatment interaction effects, were both significant for SS (P<.0001). This data suggests that selection for a high number of SS could be valuable to increase seed yield in irrigated and non-irrigated environments. Additionally, SS was the least time-consuming, and in general was the simplest phenotype to measure of all the yield components that were studied, which suggests that this trait may be a more appropriate phenotype to select for in a breeding program.

DTF appears to have the highest amount of genetic influence based on the high correlation of DTF between treatments (r=0.89, P<0.001), the high heritability estimates computed in both treatments (0.95 in the wet treatment and 0.94 in the dry treatment), and the high percent of phenotypic variation (55.11 % in the dry treatment and 68.21% in the wet treatment) explained by the QTL model. Additionally, a strong negative correlation between DTF and seed yield was observed in both treatments (r=-0.68, P<0.001 [wet]; r=-0.65, P<0.001 [dry]. The regressions of days to flowering on seed yield in the 2010 SE1 study (Figures S5 and S6) display the strong

influence of DTF on yield in this population of *B. napus*. These data indicate that in Colorado and potentially other semi-arid climates, canola should be bred for early-season maturity in order to maximize yield regardless of irrigation status.

QTL Analysis – 2010 SE1

Three QTL for DTF were detected on LGs 3, 10 and 12. Chen et al. (2011) reported a DTF QTL on LG 3 and Shi et al. (2009) reported a maturity time QTL on LG 3 which could co-locate with the DTF QTL on LG 3 observed in this study. Würschum et al. (2012) described a DTF QTL on LG 10, and Chen et al. (2011) recently reported a QTL that co-located for SS and TSW, any of which could be in similar, or the same, locations compared with this study. The QTLs on LGs 10 and 12 were detected in both treatments at similar positions, which suggests that these are stable. Furthermore, a QTL sensitivity analysis did not detect any significant QTL, which is further evidence for the stability of the QTL on LGs 10 and 12. These loci therefore have potential to benefit a plant breeder in order to improve seed yield in both irrigated and rainfed conditions.

The QTLs on LGs 10 and 12 that were detected in this study for DTF also co-located with all other yield components in most treatments. This could be evidence of the strong effect of DTF on seed yield and yield components, but could also be suggestive of tight linkage with other QTL or alternatively, a pleiotropic effect. In the event that tight linkage explains the co-location of QTL, addition of more markers to the area could shed some light on the genetic mechanisms underlying these traits, although in this population, the source of the advantageous allele remained constant for each trait, so selection for alternative alleles at tightly linked markers would be unnecessary. Other QTL that co-located include an area on LG 7 which was detected in SS and seed yield in both treatments. This QTL might also be useful in marker-assisted selection due to the high heritability estimates described for SS, along with the stability of this QTL, which was also confirmed to be stable by lack of QTL detected from a QTL sensitivity analysis.

Correlation, ANOVA and heritability analyses – 2011 DHYB

Correlation and ANOVA analyses were mostly consistent with the SE1 results as well as with several other studies (e.g. Fan et al., 2010; Zhang et al., 2011; Li et al., 2007). Effects of genotype were found to be significant (P<0.001) for yield and all yield-related traits, and treatment effects were also significant (P<0.05) for all traits excluding SS. Genotype by treatment effects were significant only for TSW (P<0.05). The significant correlation coefficient of seed yield that was observed between treatments (r=0.45, P<0.001) suggests a strong genetic influence on this trait. However, there is room in this estimate for genotype by treatment influence and random error as well, although analysis of variance results for genotype by treatment effects on seed yield were not significant (P=0.68).

Significant Pearson correlation coefficients between seed yield and yield-related traits were observed in this study (P<0.05). Specifically, SS had highly significant correlations with seed yield in both treatments (r=0.45, P<0.001 [dry]; r=0.52, P<0.001 [wet]). While other correlations of yield components with seed yield were observed to be significant (P<0.05), SS was the highest in both treatments. SS had a high correlation coefficient between treatments as well (r=0.76, P<0.001), demonstrating the highly significant influence of genetics on this trait. Other yield components had significant correlations between treatments (P<0.001), including a high estimate for DTF (r=0.77, P<0.001). DTF was negatively correlated with seed yield and SS in both treatments, which is consistent with the previous study and suggest that early maturity should be a consideration when breeding for seed yield in Colorado.

The SS data from this study suggest that this trait may be a useful candidate for selection in a breeding program. High heritability estimates in both treatments (h^2=0.65 in wet and dry) were observed for SS, which support the correlation results reported above. Additionally, SS was in general the simplest phenotype to measure of all the yield components, which substantiates

the evidence that this trait may be a suitable phenotype to select for in a canola breeding program in Colorado or other semi-arid climates.

Broad sense heritability estimates for seed yield in the irrigated treatment (h^2=0.45) are similar to those reported by other studies (e.g. Shi et al., 2009; Chen, 2011). However, the h^2 estimates were higher in the dry treatment than the wet treatment for seed yield (h^2=0.61), as well as for SMI (h^2=0.40 [dry]; h^2=0.35 [wet]), and DTF (h^2=0.84 [dry]; h^2=0.80 [wet]). This could be a reflection of the influence of bird damage on yields, which appeared to be more damaging in the wet treatment of the study. There could have also been an effect of variability in the linear-move irrigation system in the wet treatment, which at times might have at times have changed speed, and therefore irrigation amounts, over the study.

TSW and SMI analyses provided variable results in this study. Non-significant correlation coefficients were found between TSW and seed yield in both treatments. TSW correlated negatively with SS in the irrigated treatment (r=-0.20, P<0.05) and correlated negatively with SMI in the dry treatment (r=-0.19, P<0.05), but otherwise showed no effect on yield components. A similar pattern was observed for SMI, which correlated negatively with SS in the wet treatment (r=-0.15, P<0.05). The negative correlations reported between yield components correspond to results from other *B. napus* studies (e.g. Fan et al., 2010; Zhang et al., 2011), and may indicate competition for energy and resources during silique and/or seed development. This suggests that breeding for TSW or SMI in order to increase seed yield in rainfed conditions could produce variable results, and perhaps more studies need to be done to confirm the relationship between seed yield and these yield components under water stress.

DTF appears to have the highest amount of genetic influence based on the high correlation of DTF between environments reported above, the high heritability estimates computed in both treatments (0.95 [wet] and 0.94 [dry]), and the high percent of phenotypic variation (32.62% in

the dry treatment and 38.48% in the wet treatment) explained by the QTL model. Moreover, a significant negative correlation between DTF and seed yield was observed in both treatments (r=-0.33, P<0.001 [dry]; r=-0.46, P<0.001 [wet]). These data advocate that in Colorado, canola should be bred for early maturity in order to maximize yield regardless of irrigation status. Moreover, these results suggest that there would be a strong response to selection if early maturity was a goal undertaken in a breeding program.

QTL Analysis – 2011 DHYB

Several novel additive and epistatic QTL were detected in this study. Two novel epistatic interactions were observed for seed yield, one in the dry treatment and one in the wet treatment (Figure 4). An additional epistatic interaction was detected for TSW in the dry treatment. None of these epistatic interactions have been previously reported, although strictly additive QTL have appeared in some reports for seed yield and yield components (e.g. Li et al., 2007; Chen et al., 2011; Fan et al., 2010) that may co-locate with the QTL for seed yield and TSW from these epistatic QTL. These results are consistent with this study since each of the four loci involved in seed yield epistasis, and one of the two loci involved in TSW epistasis, were shown to have additive effects as well.

QTL studies of yield and yield-related traits in *B. napus* from Li et al. (2007), Zhang et al. (2011), Fan et al. (2010), Chen et al. (2011), Würschum et al. (2012) and Shi et al. (2009) support QTL that were detected in this study, although it is difficult to make exact comparisons because there is no integrated genetic linkage map publicly available for *B. napus*. Additionally, different markers were used in each study, which makes comparisons even more unreliable. However, proximity indicated from similar cM positions may be evidence for similar locations of these QTL.

Würschum (2012) and Zhang (2011) both reported a large effect QTL for DTF on LG 2 which was detected in this study in both treatments. The QTL explained 16.21% of trait variation in the dry treatment and 11.84% of trait variation in the wet treatment, which corresponds to estimates from each study referenced above.

A chromosomal region spanning 30 cM on LG 12T was detected for yield, SS and DTF in both treatments, and explained 5.65% (yield in dry treatment) to 10.14 percent (DTF in dry treatment) of the trait variation. Chen et al. (2011), Zhang et al. (2011), and Li et al. (2007) have all reported QTL for either seed yield, SS or TSW in the vicinity of this region. In this study, the QTL for these traits may have similar locations, and therefore genetic control, with the other mapping populations.

Würschum et al. (2012) reported several QTL from a DH study which included nine crosses from elite breeding lines (n=391) that were found to co-locate with many of the QTL detected in this population. On LG 17, a QTL detected in this study for seed yield in the dry treatment appeared also for seed yield in the Würschum study that could be at similar positions. Analysis of seed yield in the wet treatment uncovered a QTL close to a seed yield QTL also reported by Würschum, and a major effect QTL for FT also co-located with their study on LG 2.

Of the QTL detected in all trait-treatment combinations, there are several novel QTL, including a locus on LG 17 that explained 7.54% of trait variation for seed yield in the dry treatment and a locus on LG 16 that explained 11.41% of seed yield in the wet treatment. No other QTL have been published for seed yield or yield components that co-locate with these QTL. An additional potentially novel QTL for SS was detected on LG 7 in both treatments. However, a QTL for seed yield has been reported in a similar location by Würschum et al. (2012) which could be evidence of co-location. Two QTL for DTF in the wet treatment on LGs 14 and 18 appear to be novel, as well as two QTL for SMI in the dry treatment.

