THESIS

QTL MAPPING OF ROOT AND LEAF TRAITS ASSOCIATED WITH DROUGHT TOLERANCE IN A CANOLA (*BRASSICA NAPUS* L.) DOUBLED HAPLOID POPULATION

Submitted by

Melaku Degefu Mekonnen

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 2013

Master's Committee:

Advisor: Patrick Byrne

John McKay
Arathi Seshadri

ABSTRACT

QTL MAPPING OF ROOT AND LEAF TRAITS ASSOCIATED WITH DROUGHT TOLERANCE IN A CANOLA (*BRASSICA NAPUS* L.) DOUBLED HAPLOID POPULATION

Drought stress is one of the major constraints to canola production in Colorado, therefore, improved understanding of the inheritance and genetic variation for drought tolerance will help to develop cultivars that are adapted to the state. To learn more about traits associated with drought tolerance in canola we focused on root pulling force and carbon isotope discrimination with the objectives of:

- Detecting the location, number, and effects of quantitative trait loci (QTL) associated with root pulling force and carbon isotope discrimination
- Understanding the association of root pulling force and carbon isotope discrimination with yield components in well watered and water limited environments

We used 148 doubled haploid lines of the DHYB population developed from the cross of a black seeded (DH12075) and a yellow seeded (YN01-429) parent. The experiment was conducted as a split-plot design with three replications in the 2011 and 2012 summer seasons. Two moisture regimes (wet and dry) constituted the main plot factor, with genotype as the subplot factor. A single individual plant per replication represented each genotype. Days to flowering, leaf relative water content, $\delta^{13}C$ (the ratio of ^{13}C to ^{12}C), plant height, lateral branch number, lateral root number, thousand seed weight, seed yield per plant, root pulling force, and proportion of aborted siliques were measured, though not every trait was evaluated in each treatment or year. Phenotypic correlation among all pairs of traits and heritability of the traits under both treatments were estimated. QTL analysis was conducted for each trait and environment with R/qtl software.

Analysis of variance revealed significant differences (P<0.001) among genotypes for days to flowering, plant height, and $\delta^{13}C$ in both treatments and years. Transgressive segregation was observed for root pulling force, $\delta^{13}C$, days to flowering and plant height in both treatments and years. Root pulling force was significantly correlated with plant height (r = 0.32 to 0.54, P<0.001), fresh biomass (r = 0.17 to 0.58, P<0.001), and lateral root number (r = 0.21 to 0.42, P<0.001) in both years and under both moisture treatments. The strong positive correlation of root pulling force with the branch number and fresh biomass suggests that it can be used to detect genotypes with higher yield potential in drought. $\delta^{13}C$ was positively correlated with days to flowering in each experiment and negatively correlated with seed yield per plant and thousand seed weight.

In 2011 QTL were detected for days to flowering on linkage group 1, 2, 12T, and 16, and for the interaction between loci on linkage groups 1 and 16; some of the same QTL were also detected in 2012. In 2011, QTL for root pulling force were detected on linkage groups 3, 5, 11, 14T, and 18 and for the interaction between QTL on linkage groups 3 and 18. In 2012, consistent QTL were detected on linkage groups 11 and 18. The QTL for root pulling force co-localized with a fresh biomass QTL on linkage group 11 and with plant height on linkage group 14T. Five QTL for $\delta^{13}C$ were detected on linkage groups 2, 9, 18, and 19 in different environments. In general QTL for $\delta^{13}C$ were associated with QTL for days to flowering. No epistatic interactions were detected for the QTL detected in 2011 and 2012 for $\delta^{13}C$, suggesting strong additive gene action for $\delta^{13}C$. We found high heritability and relatively low QTL x environment interaction for root pulling force and $\delta^{13}C$; therefore, we suggest these traits can be used to select genotypes with a higher yield and biomass in dry environments. The study provides insights about root pulling force, $\delta^{13}C$ and their relationships with yield, and yield related traits in canola. In

order to utilize these traits in breeding for drought tolerance and marker assisted breeding further research on the relationship among these traits is imperative.

ACKNOWLEDGMENT

My deepest thank goes to my advisor Dr. Patrick Byrne who has helped in all aspects of achieving my degree. Drs. John McKay and Arathi Seshadri who served as members of my committee also deserve my hearty thanks and appreciation for their logistic, technical and financial support for my thesis project. I would also sincerely like to thank Dr. Jack Mullen for all his support in data analyses, field help and genuine unreserved support whenever I need.

Special thanks to friends who helped me when I first came to Fort Collins: Dr. Gordon Cisar, Mrs. Kathy Cisar, Mrs. Katherine Doesken, Mr. Nolan Doesken, Dr. Carol Boxley, Mr. Donald Boxley, Peter and Ethan Boxley.

I would like to acknowledge my previous coworkers and colleagues at Cargill, Dr. Wally Palm, Mr. Phil Phelan, Mrs. Kristina Tiedeman, Dr. Zhizheng Chen, Dr. Honggan Zheng, Dr. Benyuan Dang, Miss Jenine Bryant, Miss Diana Berger, Mr. Patrick Hughes, Mr. Ryan Paterson, Mr. Steve Stadelmaier, Mr. Dave Herrmann, Mrs. Diane McDonald and Mr. Duane Sinning. I would like to thank Mr. Scott Reid, members of the Byrne and McKay lab staff and graduate students for all their support during my field seasons. My gratefulness for Cargill's support will always be in my heart.

Finally, I would like to thank friends from the Ethiopian community: Mr. Debebe Masresha, Dr. Daniel Desta, Dr. Mekonnen Lema, Miss Meaza Belay, Dr. Erena Edae, Dr. Ferdu Azerefegne, Mrs. Almaz Bulcha, and other friends and families in Denver. Finally yet importantly, my deepest gratitude goes to Mr. Naod Beteselassie for his guidance from my first day in the US.

DEDICATION

I would like to dedicate this work to my grandmother Zenebech Hassen Sedecha, uncles Alemayehu Degefu Mekonnen and Workneh Degefu Mekonnen (rest in peace) and my aunt Tsehay Degefu Mekonnen (rest in peace) who played a significant role and contributed to my pursuit of education. Though my grandmother does not have a formal education, she helped with all her power by making sure I went to school, pursued my education, and succeeded. Mazuke I love you!

TABLE OF CONTENTS

1. INTRODUCTION

Brassica napus L., also known as oilseed rape, belongs to the family *Brassicaceae,* which is known to contain greatly diversified plant species (Gomez-Campo, 1999). The *Brassica* genus provides edible plant parts that range from the flower (as in cauliflower), to leaves (as in cabbage), seed extracts as condiment (as in *B. juncea),* and seed oil (as in *B. napus, B. juncea, B. carinata*, and others) to name a few (Gomez-Campo, 1999; Rakow, 2004). Cabbage, kale, cauliflower, broccoli, and Brussels sprouts are all members of the species *B*. *oleracea* and are commonly available vegetables in an ordinary grocery store.

B. napus (AACC genome, 2n=4x=38) is an allotetraploid species derived from the inter-specific hybridization between the diploid species *B. rapa* (AA genome, 2n=2x=20) and *B. oleracea* (CC genome, 2n=2x=18). This botanical relationship and relationships among other important oilseed rapes has been described by Nagaharu (1935) as the famous triangle of U (Figure 1). The origin of *B. napus* is not known with certainty; it does not occur wild in nature and has at least a few hundred years of history (Gomez-Campo, 1999). It is believed to have originated somewhere in the Mediterranean region of South Western Europe where there is an overlap in the origin and geography of the two contributing parents *B. rapa* and *B. oleracea* (Tsunoda, 1980; Gomez-Campo, 1999).