An area on LG 9 near cM position 106 was detected for both TSW in the wet treatment and seed coat color. The coat color QTL was of major effect, accounting for 40.1% of the phenotypic variance. Yellow seed coat color has been reported to be associated with thinner seed coat and higher oil content in *B. napus* (Jonsson, 1977; Tang et al., 1997; Fu et al., 2007; Zhi-wen et al., 2005; Delourme, 2006; Nesi et al., 2008; Abbadi and Leckband, 2011). Co-location of the seed coat color QTL with a TSW QTL affirms this observation, since TSW and oil content have been previously reported to be significantly correlated (Shirzadegan and Robbelen, 1985; Tang et al., 1997). Fu (2007) reported this QTL on LG 9 for seed coat color in a study that was tested in multiple environments. The QTL was detected in a total of three environments across two years of the study and explained approximately 39 to 52 percent of the variance, which is similar to the 40 percent explained variation revealed in this study. Fu suggests a candidate gene *TT10* involved in the flavonoid metabolic pathway based on synteny analysis with *Arabidopsis thaliana*. Although more work needs to be done to verify this claim, the co-localization the QTL detected by Fu and colleagues with the QTL detected in this study suggests that there is a large effect seed coat color QTL that may also affect seed oil content. This LG 9 QTL has also been previously reported by Delourme (2006), who detected a similar QTL region for oil content that explained 7.1% of the phenotypic variation. However, the QTL was only detected in one of several environments from the study.

Seeds per silique QTL

Four QTL were detected for SS, a trait that did not appear to be affected by genotype by treatment interaction or treatment (Table 6); the QTL mapped to similar positions in both treatments. In addition, the amount of variation explained by the models that include these QTL is similar for both treatments (29.92% in the dry treatment and 28.38% in the wet treatment), indicating stability of the QTL. A QTL by treatment analysis did not reveal any significant QTL,

which adds more evidence to this conclusion. Furthermore, taken together, the effect of the four QTL summed to nearly five seeds per silique for both treatments, where the population means were approximately 19 seeds per silique. The results from the correlation, ANOVA, heritability and QTL analyses all demonstrate that selection for SS in a breeding program could be very effective for improving seed yield in both irrigated and rainfed conditions.

Interestingly, fruit and flower abortion rates were found to be significantly different ($P<0.001$) for the parents in this population in the dry treatment (A. Seshadri, Colorado State University, personal communication). The rates for the DH lines are currently being investigated, and will be an interesting addition to this data set. It is possible that the QTL for SS may co-locate with QTL for seed or flower abortion rates, which could provide a biological cause for the variation observed in this trait.

Performance of Line 118

As mentioned previously, in the dry treatment of the 2011 DHYB study, four lines had higher seed yield than in the wet treatment for seed yield, yet only one line (line 118) performed wet significantly ($P<0.01$) better than in the wet treatment. Line 118 had higher seed yield than any other line that flowered at the same time in the dry treatment, but had nearly average seed yield for lines that flowered at the same time in the wet treatment (Figure S6). In terms of yield components, this line had larger TSW and SMI in the dry treatment than the wet treatment, but had fewer SS in the dry treatment versus the wet. Investigation of yield component QTL revealed that this line possessed the favorable allele at one of the two additive QTL that were detected for SMI in the dry treatment. The line also had the favorable allele at all three additive QTL that were detected for TSW in the dry treatment. This is particularly interesting given the magnitude of the yield advantage in the dry treatment. Other researchers are investigating water use efficiency (WUE) and root characteristics in this population; these studies will be

interesting to look at comparatively with this study in order to determine what other characteristics the lines that performed better in the dry treatment might possess.

Timing and intensity of water stress

The effect of treatment on seed yield and most yield-related traits was significant in both 2010 and 2011 ($P<0.05$) indicating that the lines in the rainfed treatment were subjected to water stress during both years of the study. Comparing climatic data (Figures S1 and S2), it is apparent that there was less total rainfall and a higher frequency of high temperature days during the 2010 growing season. Based on climatic data as well as personal observation, the drought stress began in 2010 during the last 2 weeks of May. The average weekly high rose from 19^{o}C to 26^{o}C during the two-week period from May 14 to May 27, 2010; during the same time period, less than 2 cm of precipitation fell on the rainfed treatment. The trend of persistent heat and limited precipitation continued for much of the rest of the growing season. For all of the lines and the parents in the 2010 SE1 population, this stress onset occurred prior to flowering. During the range of flowering for all of the lines in this population, less than 2 cm of precipitation fell on the study.

In contrast with 2010, in 2011 there was more precipitation, especially near the beginning of the growing season. During the time of flowering for all of the lines in the DHYB population, almost 9 cm of precipitation was received. Therefore, in the 2011 DHYB study, most of the drought stress occurred after flowering, during silique development. Soil samples taken near the end of the study confirmed that water potential for the wet treatment was approximately -1.0 MPa versus approximately -2.5 MPa for the dry treatment (Figure S6), indicating that the plants in the dry treatment were subjected to water stress at that time.

Comparison of this study with a previous study from Richards and Thurling (1978) indicates that the resulting decrease in seed yield and yield components observed from the wet to the dry

treatment are consistent with their results. In their study, commencement of drought stress before or during flowering resulted in a decrease of 80% in seed yield, and commencement of drought stress after flowering resulted in an approximate 40% decrease in seed yield, which is consistent with the results that we observed in both years of this study: In 2010, when the majority of the drought stress occurred before and during flowering, mean seed yield decreased 85% from the wet treatment to the dry treatment. In 2011, when the majority of the drought stress occurred post-anthesis, mean seed yield decreased 58% from the wet treatment to the dry treatment.

In this study, we discovered four QTL that appear to be stable across environments for SS, as well as several QTL for DTF which show potential for use in marker-assisted selection techniques in order to increase seed yield under full, limited, or no irrigation. Although all detected QTL need to be validated with repeat studies or near-isogenic lines, the data from this study indicate that SS and DTF are highly correlated with seed yield and both have high heritability estimates, indicating minimal genotype by environment interactions which could complicate breeding results in different environments. Several other studies have shown that SS and seed yield are positively and significantly correlated in *B. napus* (Chi et al., 2007; Chen et al., 2007), which supports the results presented from this research. These novel QTL could serve as a basis for *B. napus* dryland breeding efforts in Colorado and the High Plains area of the U.S. in order to release a high-yielding cultivar for biodiesel or food oil use.

REFERENCES CITED

Abbadi A and Leckband G (2011) Rapeseed breeding for oil content, quality, and sustainability. *Eur J Lipid Sci Techol* 113: 1198-1206.

AGI (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408: 796–815.

Altenbach SB, Kuo CC, Staraci LC, Pearson KW, Wainwright C, Georgescu J, Townsend J (1992) Accumulation of a Brazil nut albumin in seeds of transgenic canola results in enhanced levels of seed protein methionine. *Plant Mol Biol* 18: 235-245.

Angadi SV, Cutforth HW, Miller PR, McConkey BG, Entz MH, Brandt, SA, Volkmar KM (2000) Response of three Brassica species to high temperature stress during reproductive growth. *Can J Plant Sci* 80: 693-701.

Bell JM and Keith MO (1982) Gross energy values of oils from high and low erucic acid rapeseed oils and of rapeseed grains. *Can Inst Food Sci Tech J* 15: 221-224.

Bell JM (1993) Factors affecting the nutritional value of canola meal: A review. *Can J An Sci* 73(4) 689-697.

Boyer JS (1982) Plant productivity and environment. *Science* 218: 443-448.

Branca F and Cartea E (2011) Brassica In C Kole (ed.), *Wild Crop Relatives - Genomic and Breeding Resources: Oilseeds* 17-36. New York USA: Springer Science.

Broman KW and Sen S (2009) A Guide to QTL Mapping with R/qtl. Springer Science, New York, USA.

Broman KW, Wu H, Sen S, Churchill GA (2003) R/qtl: QTL mapping in experimental crosses. *Bioinformatics,* 19: 889-890.

Canola Council of Canada (2009) Canola Meal: Feed industry guide (4th ed.)[Brochure] Available at http://www.canolacouncil.org (verified January 2012)

Canola Council of Canada (2001) Canola Properties and Uses. Available at http://www.canolacouncil.org (verified January 2012).

Cavell AC, Lydiate DJ, Parkin IA, Dean C, Trick M (1998) Collinearity between a 30-centimorgan segment of *Arabidopsis thaliana* chromosome 4 and duplicated regions within the *Brassica napus* genome. *Genome* 41(1): 62-9.

Cermankova L, Sharpe A, Trick M, Bechyne M, Lydiate D (1999) Genetic analysis of quantitative traits in *Brassica napus* using substitution lines. *10th Intl Rapeseed Congress*, Canberra.

Chakravorty U, Hubert MH, Nøstbakken L (2009) Fuel versus food. *Ann Rev Res Econ* 1: 645-663.

Champolivier L and Merrien A (1996) Effect of water stress applied at different growth stages to *Brassica napus* L. var Oleifera on yield components and seed quality. *Eur J Agron* 5: 153-160.

Charlton KM, Corner AH, Davey K, Kramer JK, Mahadevan S, Sauer FD (1975) Cardiac lesions in rats fed rapeseed oils. *Can J Comp Med.* 39(3): 261–269.

Chay and Thurling (1989) Identification of genes controlling pod length in spring rapeseed, *Brassica napus* L., and their utilization for yield improvement. *Plant Breed* 103: 54-62.