Canola is a specific type of *B. napus* whose name came from **CAN**adian **O**il **L**ow **A**cid, referring to a quality standard of "double low", *i.e.,* low erucic acid (less than 2% or 20 g/kg) and low glucosinolate (less than 30 µmol/kg) content in oil extracted from seeds (Robbelen and Downey, 1989). Canola exists as both spring and winter types, which gives it a wide range of environmental adaptation. Spring canola is grown mostly in temperate regions in areas with dry weather and shorter growing seasons such as the Canadian Prairie Provinces. Canada is the major producer of canola in the world (FAO, 2007 http://faostat.fao.org, Wittkop et al., 2009). Canola is used mainly for production of vegetable oils for human consumption, industrial applications (bio-fuels and lubricants), and animal feeds. Winter canola

(European rapeseed) is grown mostly in Western Europe with mild winter conditions and is used for industrial applications (Galili et al., 2002). In the US, canola production is concentrated in the Great Plains (North Dakota, Minnesota, Montana, South Dakota, Colorado Kansas and Oklahoma). The production is mainly during drier and shorter growing seasons in rotation with small grains, such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.*)*, and flax (*Linum usitatissimum* L.). North Dakota provides the lion's share of the US production (Shahidi, 1990; USDA, 2012). The production of canola in the US increased after the Food and Drug Administration granted the oil a status of Generally Recognized as Safe (GRAS) in 1985. The recognition allowed canola oil to be used in food industries in the US (Raymer; 2003). Close to 99 % of canola oil in the US is imported from Canada, which seems economically reasonable given the proximity of the two countries, thus minimizing the transportation and other overhead costs to the end users in the US (Brooks, 2009).

Canola has become an economically important oil crop as it is the third most consumed edible oil in the world next to soybean and palm. High oil content per seed (40-50%), together with oil that is less saturated, has zero trans-fats, and high oleic acids (monounsaturated fatty acid) signify a potentially growing market for canola oil (Wittkop et al., 2009; USDA, 2012). The neutral flavor of canola oil has also helped increase demand in food industries such as for frying, and as an ingredient in baking, dressings, margarine, and many other food applications (Sakurai and Pokorny, 2003). The high oleic acid content provides more stability that makes canola oils suitable for high temperature frying. The increase in demand for healthier oil has opened a specialty market for canola in the edible oil industry.

In Europe commodity rapeseed is being used as one of the major raw material sources for the biodiesel industry. European Union biodiesel policy that envisions a 10% target for use of renewable energy in road transport fuels by 2020, favors the use of rapeseed oil (Londo et al., 2010). The low saturated fatty acid content that lowers freezing temperature for engines also favors the use of canola oil for biodiesel (Luo et al., 2010). In contrast, it is less likely that it will be used as a biodiesel feedstock

in the US, mainly due to increasing demand for canola oil for use as edible oil. High-erucic acid rapeseed (HEAR) oil produced in the US is used in lubricants, hydraulic fluids, soap, and paints indicating an effort to employ rapeseed products for industrial applications (USDA, 2012).

Canola meal or rapeseed extraction meal (REM), a leftover product after extracting oil, is the second largest protein meal produced in the world. Used as feed for animals and a potential protein source for human nutrition, the meal is 40% protein from total extract (Wittkop et al., 2009).

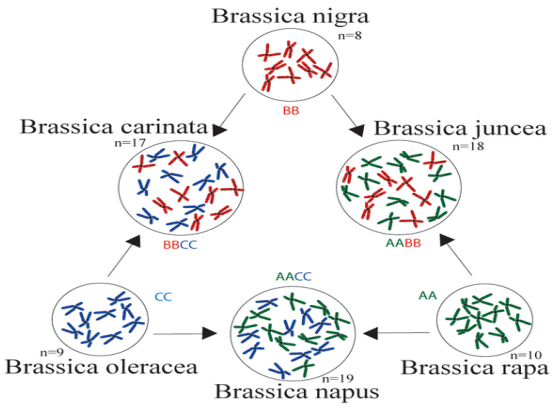


Figure 1.1 Triangle of U depicts genomic relationships of six *Brassica* species. The Letter n indicates the number of chromosomes in the gametes of each species. Diploid species found in the corners hybridized with one another to create the allotetraploid species in-between. Adapted from the Wikimedia commons file "Image: Triangle of U Simple.PNG" http://commons.wikimedia.org/wiki/File:Triangle_of_U_Simple.PNG

1.1 Canola production constraints

Canola production has been threatened by both biotic and abiotic stresses, with a wide range of severity and damage. Among biotic constraints, broadleaf weeds are of prime importance because of very intense competition that depresses yield. Most canola varieties grown in Canada and the US include either a Roundup Ready gene for glyphosate tolerance (Monsanto Company) or a Liberty-Link

gene for glufosinate tolerance (Bayer Crop Science) (Roush, 2002). This has contributed to a reduced use of herbicides which otherwise would be applied to manage weeds in canola production fields.

Flea beetles (*Phyllotreta cruciferae)* and false chinch bug (*Nysius raphanus*) are common insect pests on canola in Colorado (Sediqi, 2012). Black leg (*Leptosheina maculanus*), club root (*Plasmodiophora brassicae),* downy mildew (*Peronospora parasitica),* Sclerotinia stem rot *(Sclerotinia sclerotiorum*), and white rust (*Albugo candida*) are major diseases which affect yield (seed and oil) and quality of canola. These diseases are also very common in major canola producing districts of Australia and Canada (Nyvall, 1979; Barbetti and Khangura, 2000; Tewari et al., 2005).

Production of canola is also affected by abiotic stresses that limit crop productivity. These include heat, water logging, drought, and frost in Australia, Canada and the US (Zhang et al., 2006; Singh et al., 2008). Canola is very sensitive to high temperature stress, especially at flowering and seed filling, with high flower and silique abortion leading to considerable yield and quality reductions (Young et al., 2004; Din et al., 2011). Water logging has been reported to result in significant yield losses (Voesenek et al., 1999; Zhang et al., 2006), even as limited moisture and drought result in serious yield and quality reductions (Cheema et al., 2004; Sinaki et al., 2007; Wan et al., 2009; Wang et al., 2009, Din et al., 2011; Shirani, 2012).

1.2 Drought in Colorado

Colorado is known to experience moisture deficits in most years. In the past few decades there have been several serious drought years that continue to pose a major threat for crop production (Schubert et al., 2002; http://droughtmonitor.unl.edu/). Major crop producing areas of Colorado receive less than 450 mm (18 inches) annual rainfall, which is inherently erratic, leading to drought becoming the major recurring problem in the region. Consequently, canola production in Colorado is mainly under irrigation as the sporadic rainfall adversely affects the dryland (non-irrigated) crop production. This situation has forced agricultural research in Colorado to focus on efficient utilization of limited water

resources by developing and applying agricultural technologies relating to breeding varieties tolerant to drought stress.

Canola has been a minor crop in Colorado but has the potential to become a more important oilseed crop for either edible oil or biofuel. The development of nearby oilseed crushing facilities will be required to encourage canola production by reducing transportation costs (Nielsen, 1997; Johnson et al., 2009). The existence of genetic potential for drought tolerance as indicated in Cheema et al. (2004) encourages the exploration and development of canola genotypes with better drought adaptation for Colorado farmers. In addition, both winter and spring canola can potentially fit into wheat-based cropping systems as a rotation crop in Colorado (Johnson et al., 2009). Johnson et al. (2009) also addressed the possibility of integration of winter canola into both dry and irrigated cropping systems in Colorado. Spring canola production in Colorado could be affected by the high temperatures that reduce yield and quality (Johnson et al., 2009, Heiliger, 2012). The practicality of introducing spring canola depends on planting and harvesting time (planting in early spring and harvesting in July), followed by winter wheat planting in September (Johnson et al., 2009). Canola can also be beneficial as a rotation crop because it helps in breaking pest cycles for wheat and improving soil structure (Soon and Clayton, 2002).

1.3 Plant traits associated with drought tolerance

Drought tolerance is a relative term, which describes the ability of a plant to withstand a period of dryness from insufficient water supply (Passioura, 1997). Blum (2005) defined drought avoidance as the capacity of a plant to maintain high plant water status or cellular hydration despite the occurrence of drought. Understanding the avoidance and tolerance mechanisms and associated traits helps in breeding and selecting genotypes with better drought tolerance genetics.

Traits that are associated with drought tolerance/avoidance in plants could be above ground traits, such as leaf morphology, leaf number, water use efficiency, transpiration efficiency, and flowering

time, or below ground traits such as root architecture, morphology, length, thickness, and numbers (Reynolds et al., 2008). Most of these traits are also associated with the physiology of plant water relations and therefore, targeting them can potentially facilitate better strategies for drought tolerance improvement in plants. For instance, early flowering canola genotypes may escape from drought in some crop producing areas of the Great Plains (Chen, et al., 2005), including Colorado where such early maturity could be potentially beneficial as the reduced life span may minimize susceptibility to reduced soil water levels later in the season.

1.3.1 Leaf and carbon isotope discrimination (water use efficiency)

Studying the genetics of phenotypic and physiological traits associated with leaves would help researchers to better understand the genetic basis that may lead to utilization of the traits for breeding and improvement of drought tolerance. Plant leaf morphological and physiological traits and their response to water loss/gain and drought tolerance have been studied in many field crops and trees species. Leaf traits have been used as tools in the development of genotypes with better drought tolerance/adaption in plant systems (Martin, 1988; Condon et al., 2004). Traits such as leaf stomatal conductance, which measures the rate and passage of carbon dioxide (CO_2) entering or water vapor exiting the stomata, play a significant role in water stress avoidance. For example, stomatal conductance decreases under stress, i.e., plants close stomata to reduce water loss. Reduction in stomatal conductance results in reduction in photosynthetic rate that in turn has a direct impact on yield and yield components under stressful conditions (Lawlor and Cornic, 2002). Because of the difficulty of accurately quantifying and measuring leaf stomatal conductance in field conditions, more often associated traits that are easier to quantify and measure such as carbon isotope discrimination and relative water content are studied. Genetic variation has been detected for leaf water content in quantitative trait loci (QTL) studies in different crop species and mentioned as a selection criterion for drought tolerance as it is known to have a positive correlation with yield under drought stress in barley

and wheat (Teulat et al., 1997). However, there is limited information with regard to the leaf traits associated with drought tolerance in canola.

Water use efficiency (WUE) can be defined as an instantaneous measurement of the efficiency of carbon gain for water loss, or an integral of this efficiency over time (as a ratio of water use to biomass or yield accumulation, or the ratio of carbon assimilation to water loss). It is usually associated with stomatal conductance, transpiration and mesophyll photosynthetic capacity (Condon and Richards, 1992; Condon et al., 2004). WUE is a measure of how a plant adapts to limited moisture stress and is associated with crop improvement under conditions of limited water availability (Blum, 2005; Condon and Richards, 1992). Quantification of relative carbon isotope discrimination (CID) between ^{12}C and ^{13}C helps to estimate photosynthetic efficiency and rate. It is related to biomass accumulation and can also be a surrogate measure for WUE (Hall et al., 2005). WUE integrates the ratio of net photosynthesis to transpiration (Farquhar et al., 1982, 1989; Farquhar and Richards, 1984).

The tremendous genetic diversity and variation with respect to WUE in plants provides researchers with opportunities to use the trait for selection. For instance, Hall et al., (2005) reported a number of QTL associated with WUE in *B. oleracea*. These QTL have a range in magnitude of their control over phenotypic variation for WUE explained by carbon isotope discrimination. Blum (2005) explained that crop yield under water-limited conditions can be determined by genetic factors controlling yield potential, drought resistance and WUE. Existence of genetic variation for carbon isotope discrimination was first detected in the 1930's (Wickman, 1952; Farquhar, 1989). Heterosis for carbon isotope discrimination was also detected in *B. oleracea* (Hall et al., 2005). Discrimination against ^{13}C in plant systems is due to RUBISCO (ribulose-1, 5-biphosphate carboxylase/ oxygenase). It takes place at carboxylation and is moderated by relative pressure of CO_2 at carboxylation (P_C) site to ambient air (P_I) (Le Roux et al., 2001). Estimating the discriminating capacity of genotypes is very important to detect the existing genetic variation for further use in breeding, physiology or both.

Precisely estimating the existing isotope ratio in the plant system is essential; hence, scholars agreed to quantify the relative difference in isotope ratio between sample plant tissue and a standard through combusting the sample and using techniques in mass spectrometry (Sharp, 2007). Carbon isotope value is designated with a symbol $\delta^{13}C$. It is calculated as the ratio of the difference in carbon isotope concentration ($^{13}C/^{12}C$) between a sample and a standard to the standard PeeDee Belemnite[1] (PDB) as shown in the formula.

Formula 1

$\Delta = (Rx - Rstd)/Rstd) * 1000$ (Sharp, 2007)

R_x = ($^{13}C/^{12}C$) in a plant tissue sample

R_{std} (PDB) = ($^{13}C/^{12}C$) of the universal standard

Sharp (2007) explained that $\delta^{13}C$ value is expressed as parts per million or parts per thousand and designated by percentile (‰). The values could either be negative or positive. For example, if $\delta^{13}C$ is negative, it means the ratio of $^{13}C/^{12}C$ in the plant tissue sample is less than the standard and if positive, the ratio is greater in the sample than the standard (PDB). The $\delta^{13}C$ value for the standard is always zero and it is the only universally accepted way for reporting $\delta^{13}C$ values (Sharp, 2007). The relatively higher value of the sample compared to the PDB means there is higher $^{13}C/^{12}C$ in the plant sample and that means the plant had higher water use efficiency.

The application of carbon isotope discrimination in breeding and selection programs requires the knowledge of the relation between leaf carbon isotope discrimination and plant growth in field conditions (Condon and Richards, 1992; Lawrence, 1995). The information provided by carbon isotope discrimination helps in an integrative assessment of genotypic variation in leaf transpiration efficiency (Condon and Richards, 1992). The relatively higher heritability of the trait compared to highly variable

[1] Standard used to report relative carbon isotope ratio in most laboratories in the world (Sharp, 2007)

traits such as osmotic adjustment in crops (Kerry, 1988) make the feasibility of utilizing carbon isotope discrimination for drought associated studies which could help breeders in selecting for plants with better adaptation/tolerance to drought. For example, drought tolerant wheat genotypes were selected using carbon isotope discrimination in Australia under favorable environments during plant growth and development (Rebetzke et al., 2002). Therefore, studying the genetic structure of carbon isotope discrimination in canola would aid in understanding the genetic basis of the trait, information that could further help in its use for breeding.