Chen W, Zhang Y, Yao J, Ma C, Tu J, Tingdong F (2011) Quantitative trait loci mapping for two seed yield component traits in an oilseed rape (*Brassica napus*) cross. *Plant Breed* 130: 640-646.

Chen W, Zhang Y, Liu X, Chen B, Tu J, Fu T (2007) Detection of QTL for six yield-related traits in oilseed rape(*Brassica napus*) using DH and immortalized F2 populations. *Theor Appl Gen* 115: 849–858.

Churchill GA and Doerge R (1994) Empirical threshold values for quantitative trait mapping. *Genetics,* 138: 963-971.

Clarke JM and Simpson GM (1978) Influence of irrigation and seeding rates on yield and yield components of *Brassica napus* cv. Tower. C*an J Plant Sci* 58: 731-737.

CO Climate Center (2011) http://www.ccc.atmos.colostate.edu. Verified February 2011.

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* 142: 169-196.

Coonrod D (2005) Inheritance of oleic, eicosenoic and erucic acid in canola. (M.S. thesis) Colorado State University, Fort Collins, CO.

Delourme R, Falentin C, Huteau V, Clouet V, Horvais R, Gandon B, Specel S, Hanneton L, Dheu JE, Deschamps M, Margale E, Vincourt P, Renard M (2006) Genetic control of oil content in oilseed rape (*Brassica napus* L.). *Theor Appl Genet* 113: 1331-1345

Duan JK and DeClerq DR (1988) Quality of yellow and dark seeds in *Brassica campestris* canola varieties Candle & Tobin. *J Amer Oil Chem Soc* ,65: 122-126.

Energy Independence and Security Act of 2007. HR 6, Title II, Subtitle A, Section 202.

Energy Policy Act of 2002. HR 4, Division C, Title VIII, Subtitle A, Section 820.

Energy Policy Act of 2005. HR 6, Title XV, Subtitle A, Section 1501.

Falconer DS and Mackay TFC (1996) Introduction to Quantitative Genetics. Longman Group Ltd, Harlow, Essex, England.

Fan C, Cai G, Qin J, Li Q, Yang M, Wu J, Fu T, Liu K, Zhou Y (2010) Mapping of quantitative trait loci and development of allele-specific markers for seed weight in *Brassica napus*. *Theor Appl Genet* 121: 1289-1301.

Farahani HJ, Peterson GA, Westfall DG, Sherrod LA, Ahuja LR (1998) Soil water storage in dryland cropping systems: The significance of cropping intensification. *Soil Sci Soc Am J* 62: 984-991.

Federal Register (2007) Regulation of fuels and fuel additives: Renewable Fuels Standard Program. *Rules and Regulations,* 72 (83).

Fischer RA and Maurer R (1978) Drought resistance in spring wheat cultivars I: Grain yield responses. *Aust J Agric Res.,* 29: 897-912.

Fore S, Lazarus P, Porter P, Jordan N (2011). Economics of small-scale on-farm use of canola and soybean for biodiesel and straight vegetable oil biofuels. *Biomass Bioenerg* 35 (1): 193-202.

Fore S, Porter P, Lazarus P (2011) Net energy balance of small-scale on-farm biodiesel production from canola and soybean. *Biomass Bioenerg* 35 (5): 2234-2244.

Fu FY, Liu LZ, Chai YR, Chen L, Yang T, Jin MY, Ma AF, Yan XY, Zhang ZS, Li JN (2007) Localization of QTLs for seed color using recombinant inbred lines of Brassica napus in different environments. *Genome* 50: 840-854.

Geritz S, Meijdenb E, Metz J (1999) Evolutionary dynamics of seed size and seedling competitive ability. *Theor Popul Biol* 55: 1324-1343.

Gunasekera CP, Martin LD, Siddique KHM, Walton GH (2006) Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*Brassica napus* L.) in Mediterranean-type environments II. Oil and protein concentrations in seed. *Eur J Agron* 25: 13–21

Hall AE, Fiebig A, Preuss D (2002) Beyond the Arabidopsis genome: opportunities for comparative genomics. *Plant Physiol* 129: 1439–1447

Hammond EG (1991) Organization of rapid analysis of lipids in many individual plants. p. 321-330. *In* H.F. Linskens and J.F. Jackson (ed.) Modern methods of plant analysis. Vol. 12: Essential oils and waxes. Springer-Verlag, Berlin, Germany.

Heaton EA, Dohleman FG, Long SP (2008) Meeting US biofuels goals with less land: the potential of Miscanthus. *Global Change Biol,* 14: 2000-2014.

Hergert GW, Margheim J, Pavlista A, Burgener P, Lyon D, Hazen A, Martin D, Supalla R, Thomson C (2011) Yields and ET of deficit to fully irrigated canola and camelina. *Proc 23rd Ann Cent Pl Irr Conf*

Howard HK and Daun JK (1991) Oil content determination in oilseeds by NMR, Method of the Canadian Grain Commission Grain Research Laboratory. Agriculture Canada, Winnipeg, pp.5.

IPCC (2001) Summary for Policy Makers. In *Climate Change 2001: The Scientific Basis. Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel on Climate Change*. Eds. J T Houghton, Y Ding, D J Griggs, M Noguer, P J van der Linden, X Dai, K Maskell and C A Johnson. Cambridge, UK, and New York, USA: Cambridge University Press.

Jain SM, Sopory SK, Veilleux RE (1996) *In vitro* haploid production in higher plants, Vol. 1, 125-144. Netherlands: Kluwer Academic Publishers.

Johnston AM, Tanaka DL, Miller PR, Brandt SA, Nielsen DC, Lafond GP, Riveland NR (2002) Oilseed crops for semiarid cropping systems in the Northern Great Plains. *Agron J* 94: 231-240.

Jonsson R (1977) Breeding for improved oil and meal quality in rape (*Brassica napus* L.) and turnip rape (*Brassica campestris* L.). *Hereditas* 87: 205-218.

Kimber D and McGregor DI (1995) *Brassica* oilseeds: Production and utilization. Wallingford, UK: CAB International.

Kole C (2011) Wild Crop Relatives - Genomic and Breeding Resources: Oilseeds. Springer Science New York USA.

Kosambi DD (1944) The estimation of map distances from recombination values. *Ann Eugen* 12: 172-175.

Lagercrantz U (1998) Comparative mapping between *Arabidopsis thaliana* and *Brassica nigra* indicates that Brassica genomes have evolved through extensive genome replication accompanied by chromosome fusions and frequent rearrangements. *Genetics* 150: 1217–1228.

Lukens L, Zou F, Lydiate D, Parkin I, Osborn T (2003) Comparison of a *Brassica Oleracea* genetic map with the genome of *Arabidopsis thaliana*. *Genetics* 164: 359–372.

Marjanovic-Jeromela A, Marinkovic R, Ivanovska S, Jankuovska M, Mijic A, Hristov N (2011) Variability of yield determining components in winter rapeseed (*Brassica napus* L.) and their correlation with seed yield. *Genetika* 43(1): 51-66.

McCouch S (2004) Diversifying Selection in Plant Breeding. *PLoS Biol* 2(10).

Melchinger AE, Utz HF, Schön CC (1998) Quantitative Trait Locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. *Genetics,* 149: 383-403.

Moghaddam MJ and Pourdad SS (2011) Genotype x environment interactions and simultaneous selection for high oil yield and stability in rainfed warm areas rapeseed (*Brassica napus* L.) from Iran. *Euphytica* 180: 321-335.

Mohan M, Nair S, Bhagwat A, Krishna TG, Yano M, Bhatia CR, Sasaki T (1997) Genome mapping, molecular markers and marker-assisted selection in crop plants. *Mol Breeding* 5: 87-103.

Naylor RL, Liska AJ, Burke MB, Falcon WP, Gaskell JC, Rozelle SD, Cassman KG (2007) The ripple effect: Biofuels, food security, and the environment. *Environment* 49(9): 30-43.

Nesi N, Delourme R, Bregeon M, Falentin C, Renard M (2008) Genetic and molecular approaches to improve nutritional value of *Brassica napus* L. seed. *C R Biol* 331: 763-771

Nielsen DC (1997) Water use and yield of canola under dryland conditions in the central great plains. *J Prod Agric* 10(2) 307-313.

Nielsen DC (1998) Comparison of three alternative oilseed crops for the central Great Plains. *J Prod Agric* 11(3) 336-341.

Nielsen DC, Unger PW, Miller PR (2005) Efficient water use in dryland cropping systems in the Great Plains. *Agron J* 97: 364-372

Palmer WC (1965) Meteorologic drought. US Weather Bureau, *Res Pap* 45: 1-58.

Panjabi PJA, Bisht NC, Padmaja KL, Sharma S, Gupta V, Pradhan AK, Pental D (2008) Comparative mapping of *Brassica juncea* and *Arabidopsis thaliana* using intron polymorphism (IP) markers: homoeologous relationships, diversification and evolution of the A, B and C Brassica genomes. *BMC Genomics* 9: 113

Parkin IAP, Gulden SM, Sharpe AG, Lukens L, Trick M, Osborn TC, Lydiate DJ (2005) Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. *Genetics* 171 (2): 765-781.

Pavlista AD, Santra DK, Isbell TA, Baltensperger DD, Hergert GW, Krall J, Mesbach A, Johnson J, O'Neill M, Aiken R, Berrada A (2011) Adaptability of irrigated spring canola oil production to the US High *Plains Ind Crop Prod* 33: 165-169.

Pennisi E (2008) The blue revolution, drop by drop, gene by gene. *Science* 320: 171-173.

Peterson GA, Unger PW, Payne WA (2006) Dryland Agriculture. ASA-CSSA-SSSA Publishing, Madison, Wisconsin, USA.

R Development Core Team (2011) R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria.

Rafalski JA (2010) Association genetics in crop improvements. *Curr Opin Plant Biol* 13: 174-180.

Rakow G and Relf-Eckstein J (2005) YN01–429. *Plant Var Seeds* 55: 40-41 .

Richards RA and Thurling N (1978) Variation between and within species of rapeseed (*Brassica campestris* and *B. napus*) in response to drought stress. I. Sensitivity at different stages of development. ***Aust J Ag Res*** 29, 469–477.

Richards RA and Thurling N (1979) Genetic analysis of drought stress response in rapeseed (*Brassica campestris* and *B. napus*). II. Yield improvement and the application of selection indices. *Euphytica* 28: 169-177.

Rife C, Auld DL, Sunderman HD, Heer WF, Baltensperger DD, Nelson LA, Johnson DL, Bordovsky D, Minor HC (2001) Registration of 'Wichita' winter rapeseed. *Crop Sci* 41: 263-a-264.

Scarth R and Tang J (2005) Modification of Brassica oil using conventional and transgenic approaches. *Crop Sci* 46 (3): 1225-1236.

Semagn K, Bjornstadt A, Ncjiondjop MN (2006) An overview of molecular marker methods for plants. *African J Biotech* 5 (25): 2540-2568.

Sen S and Churchill GA (2001) A statistical framework for quantitative trait mapping. *Genetics* 159, 371-387.

Shahidi, F (1990) Canola and Rapeseed: Production, Nutrition, and Processing Technology. New York USA: Van Nostrand Reinhold Publishing.

Shi J, Li R, Qiu D, Jiang C, Long Y, Morgan C, Bancroft I, Zhao J, Meng J (2009) Unraveling the complex trait of crop yield with quantitative trait loci mapping in *Brassica napus*. *Genetics* 182: 851–861

Shirzadegan M and Robbelen G (1985) Influence of seed colour and hull proportion on quality properties of seeds in *Brassica napus* L. *Fette Seifen Ansstrichmit,* 87: 235-237.

Si P and Walton GH (2004) Determinants of oil concentration and seed yield in canola and Indian mustard in the lower rainfall areas of Western Australia. *Aust J Agr Res* 55: 367-377.

Si P, Mailer RJ, Galwey N, Turner GW (2003) Influence of genotype and environment on oil and protein concentrations of canola (*Brassica napus* L.) grown across Southern Australia. *Aust J Agr Res* 54: 397-407.

Somerville C, Youngs H, Taylor C, Davis SC, Long SP (2010) Feedstocks for lignocellulosic biofuels. *Science* 329: 790-792.

Song K, Tang K, Osborn TC (1993) Development of synthetic Brassica amphidiploids by reciprocal hybridization and comparison to natural amphidiploids. *Theor Appl Genet* 86: 811-821.

Sosulski FW, Cadden AM (1982) Composition and Physiological Properties of Several Sources of Dietary Fiber. *J Food Sc,* 47(5)**:** 1472–1477.

Tang ZL, Li JN, Zhang XK, Chen L, Wang R (1997) Genetic variation of yellow-seeded rapeseed lines (*Brassica napus* L.) from different genetic sources. *Plant Breeding* 116: 471-474.

Tenenbaum DJ (2008) Food vs. fuel: Diversion of crops could cause more hunger. *Environ Health Perspect* 116(6): A254–A257.

Thurling N (1974) An evaluation of an index method of selection for high yield in turnip rape*, Brassica campestris* L. ssp. Oeleifera Metzg. *Euphytica* 23: 321-331.

Trick M, Y Long, JL Meng, I Bancroft (2009) Single nucleotide polymorphism (SNP) discovery in the polyploidy *Brassica napus* using Solexa transcriptome sequencing. *Plant Biotech J* 7: 334-346.

United Nations (2010) World Population Prospects: The 2010 revision. Department of Economic and Social Affairs, Population Division, United Nations, New York.

USDA (2010) A USDA Regional Roadmap to Meeting the Biofuels Goals of the Renewable Fuels Standard by 2022. USDA Biofuels Strategic Production Report June 23, 2010.

USDA (2011) www.ers.usda.gov/Briefing/SoybeansOilcrops/Canola.htm Accessed February 2012.

U.S. Energy Information Administration (2011), ***Annual Energy Review 2010***.

Van Ooijen J.W. and Voorrips RE (2001) JoinMap® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, the Netherlands.

Voorrips, RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. *J Heredity* 93 (1): 77-78.

Wittkop B, Snowdon RJ, Friedt (2009) Status and perspectives of breeding for enhanced yield and quality of oilseed crops for Europe. *Euphytica* 170: 131–140.

Xiao DR and Liu HL (1982) Correlation analysis of seed colour and seed oil in *Brassica napus* L. *Acta Agronomical Sin* 8: 24-27

Zhang L, Liu P, Hong D, Huang A, Li S, He Q, Yang G (2010) Inheritance of seeds per silique in *Brassica napus* L. using join segregation analysis. *Field Crops Res* 116: 58-67.

Zhang L, Yang G, Liu P, Hong D, Li S, He Q (2011) Genetic and correlation analysis of silique-traits in *Brassica napus* L. by quantitative trait locus mapping *Theor Appl Genet* 122: 21-31.

Zhao J, Becker HC, Zhang D, Zhang Y, Ecke W (2005) Oil content in a European x Chinese rapeseed population: QTL with additive and epistatic effects and their genotype-environment interactions. *Crop Sci* 45: 51-59.

Zhi-wen L, Ting-dong F, Jin-xing T, Bao-yuan C (2005) Inheritance of seed colour and identification of RAPD and AFLP markers linked to the seed colour gene in rapeseed (*Brassica napus* L.) *Theor Appl Genet* 110: 303-310.

Zou J, Jiang C, Cao Z, Li R, Long Y, Chen S, Meng J (2010) Association mapping of seed oil content in *Brassica napus* and comparison with quantitative trait loci identified from linkage mapping. *Genome* 53: 908-916.

SUPPLEMENTARY TABLES AND FIGURES

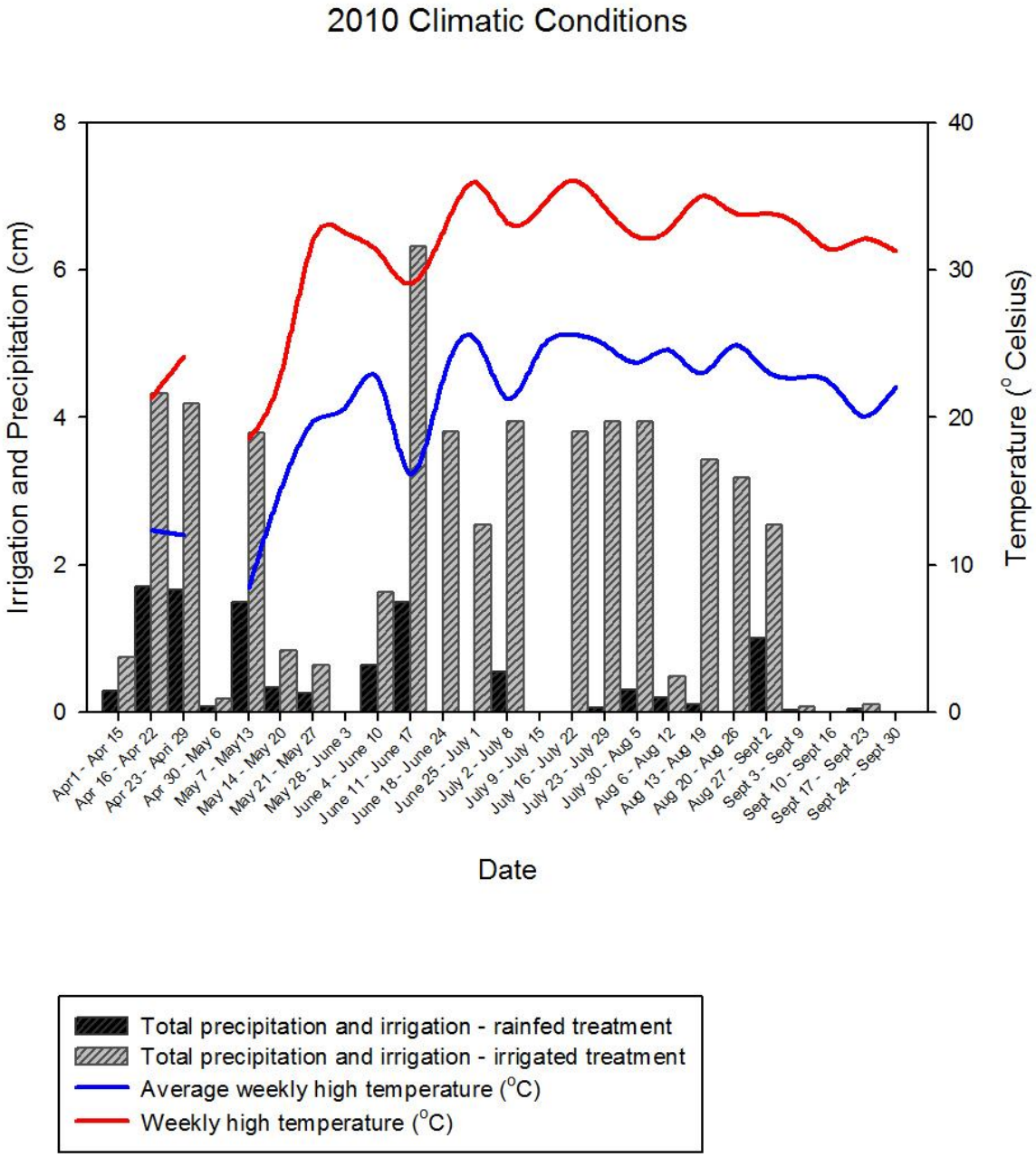


Figure S1. 2010 average high, weekly high and total precipitation and irrigation amounts for irrigated and rainfed treatments.