1.3.2 Root traits

Plant roots are among the plant organs that directly interact with water under the soil surface. Root length, structure, thickness and number can affect a plant's ability to adapt to limited soil moisture. Plants with longer roots may access moisture by growing deep into soil during periods of moisture stress. Genetic variation underlying root traits in response to moisture deficiency has been observed in many crops (Sharp et al., 2004; Xiong et al., 2006). Hence, studying root traits to estimate the effects of genes and genomic regions is one strategy to aid in selecting genotypes with better tolerance to prevailing moisture stress (Ekanayake et al., 1985; Henry et al., 2011; Gowda et al., 2011).

Water uptake by plants from soil depends on the root density, number, length, and architecture as well as soil properties. The existence of a vigorous root system provides better tolerance to drought or nutrient stress during plant growth and development. Root traits play a critical role for increasing crop yield under moisture stress (Tuberosa et al., 2002; Kashiwagi et al., 2005; de Dorlodot et al., 2007). Most root traits are quantitatively inherited (Champoux et al., 1995). QTL mapping studies on drought tolerance and yield related traits in *Brassica* spp., maize rice (*Oryza* sativa L.), barley, wheat, and melon (*Cucumis melo* L.*),* have demonstrated that root traits are controlled by several loci, each of which has a relatively small effect (Hall et al., 2005; Xiong et al., 2006; Maccaferri et al., 2008; Pico, 2008; Landi et al., 2010). Many studies have identified a number of QTL associated with root phenotypic traits, along with

their effects on yield under varying moisture regimes (MacMillan et al., 2006; Landi et al., 2010). De Dorlodot et al. (2007) described increased interest in conducting QTL studies associated with drought tolerance conditioned by root phenotypes.

Gowda et al. (2011) explained the importance of the detailed understanding of root structure and physiology with reference to breeding for drought tolerance. Utilizing the genomic regions detected in QTL studies in marker assisted selection is the main purpose behind studying these genetic variations and dissecting the genetic basis of root traits. In contrast to other field crops such as rice, detailed QTL studies on canola root traits associated with drought are lacking.

As indicated by Champoux et al. (1995) many root phenotypic traits are considered to have be associated with drought tolerance and have been evaluated to quantify root-water relationships. Among these traits, root pulling force, the vertical force required to pull out the root system from the ground could be an important trait in a drought-associated study. Root pulling force is an integrated, quantitative measure of root system development. It has a high correlation with root number and other root morphological characters such as root thickness that help plants to penetrate soil and access water from the bottom (Ekanayake et al., 1985). Root pulling force has also been associated with drought tolerance and higher yield under moisture and nutrient stress in rice and maize (Ekanayake et al., 1985; Nguyen et al., 1997; Kamara et al., 2002). For example, Kamara et al. (2002) reported a positive correlation between root pulling force and nitrogen uptake in maize under drought stress. Studies on many root traits and morphology are destructive for the root if measured on plants before harvest, but measuring root pulling force is non-destructive if it is conducted post-harvest at the end of the plant's life cycle.

1.3.3 Yield component traits and drought

Blum (2011) explained differential expression of genes between genotypes subjected to drought stress and well-watered environments that has led to the detection of expressed gene(s) and QTL.

Studying genotypes under different moisture treatments helps understand the underlying factors contributing to the differential performance.

Yield is an integrated measure of several components that can be evaluated and used in selection in any environment, including drought. However, heritability for yield under drought stress is typically very low (Blum, 2011). Canola yield (seed and oil yield) can be dissected into components (Din et al., 2011) that include number of seeds per silique, number of siliques per stem, silique length, and thousand seed weight (Heiliger, 2012). In addition, there are many agronomic traits in canola associated with drought tolerance and avoidance including days to flowering, number of branches per plant, harvest index, root number and root distribution (Din et al., 2011). The yield components mentioned above are known to be controlled by QTL, and some of these traits have been correlated with root traits under drought stress (Bahrani, 2009).

Stem branch number, which has been reported to be controlled by QTL, is among the agronomic traits which contribute to yield in dry environments (Doust et al., 2004). Number of branches per main stem is highly correlated with yield and is significantly affected by water stress (Bahrani, 2009, Frederick et al., 2001). In addition, increased branch number results in increased biomass, which in turn may have an impact on root pulling force.

Learning the nature of yield and yield component traits associated with drought tolerance in *Brassica* spp. has helped to understand the underlying genetic structure and inheritance (Hall et al., 2005; Wang et al., 2009; Wan et al., 2009). Similar studies of dissecting QTL associated with osmotic adjustment and stay green, as in Robin et al. (2003) and Jiang et al. (2004) were reported in Cattivelli et al. 2008. These aforementioned studies highlighted the importance of understanding the genetic basis of traits to utilize the QTL underlying the trait.

1.4 Quantitative traits and QTL mapping

Biological traits are typically controlled either by many genes with small effects (polygenic inheritance) termed as quantitative traits, or by single genes with major effects (monogenic inheritance) termed as qualitative traits. The main feature of qualitative traits is the existence of a distinct difference between the presence (expression) or absence of the gene. For example, some forms of black leg resistance in canola are conferred by a few major genes that act individually (race specific) or in a group (Li et al., 2005). Resistant plants show either a hypersensitive reaction against the pathogen or a completely susceptible reaction in the absence of the gene, but there is no intermediate reaction between the two. Qualitative traits, in most cases do not interact to a major extent with the environment and they are inherited as a single dominant or recessive gene (Falconer and Mackay, 1996).

Quantitative traits, on the other hand, have a continuous distribution that indicates the presence and expression of some or all of the genes underlying the trait. The range of phenotypes is therefore distributed normally in most cases. For instance, grain yield in plants, usually exhibits a wide range of yield related phenotypes from the low yielder to an intermediate yielder to a high yielder depending on the presence and expression of the responsible genes. Genes controlling quantitative traits interact with each other (epistasis) and the expression of these traits is often influenced by environmental conditions (Falconer and Mackay, 1996). Quantitative traits in plants have been targets of crop improvement for yield, quality and pest resistance (Falconer and Mackay, 1996). Understanding the influences of genes and environment on quantitative traits is a major challenge in biological studies. Enhanced understanding of these traits leads to utilizing them in a way that benefits the scientific community (Bernardo, 2008).

Quantitative traits are associated with loci containing gene(s) on a chromosome known as QTL. Many studies have been conducted to learn the genomic regions associated with phenotypic effects and

controlled by QTL. These studies began in the early 20th century when Sax (1923) conducted his studies on seed size and color of beans. QTL studies help in identifying locations and effects of genes underlying quantitative variation (Tanksley, 1993; Mohan et al., 1997; Crosses, 2001; Sen and Churchill, 2001; Collard et al., 2005).

Detecting QTL and locating genes on a chromosome requires a set of statistical analyses and adequate computing power. The end of the 20th century is considered a time of rapid development in science and technology where remarkable achievements were made, for instance, advances in computer science, computing power, molecular biology and biotechnology (Bernardo, 2008). The development of molecular biology gave birth to identification of molecular markers on chromosomes and helped in construction of linkage maps. Linkage maps can be used to identify the relative positions of genes on a chromosome, as genetic markers are milestones for presence of genes associated with the traits. QTL mapping and the use of molecular-marker technology have made it possible to explore and learn about the inheritance of single gene and polygenic traits. This helps to locate and use individual genetic factors associated with traits of interest (Tanksley, 1993).