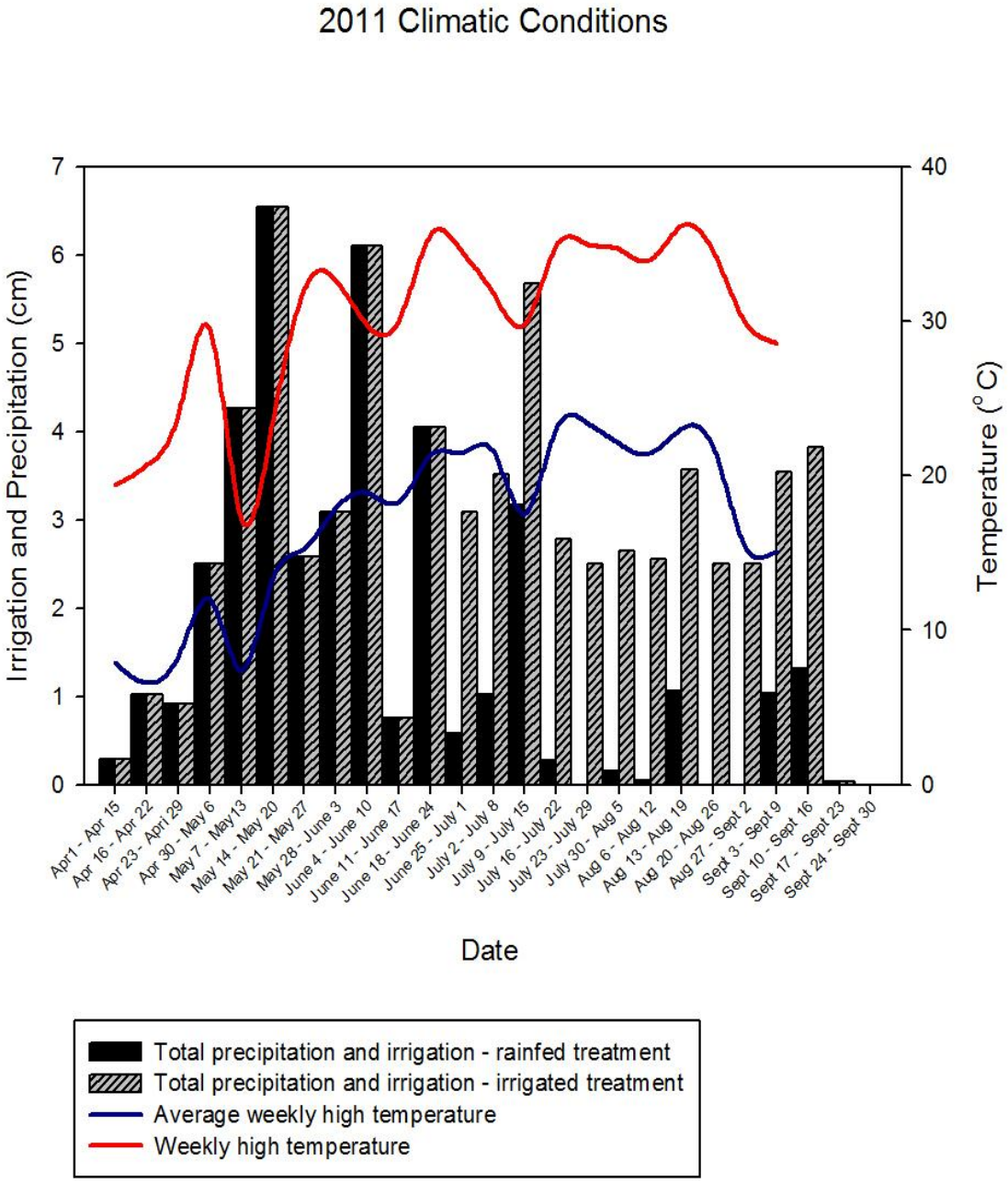


Figure S2. 2011 average high, weekly high and total precipitation and irrigation amounts for irrigated and rainfed treatments.

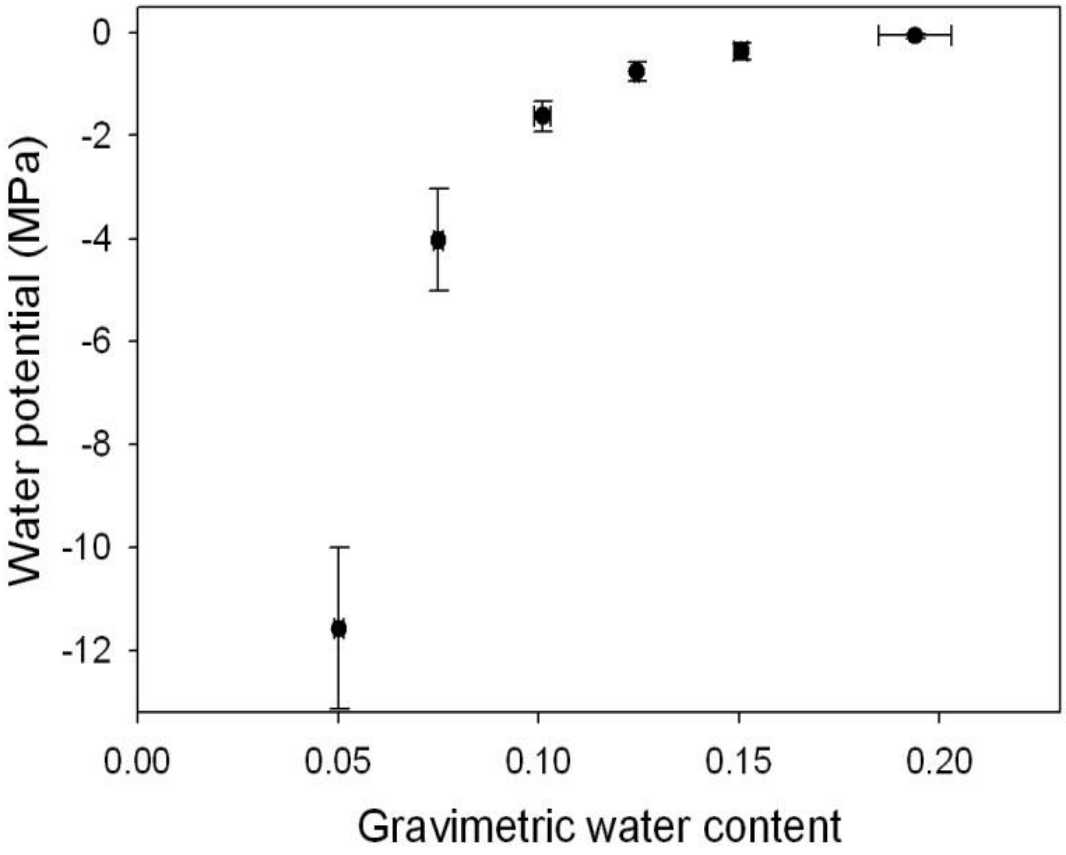


Figure S3. Water potential curve of field soil samples taken in 2011 (n=6).

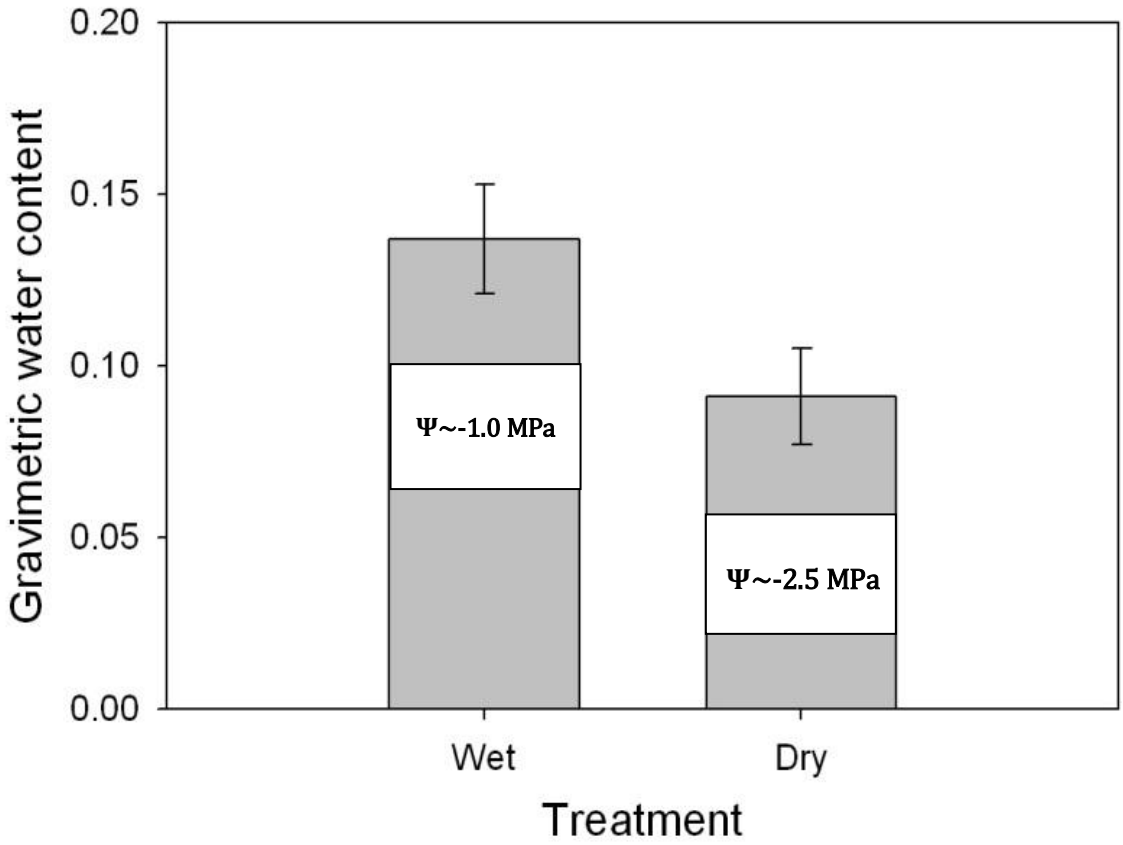


Figure S4. GWC and corresponding water potential estimates (MPa) of field soil samples taken from irrigated (wet) and rainfed (dry) treatments on August 4, 2011.