The current QTL mapping studies utilize genetic markers, especially DNA markers such as Single Nucleotide Polymorphism (SNP), Simple Sequence Repeats (SSR), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restricted Fragment length Polymorphism (RFLP) and others to locate the genomic regions (gene location on chromosome) with which the trait is associated (Collard et al., 2005).

QTL mapping studies are mainly conducted on populations developed from two parents (also known as bi-parental mapping populations) that have distinct differences for the trait of interest. The divergence of parents for the trait, and also distinction of their genetic background increases the chances of detection of polymorphic markers and QTL in the population (Collard et al., 2005; Mohan et al., 1997). Before constructing the linkage map and mapping analysis, genotyping of identified

polymorphic markers needs to be conducted across the population. Mapping studies can be carried out in an F_2 segregating generation, backcross population (crossing F_1 with the one of the parents termed the recurrent parent), or homozygous individual lines such as recombinant inbred lines (RIL, developed by repeated selfing for a number of generations) and doubled haploid (DH, developed from pollen grain of an F_1 plant or other method through tissue culture (Collard et al., 2005)). Depending on the resources, time and goal of the experiment, QTL mapping studies can be conducted in any of the above population types.

Commonly used methods for QTL mapping are single marker analysis, simple interval mapping and composite interval mapping analyses (Tanksley, 1993; Kearsey and Farquhar, 1998; Liu, 1998; Collard et al., 2005). The single marker technique does not require the existence of a complete linkage map. The main disadvantage of this method is lack of power to detect QTL greater than 15 cM (centi-Morgan) away from the marker (Tanksley, 1993; Collard et al., 2005). Statistical methods used to detect QTL based on single markers included the t-test, linear regression and analysis of variance (ANOVA).

Simple Interval Mapping (SIM) uses the interval between two markers to detect the likely site for QTL. It requires a linkage map, which is statistically more powerful than single marker analysis for QTL detection at a distance greater than 15 cM from the marker. The result of the statistical test is presented as LOD (logarithm of odds) or LR (likelihood ratio). For example, an LOD of 3 means there is a 10^{-3} probability of a false QTL detection in the chromosomal region. An appropriate LOD/LR threshold for detecting a QTL is generated by a permutation analysis of 500 to 1000 runs for each marker-phenotype association by keeping markers and genotype constant and shuffling the phenotypes to detect the rate of false positive QTL (Collard et al., 2005).

Composite Interval Mapping (CIM) attempts to separate and isolate individual QTL effects by combining interval mapping with multiple regression; confining the test to one region at a time reduces a multi-dimensional search problem (for multiple QTL) to a one dimensional search (Zeng, 1994). CIM

controls genetic variation in other regions of the genome which reduces background "noise" that could affect QTL detection (Zeng, 1994). Improvement in statistics and computer science supported by efforts from quantitative genetics resulted in the development of many software programs to aid in QTL detection. Among the available programs, R/qtl, Map QTL, QTL-Cartographer, and Q Gene are known to be used commonly by students and researchers (Joehanes and Nelson, 2008).

1.5 Plant breeding and application of QTL in marker assisted selection

Domestication of crops by the first breeders (farmers) in prehistoric times targeted plant morphology, which contributed to a relative increase in produce (yield). Selection of non-shattering cereal grains such as wheat and barley, and selection for leafiness and early maturity in *Brassica* are a few such domesticated traits (Diamond, 2002). Modern plant breeding is based on improvement of yield and quality, which is a continuation of the process that began during domestication.

Plant breeding, the art and science of selecting plants with desirable traits, played a vital role in increasing crop productivity through genetic improvement feeding a fast growing human population (Brummer et al., 2011). Development of canola from a rapeseed through conventional breeding in the mid-20th century is one of the countless achievements of plant breeding (Harvey and Downey, 1964; Shahidi, 1990). It has contributed to quality of life and economic development in the oilseed industry.

Conventional plant breeding was effective in selecting and developing germplasm with higher yield, that is stable across different agro-ecologies and tolerant to stress environments. Selection for low heritability traits such as yield is very cumbersome. The length of time to develop improved varieties using the conventional plant breeding methods motivated breeders to find tools that help them achieve goals faster. The progress in molecular marker technology accelerated discoveries of QTL associated with traits of interest (Collard, et al., 2005; Bernardo, 2008). For instance, QTL for oil content and drought tolerance related traits in canola and other *Brassica spp*. were detected in several studies (Hall et al., 2005; Delourme, et al., 2006; Zou et al., 2010). The QTL reported in studies of most major crops

usually account for a significant percent of the phenotypic variation. QTL alleles associated with drought related traits have been identified and used in breeding in many crops, including root architecture in rice (Steele et al., 2006) and maize (Tuberosa et al., 2002, 2003), leaf relative water content in barley (Teulat et al., 1997), stay green in sorghum (Borrell et al., 2000; Harris et al., 2007), and carbon isotope discrimination in *Brassica* (Hall et al., 2005), among others.

Marker Assisted Selection (MAS) for quantitative traits includes the use of DNA marker technology and QTL analysis to select plants with desired traits. In plant breeding MAS potentially helps in shortening the cycle of selection and enhancing genetic gains (Xu and Crouch, 2008; Moose and Mumm, 2008). There was a relatively low rate of converting QTL detected for application in MAS due to the unstable nature of QTL, dependence on genetic background, and high QTL X environment interaction. However, advancement in discoveries of molecular markers leading to huge marker databases promoted application of MAS as routine in public breeding institutions (Bernardo, 2008).

There are several success stories across multiple crops using MAS for introgression of favorable QTL alleles associated with drought tolerance. For instance, root length and thickness associated QTL have been successfully introgressed in rice (Steele et al., 2006). Other successes include introgressing QTL alleles associated with drought tolerance such as early maturity in rice (Steele, 2006), high grain yield in rice (Bernier et al., 2009), and high yield under drought in maize (Ribaut and Ragot, 2007), barley (Tuberosa and Salvi, 2006), sorghum (Harris et al., 2007), and wheat (Dubcovsky, 2004). A review of literature revealed no MAS publication in canola, although many multinational breeding companies use MAS strategy to enhance genetic gains and shorten the cycle of varietal development (Xu and Crouch, 2008).

The progress made in molecular biology leading to identification and use of high throughput DNA markers such as SNPs and the decline in cost of sequencing will certainly accelerate the detection,

dissection and understanding of the genetic basis of QTL. This knowledge may facilitate the cloning of identified QTL and their use in MAS programs, which may be most helpful for low heritability traits. Therefore the objectives of this experiment are

- To detect the location, number, and effects of QTL associated with root pulling force, carbon isotope discrimination, and yield related traits in a *Brassica napus* L. DH population
- To better understand the association of root pulling force and carbon isotope discrimination with yield component traits in well watered and water stressed environments
- To compare and validate QTL detected in this study based on a single plant analysis with QTL detected in a previous field plot experiment of the same DH population

2. MATERIALS AND METHODS

2.1 Plant materials

A *Brassica napus* L. DH population derived from a cross between two spring genotypes was used in this study. The parents were DH12075, a black seeded line derived from a cross between 'Westar' and 'Cresor' by the research group at Agriculture and Agri-Food Canada (AAFC) (Hegedus et al., 2003), and YN01-429, a yellow seeded line (Rakow and Relf-Eckstein, 2005). Pollen from F_1 progenies was developed into the DHYB population using microspore culture. The DHYB population used for this study had 148 lines genotyped with 321 SSR markers. The population and marker data were obtained from the Plant Biotechnology Institute, Saskatoon, Saskatchewan in Canada.

2.2 Greenhouse experiment

In December 2010, the seeds of each DH line along with the parents (DH12075 and YN01-429) were grown in a research greenhouse at Cargill Specialty Seeds and Oils Innovation Center, Fort Collins, CO for seed increase and evaluation of carbon isotope discrimination. The seeds were planted in three replications in 15 cm x 15 cm x 15 cm plastic pots (Greenhouse Megastore, Denver, CO) using a general purpose growth soil medium with bio-fungicide (Pro-Mix BX, http://www.jrjohnson.com, St. Paul, MN). Three seeds were planted in each pot; eight days after planting, pots were thinned to one seedling per pot and grown to maturity. Just before the beginning of flowering, a sample of 1-2 cauline leaves (leaves that grow directly from the stem just below the inflorescence) was collected from each DH line for carbon isotope discrimination analysis.

Flowering dates were recorded as the day on which the first flower appeared. At flowering each plant was covered with a plastic bag (sealed air bag Cryovac® www.sealedair-emea.com, Sturtevant, WI) to facilitate self-pollination. At maturity, each plant was harvested individually and threshed at Cargill Specialty Seeds and Oils seed handling and preparation unit.

2.3 Field experiment

During summer of 2011 and 2012 the trial was conducted at the Agricultural Research, Development and Education Center (ARDEC) of Colorado State University located six miles North East of Fort Collins, CO (40.6˚N/105.1˚W; Elev. 1557 m). The location is characterized by a clay loam soil texture (www.coagmet.com). The field was tilled to a very fine tilth before planting, providing a suitable seed bed for planting the small sized seeds of canola and allowing for germination within the expected range (10-15 days) after planting. Prior to planting, the seeds were treated with Prosper FX (Bayer Crop Science Inc., Alberta, Canada; EPA registration number 29159) at the rate of 1 mL and 1 g of talcum powder per 5 g of seed, for protection against flea beetles (*Phyllotreta cruciferae* Goeze) (Heiliger, 2012). The experiment was conducted in a split-plot design with three replications repeated over two years at a single location. The two moisture regime treatments, irrigated (wet) and moisture stressed (dry) were used as the main plot factor and genotypes (the DH lines and parents) were used as the subplot factor. The DH lines were randomized within the main plots. Both parents and 148 individual lines from the DH population were planted at ARDEC on April 18 of each experimental year. A block (replication) that contained two contrasting water treatments (main plots) was set up for a uniform irrigation from a pump assigned exclusively for each block. About 5-10 seeds of each genotype were hill planted at a depth of 2.5 cm and were thinned to a single plant two weeks after planting.

A drip irrigation emitter placed a few centimeters from the base of the plant watered each plant. The plants were watered four times a week for 45 minutes at a time, which amounted to about 25 mm of water. Plants in the wet treatment continued to receive water at this rate throughout the experimental period. In 2011, the dry treatment plots were watered from planting until six weeks by which time the plants reached the stem elongation stage and were able to survive with the available water from normal precipitation. However, the summer of 2012 was unusually dry with negligible amounts of rain and unusually high temperatures which could have had an adverse effect on the

experiment, particularly the dry treatment. Therefore, the dry treatment was supplemented with additional water once a week for an hour (31.25 mm), while the wet treatment continued to be irrigated four times per week.

As canola is a heavy feeder of nitrogen and phosphorous, supplementing with additional fertilizer was imperative. Therefore, a slow release garden fertilizer, Osmocote Plus 15-19-12 (Scott Miracle-Gro, Marysville, OH) was applied at five weeks after planting at a rate of 1 g per seedling. The fertilizer was applied by hand in an approximately 15 cm radius surrounding the seedlings. The plots were hand weeded twice a week for the first two months and as necessary thereafter.

2.4 Data collection

Due to the high temperature and very dry weather in spring of 2012, there was a very high infestation of flea beetle, despite the pre-treatment. Data were collected on the extent of damage per genotype. The damage was rated using a 0 or 1 scale, with 0 indicating no damage and 1 indicating infestation. Spot application of a garden insecticide containing carbaryl (Sevin) (Bayer Environmental Science Research Triangle Park, NC) was applied to those genotypes that were severely infested by the flea beetle.

To measure CID leaf samples were collected when the plants began flowering. Two to three cauline leaves with lengths of 5 to 10 cm were collected from each plant with care taken not to collect shaded or insect-damaged leaves. Leaves were placed in empty labeled 15 ml centrifuge tubes (33200, PPCO, US Plastic Corp, Lima, OH) that had been weighed with a scientific balance (Mettler Toledo Group PL 2202-5, Leicester, UK) at Cargill Specialty Seeds and Oils. The test tubes with the samples were placed into plastic bags and packed in a cooler with ice packs to limit the moisture loss from the leaves. The samples were placed in a cooler and taken to the Cargill Specialty Seeds and Oils facility, and the tubes were weighed again to measure fresh leaf weight. The samples were stored in a -80°C freezer and later freeze dried for three days in a freeze drier (Labconco, Kansas City, MO) at Colorado State University.

The leaf water content was calculated as the difference between the dry and fresh leaf weight; relative leaf water content was calculated as the ratio of leaf water content to the fresh leaf weight for each sample collected.

Four to six 4.5 mm steel ball BBs (www.daisy.com, Daisy® Rogers, AR) were placed into the test tube with the samples, and ground with a paint shaker (Fluid Management SK-650, Wheeling, IL) for one minute. A 2 mg quantity of the ground leaf tissue was weighed and sealed into an aluminum cup (Tin Capules 5 x 9 mm cups, Costech Analytical Technologies Inc., Valencia, CA). The cups were then placed in a 96 well-tray box, in which a single well was allocated to a control sample of ground maize leaf. The tray-box was sealed and sent to the Stable Isotope Facility at the University of California, Davis. The $\delta^{13}C$ in the tissue samples was analyzed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (http://stableisotopefacility.ucdavis.edu) following the procedures and principles indicated by Sharp (2005).

Days to flowering was recorded as the number of days from planting until the first bud opened and bloomed. Recording continued every other day until all plants had begun flowering. Plant height was measured from the soil surface to the top of the inflorescence at maturity using a measuring stick. Primary branches growing directly from the main stem were counted but secondary branches developing from the primary branches were not. Branches were counted between the end of flowering and beginning of physiological maturity.

Reproductive investment of each genotype over its lifespan was estimated by counting flowers and siliques in the 2012 experiment. Number of open flowers, number of aborted flowers (shriveled and brown flower buds), number of unopened flower buds (turgid and green flower buds), number of pollinated flowers (flowers with wilted corolla), number of developing fruits or siliques (green, turgid and elongated ovaries), and number of aborted siliques (shriveled, brown and small ovaries) were counted in the field. These counts were determined per plant within 2-3 weeks after the beginning of

flowering. Total number of siliques per plant was calculated as a sum of number of developing fruits and number of aborted siliques. Proportion of aborted siliques was calculated as a ratio of number of developing fruits or siliques to total number of siliques per plant. These variables were recorded in the field at the appropriate times during the experimental period by Dr. Arathi Seshadri (Department of Soil and Crop Sciences at CSU) and her student workers.

To evaluate root pulling force at physiological maturity, when the color of seed pods changed from green to yellow, both dry and wet treatments were watered by an emitter for 1 hour for three consecutive days so as to saturate the soil to field capacity. This watering reduces root damage during the process of root pulling. A Dynamometer (DS2-220, Imada, Northbrook, IL) was attached to a 50 cm long rope tied to the root crown at the other end. Plants were pulled vertically by a person standing straight over a plant with legs apart. The Dynamometer was adjusted to record a reading of the maximum force exerted in kilograms during the process of pulling the plant from the ground and re-set after pulling a plant.