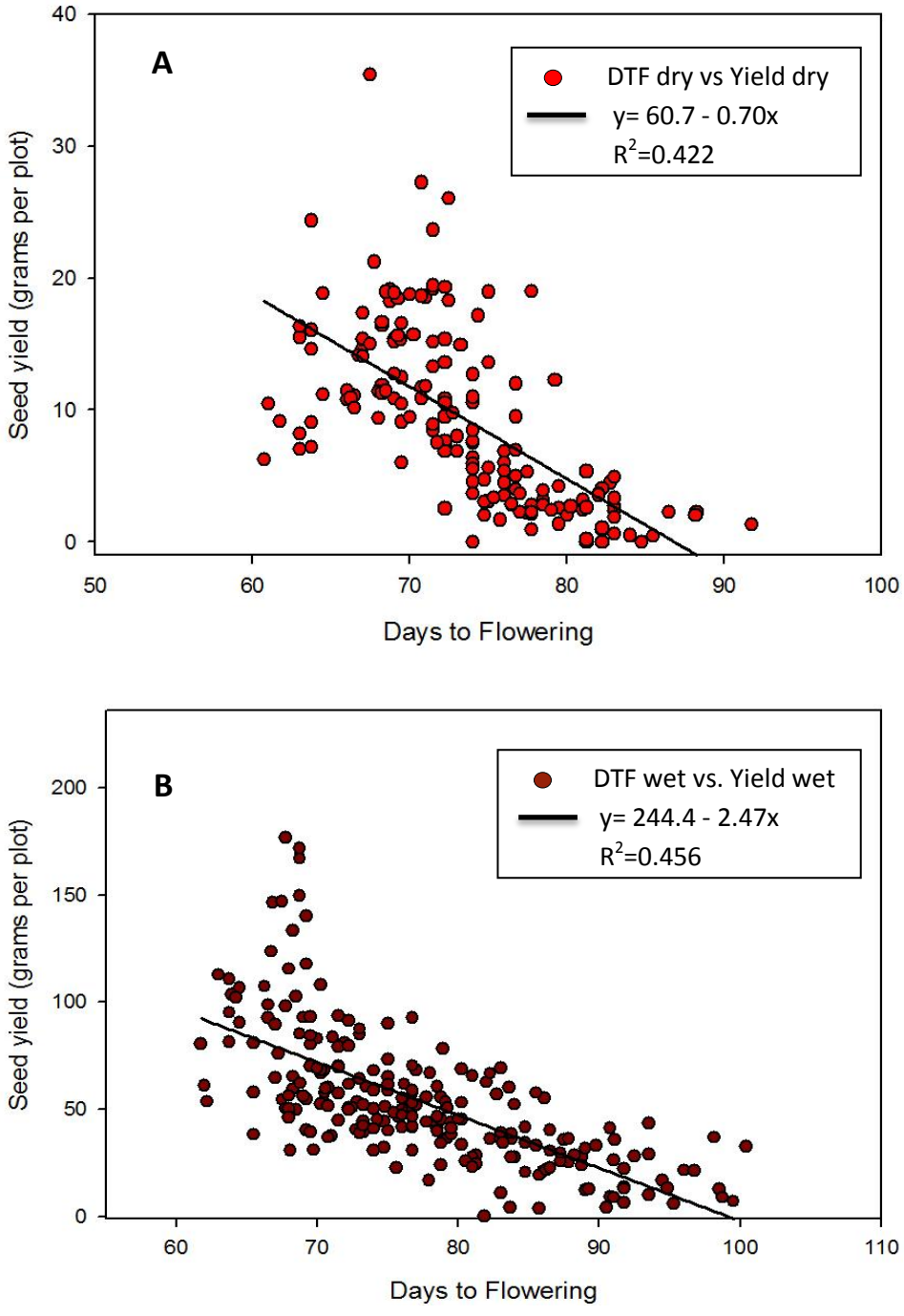


Figure S5. Regression of DTF on seed yield in the 2010 SE1 population dry (A) and wet (B) treatments.

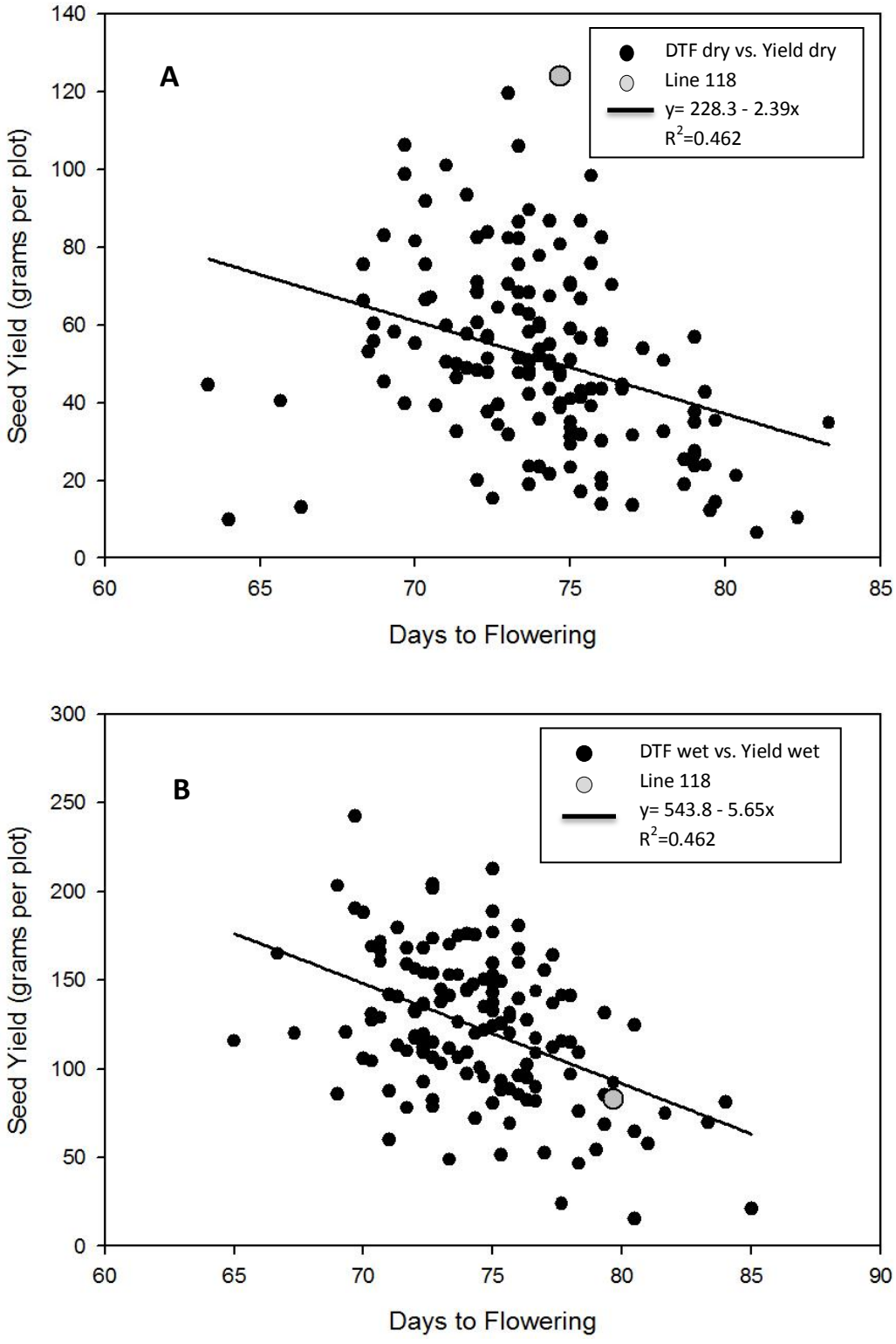
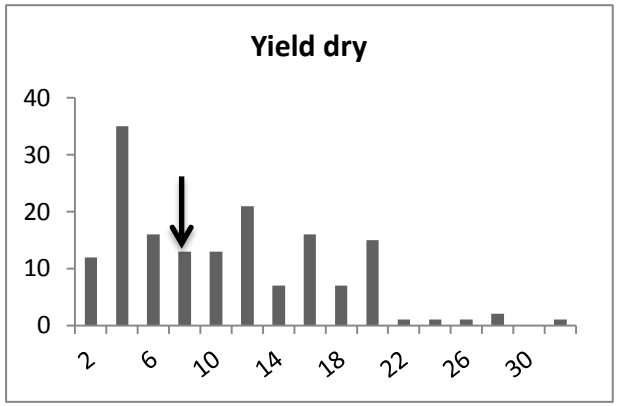
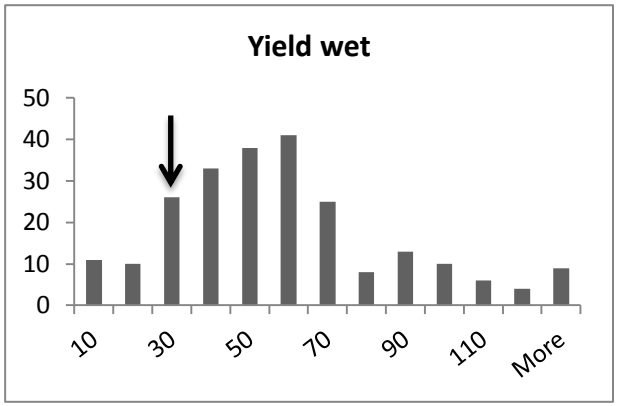
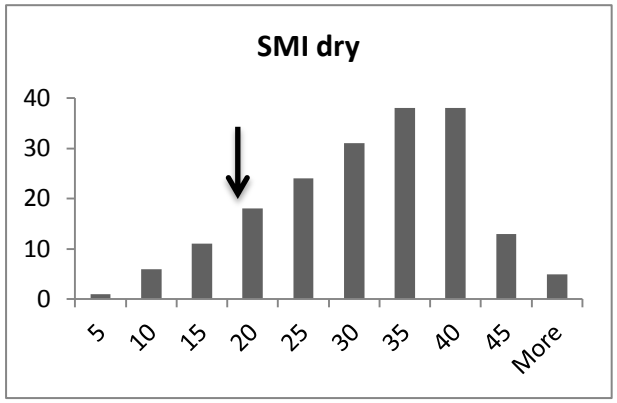
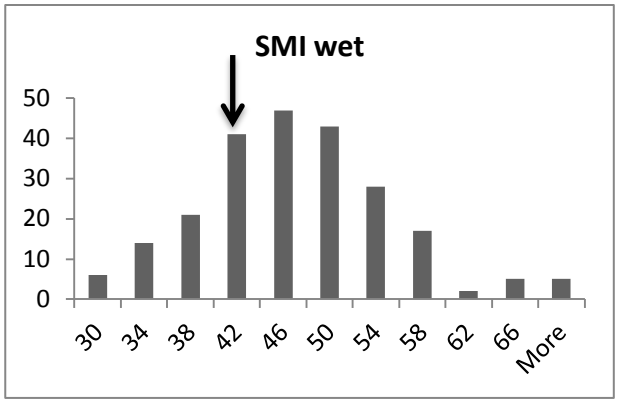


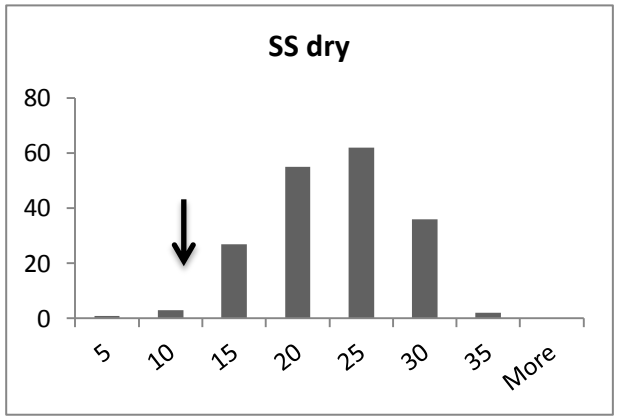
Figure S6. Regression of DTF on seed yield in the 2011 DHYB population dry (A) and wet (B) treatmens. Line 118, highlighted with the large grey circle, yielded significantly higher in the dry treatment than the wet treatment (P<0.01)

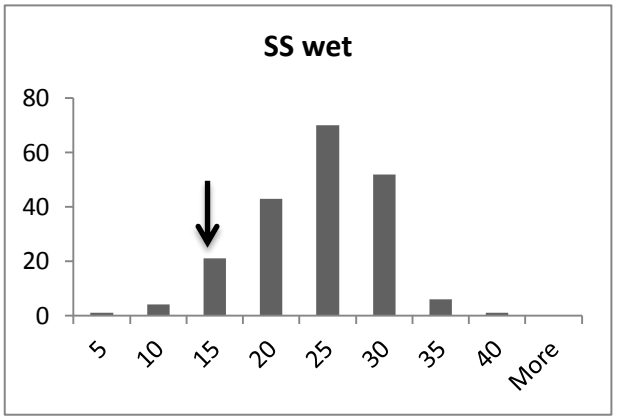

Yield dry
40
30
20
10
0
2
6
10
14
18
22
26
30


Yield wet
50
40
30
20
10
0
10
30
50
70
90
110
More


SMI dry
40
30
20
10
0
5
10
15
20
25
30
35
40
45
More


SMI wet
50
40
30
20
10
0
30
34
38
42
46
50
54
58
62
66
More


SS dry
80
60
40
20
0
5
10
15
20
25
30
35
More


SS wet
80
60
40
20
0
5
10
15
20
25
30
35
40
More

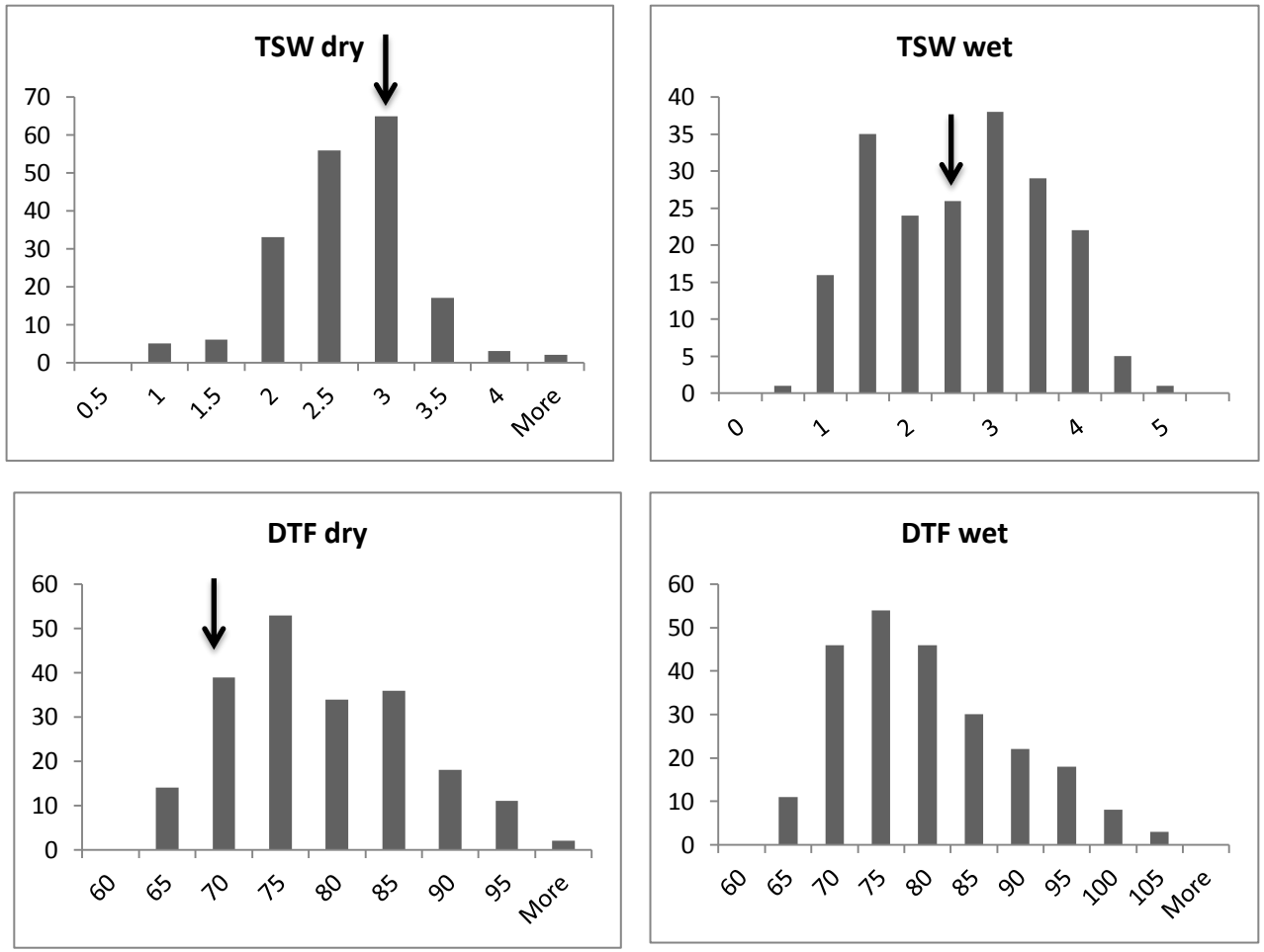
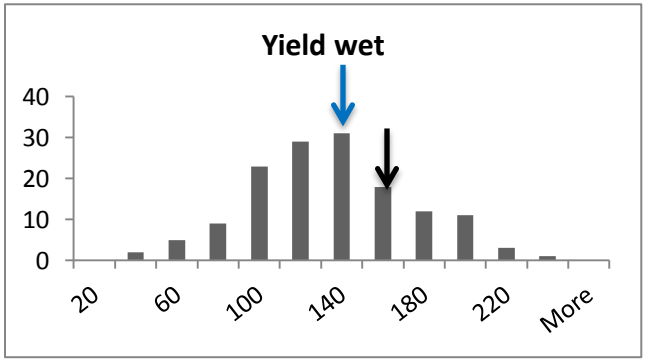
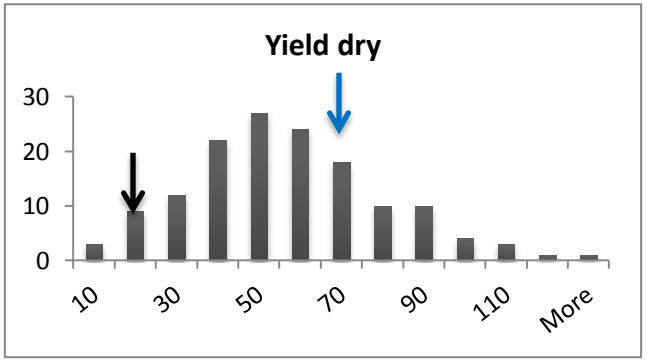
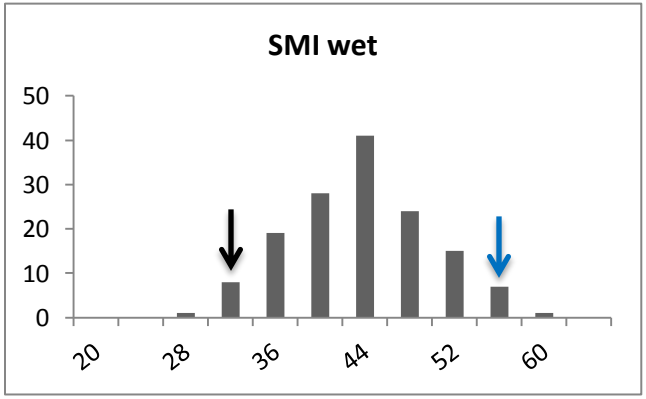
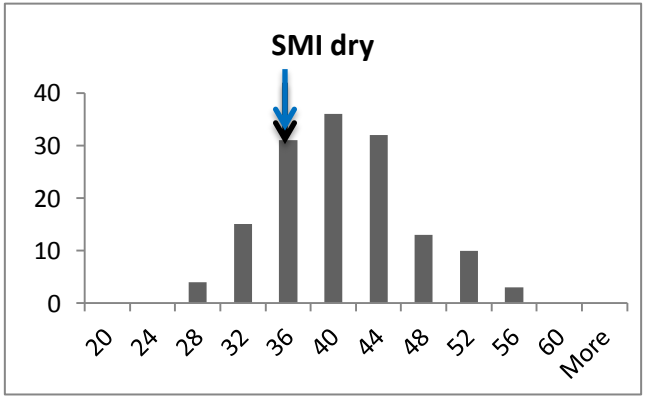