Pulled plants were cut to remove the stem just above the root crown. Soil particles and mud from the root were removed by hand and major (coarse) roots were counted. The upper part of the plants with foliage and pods were weighed to estimate the fresh weight of above ground biomass of the plant. The fresh biomass of individual plants were put separately into paper bags and placed in an oven at 40°C for five to seven days, after which the dry biomass weight was recorded.

The dry biomass was threshed by a single plant thresher (ALMACO, Special Agricultural Equipment, Nevada, IA), and the seed and chaff were separated by a column seed cleaner (www.agriculex.guelph.org Ontario, Canada). Total seed weight of each plant and weight of 200 seeds from each plant counted using a seed counter (Model U, International Marketing and Design Corp, San Antonio, TX) were recorded. Thousand seed weights were extrapolated from the 200 seed weights.

2.5 Data and QTL analysis

The data collected were summarized and tested for normality using the Shapiro–Wilk test of the Univariate procedure of SAS® 9.2 (SAS Institute Inc. 2010). Analysis of Variance (ANOVA) and least squares means of phenotypic values of all traits for both treatments and years were calculated using the Mixed procedure of SAS® 9.2 with replication and plant position as random effects. Least squares means were used to graph the frequency distribution of the phenotypic values. To visually display transgressive segregation, phenotypic values of the DH lines were plotted along with both parents for both treatments and years. Linear regression was conducted to determine the relationship between thousand seed weight and yield. A drought index was calculated as the ratio of the difference between 1 and the ratio of individual line mean of dry to wet to the difference between 1 and the ratio of the grand mean of dry to the grand mean of wet, as shown in the following formula (Fischer and Maurer, 1978) (Table A2):

$$Dx = \frac{1 - \frac{yd}{yw}}{1 - \frac{\mathrm{Yd}}{\mathrm{Yw}}}$$

y_d is mean of individual DH line under dry; y_w is mean of individual DH line under wet

Y_d is grand mean of genotypes under dry; Y_w is grand mean of genotypes under wet

D_x is drought index

Broad sense heritability estimates for traits was estimated using following formula:

$h^2 = V_G / V_P$

Where h^2 is broad sense heritability; V_G is the total genotypic variance

V_P is the total phenotypic variance

Estimates of the genetic and phenotypic variance components were calculated in SAS by using the Varcomp procedure. Phenotypic correlation coefficients between all pairs of traits were calculated

for each treatment in each year using least squares means. A T-test was used to compare phenotypic mean values of DH lines grown in the two treatments.

A genetic linkage map constructed by Dr. Jack Mullen (Colorado State University) with JoinMap 4 software (Van Ooijen 2006) was used for the QTL analysis. Markers were assigned to linkage groups based on recombination frequency, with a threshold of 0.25. A total of 321 SSR markers were mapped using the maximum likelihood algorithm, with the Kosambi mapping function. The genetic linkage of the markers was mapped onto 21 linkage groups. QTL analysis was conducted with R/qtl software (Broman et al. 2003; www.rqtl.org) using interval mapping with 1.0 cM walking distance to calculate the significance of marker-trait associations using a standard interval (EM) method and the Haley-Knott regression algorithm. Least squares means of each DH line were used to calculate the genomic region associated with variation in the trait using the *calc.genoprob* R/qtl command. A genome scan for QTL was done using the *sacnone* command. To help detect interacting QTL and to separate the QTL, a two-dimensional scan *scantwo* with 1000 permutations was conducted, to confirm the QTL detected earlier with *scanone.* Significant loci, using a 5% genome wise threshold determined from 1000 permutations, were selected using a step-wise model selection approach (Manichaikul et al., 2009) by using the *StepwiseQTL* command. A *fitqtl* model analysis was conducted to find the R^2 (proportion of phenotypic variation explained by QTL) and logarithm of odd (LOD) score of the model and the QTL. The *Effectscan* command was used to detect the gene action (additive vs. epistasis) associated with the QTL. Covariate analysis was performed in situations where QTL were not detected in either of the treatments; combining means of the two treatments helped by increasing the number of observations and the power of QTL detection. Covariate analysis was done by considering treatment (wet and dry) as covariate using the *Addcovar* command.

3. RESULTS

3.1 Growing conditions

In general, the 2011 growing season was favorable for canola growth and development. The season can be described as having cool to moderate temperatures accompanied by ample precipitation. A hail storm that removed leaves and flowers occurred on June 10. However, plants were able to recover from the damage through regrowth of leaves and inflorescences. In contrast, the growing season in 2012 was very dry and hot, less conducive for canola growth and development. Early moisture stress at planting, germination and seedling establishment, accompanied by hot and dry weather at vegetative and flowering stages, severely affected the canola genotypes in 2012. Monthly average high temperature and precipitation in June 2011, which encompassed the late vegetative and early reproductive stages, were 35.5 °C and 51.1 mm, respectively. However, in the 2012 growing season the average high temperature for June was 38.2 °C with only 15.8 mm of rainfall. Monthly average temperature and precipitation data for both growing seasons are shown in appendix Table A1.

3.2 Descriptive statistics and ANOVA

Data were checked for normality and appropriate transformations were performed as necessary prior to any statistical analysis. Most of the traits showed an approximately normal distribution; some of the frequency distribution seems normally distributed however, statistically deviated from normality (Appendix Figures A1, A2, A3, A4 and A5). Arcsine transformation was used to transform proportion of silique abortion as the data deviated from normality.

The response of genotypes differed between years, with some traits showing a significant difference among genotypes in 2011 but not in 2012 for the same moisture treatment (Table 3.1). Genotypes differed significantly (P<0.001) for days to flowering and plant height in both well-watered (wet) and moisture-stressed (dry) treatments in both 2011 and 2012. While there were highly significant differences (P<0.001) among genotypes for root pulling force in the wet treatment in both years,

significant differences among genotypes in the dry treatment were restricted to 2011. Lateral branch numbers differed significantly (*P*<0.001) among genotypes under both treatments in 2011, however, no significance was detected in 2012 under either treatment. Significant differences (*P*<0.001) among genotypes were observed for thousand seed weight in the wet treatment of 2011, but no significance was detected in 2012. Significant differences (*P*<0.001) among genotypes were detected for fresh biomass in the wet treatment of 2011, however, significance was detected only at the *P*<0.05 level in both wet and dry treatments of 2012. Differences among genotypes for both thousand seed weight and fresh biomass were non-significant in the dry treatment of 2011. While there was a significant difference among genotypes (*P*<0.001) for the proportion of aborted siliques in the wet treatment of 2012, seed yield per plant (yield) was significantly different (*P*<0.05) among genotypes in 2011 in both treatments but not in either wet or dry treatments in 2012.