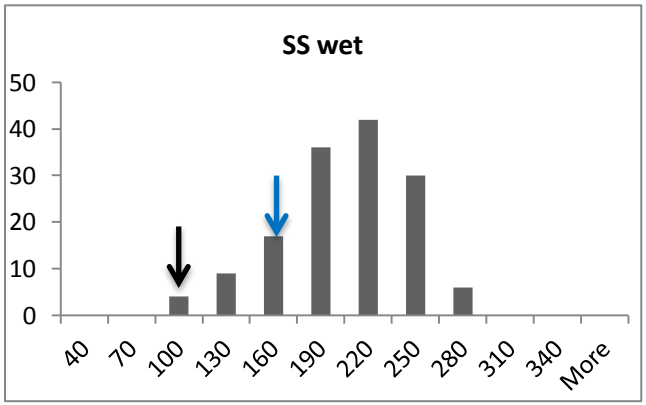
Figure S7. Frequency distributions of the DH lines in the 2010 SE1 study. Parent IMC106RR value is highlighted with a black arrow. Parent Wichita did not flower.

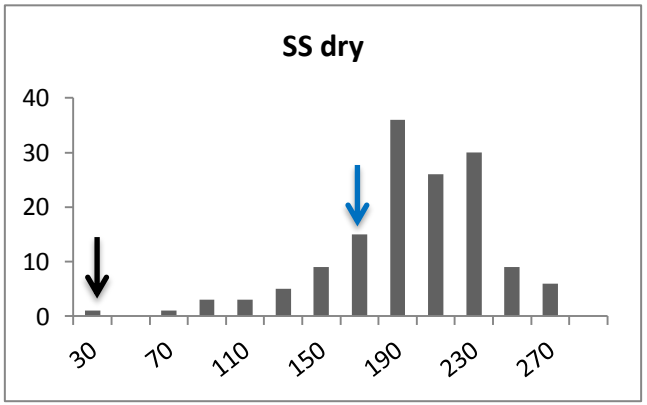

Yield wet
40
30
20
10
0
20
60
100
140
180
220
More


Yield dry
30
20
10
0
10
30
50
70
90
110
More


SMI wet
50
40
30
20
10
0
20
28
36
44
52
60


SMI dry
40
30
20
10
0
20
24
28
32
36
40
44
48
52
56
60
More


SS wet
50
40
30
20
10
0
40
70
100
130
160
190
220
250
280
310
340
More


SS dry
40
30
20
10
0
30
70
110
150
190
230
270

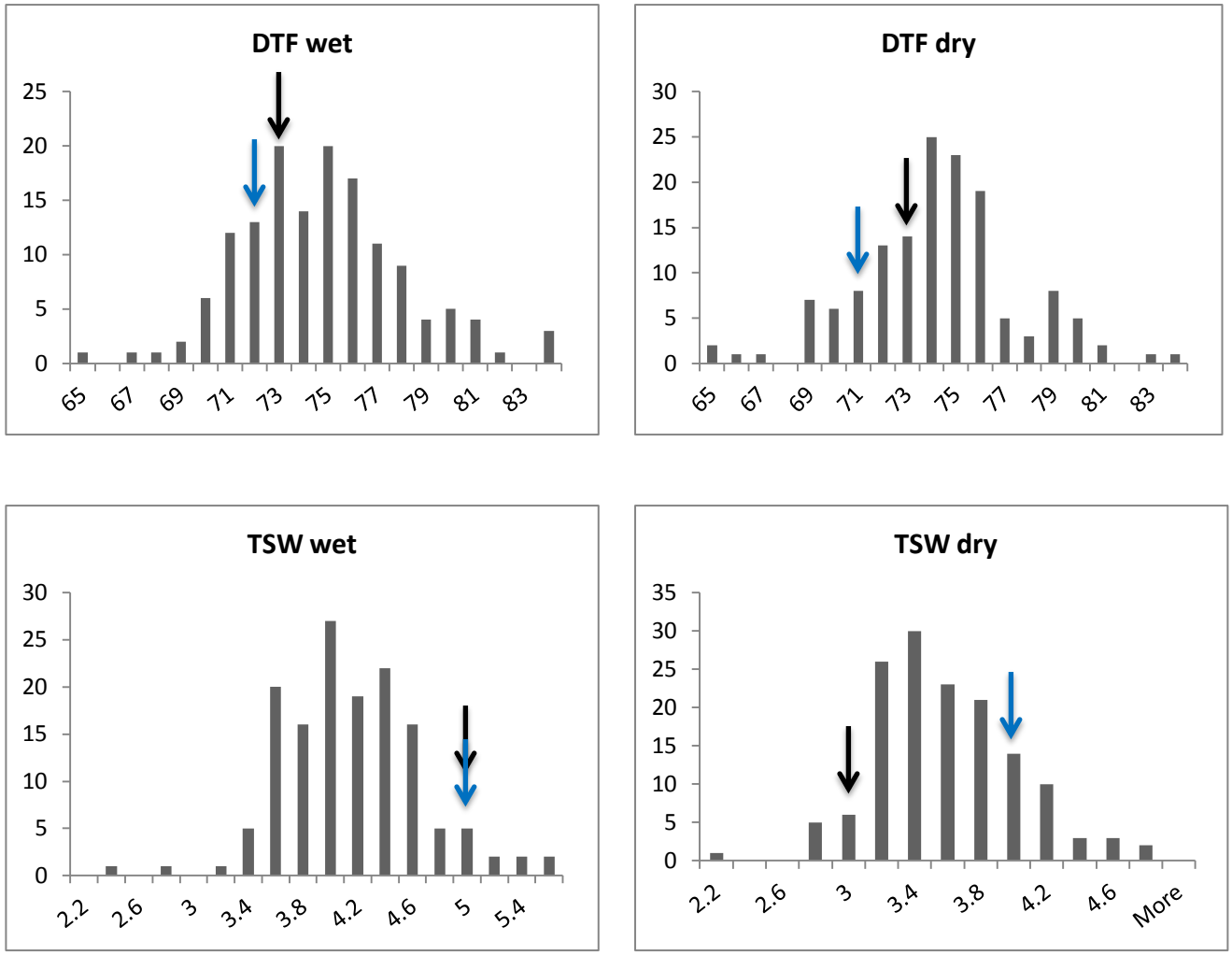


Figure S8. Frequency distributions of the DH lines from the 2011 DHYB study**.** Parent YN01-429 values are highlighted with a black arrow. Parent DH12075 values are highlighted with a blue arrow.