Table 3.1: Significance of genotypes in wet and dry treatments and of genotype by environment interaction in the analysis of variance for 10 traits measured in the DHYB canola population in 2011 and 2012 in Fort Collins, CO. Number presented are F-Values from the analysis. (N = 444)

	2011			**2012**		
Trait (units)	**Dry**	**Wet**	**GxE**	**Dry**	**Wet**	**GxE**
Days to flowering (no.)	1.64**	2.65**	14.38**	2.01**	2.74**	1.14
Plant height (cm)	2.01**	2.87**	1.25*	1.53**	2.23**	0.99
Lateral branch numbers (no.)	1.91**	2.31**	1.02	0.87	1.01	0.92
Root pulling force (kg)	1.88**	1.82**	0.77	1.12	1.53**	0.87
Lateral root number (no.)	1.03	1.18	1.07	1.01	1.19	0.83
Leaf relative water content (%)	1.29*	1.33*	1.03	na	0.87	na
δ13C (‰)	2.34**	1.56*	0.90	na	2.01**	na
Seed yield per plant (g)	1.59*	1.69*	1.16	0.98	1.10	0.76
Thousand seed weight (g)	1.14	2.43**	1.06	0.78	1.31	0.46
Fresh biomass (kg)	1.13	1.54**	0.12	1.79*	1.09*	0.77
Proportion of aborted silique	na	na	na	na	1.48**	na

*,** Significant at *P*<0.05 and *P*<0.001, respectively
na, data not available; N, number of genotypes

3.2 Trait means, correlation and heritability

3.2.1 Days to Flowering: The mean value for days to flowering was similar in both treatments and years, ranging from 69.5 to 70.6 days (Table 3.2). However, the general trend indicates the mean number of days to flowering was slightly less in the wet treatment than dry for both years. On average genotypes in the DHYB population took slightly longer to initiate flowering in 2011 as compared to the 2012 growing season. The earliest genotype to flower did so within 63 days after planting in 2011 and within 58 days after planting in 2012, both under the dry treatment. The latest flowering genotypes bloomed 80 days after planting in the dry treatment in 2011, and 92 days after planting in the wet treatment in 2012. Transgressive segregants were observed for days to flowering in both years and treatments (Figure 3.1).

In both parental lines, the mean days to flowering decreased by a few days in the dry treatment of 2012 as compared to the dry treatment of 2011. However, parental genotypes took longer to flower under the wet treatment of 2012 as compared to the wet treatment of 2011 (Table 3.3). Higher heritability for days to flowering under the wet treatment for 2011 and 2012 indicated that the wet treatment elicited less environmental variance as compared to the dry treatment in our study (Table 3.4). There was a strong negative correlation (r = -0.44 (wet), P<0.001; r = -0.56 (dry), P<0.001) between days to flowering and seed yield per plant in both treatments of 2011 but no significant association with seed yield per plant was found in 2012 (Table 3.5 and 3.6). Thousand seed weight was negatively correlated (r = -0.47 (wet), P<0.001; r = -0.25 (dry), P<0.001) with days to flowering in wet and dry treatments of 2011. Similarly a negative correlation was detected between days to flowering and thousand seed weight (r = -0.18 (wet), P<0.05; r = -0.11 (dry), P<0.05) in 2012.

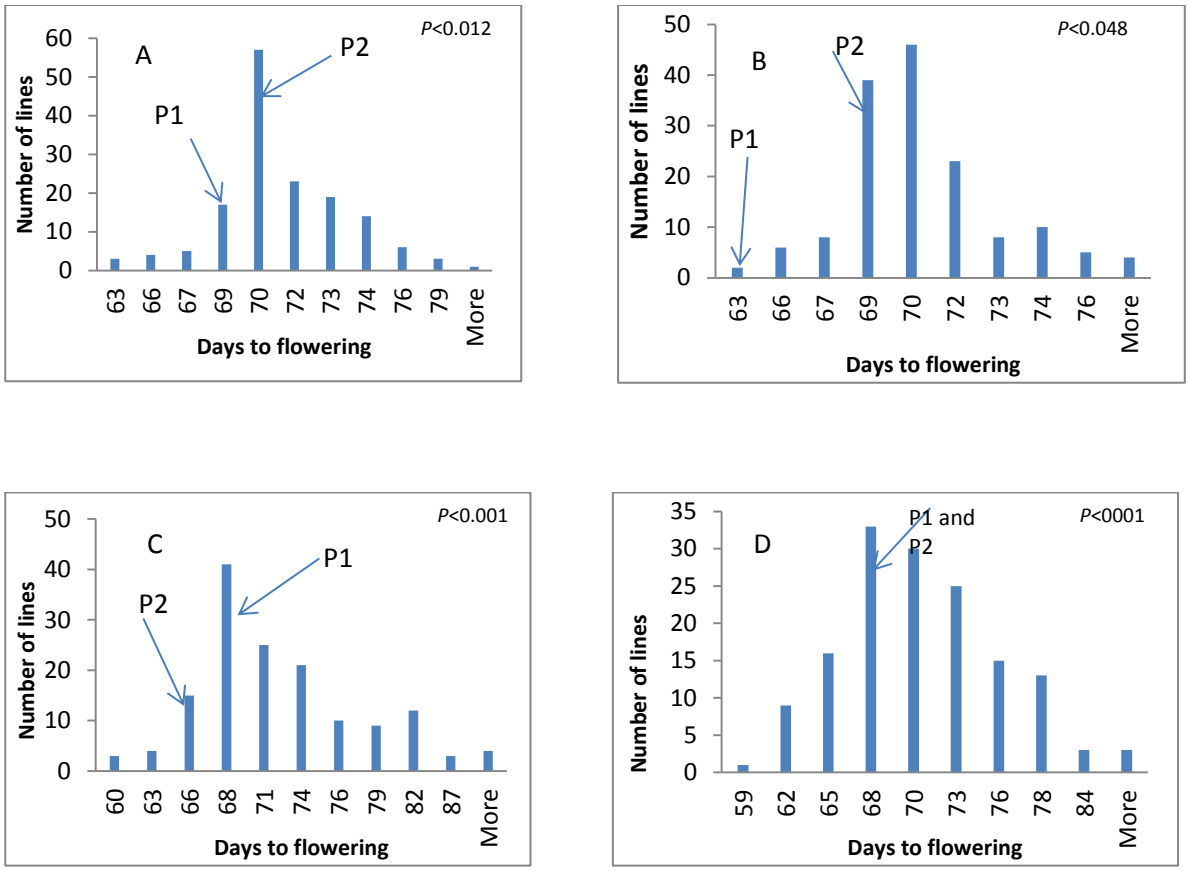


Figure 3.1: Frequency distribution of days to flowering in the DHYB canola population in Fort Collins, CO. A, dry treatment, 2011; B, wet treatment, 2011; C, dry treatment, 2012 and D, wet treatment, 2012 P1, YN01-429; P2, DH012075. The *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)

Table 3.2: Population mean, standard error, range and significance of lines for 10 traits measured in the DHYB canola population in 2011 and 2012 under two moisture treatments in Fort Collins, CO.(N = 444)

	2011				**2012**			
	Dry		**Wet**		**Dry**		**Wet**	
Traits	**Mean + SE**	**Range**	**Mean + SE**	**Range**	**Mean + SE**	**Range**	**Mean + SE**	**Range**
DTF (number)	70.1 + 0.2	63.0 to 80.1	69.9 + 0.2	61.7 to 78.7	70.6 +0.3	57.7 to 89.5	69.5 +0.3	59.3 to 92.3
PHT (cm)	72.2 + 0.5	43.3 to 96.0	91.9 + 0.7	68.9 to 119.0	76.6 +0.7	43.5 to 100.6	97.5 + 0.6	55.5 to 129.1
LRN (number)	14.3 + 0.2	6.5 to 20.7	15.6 + 0.2	9.3 to 22.0	16.8 + 0.3	6.0 to 29.7	19.5 + 0.3	11.7 to 26.7
RPF (kg)	32.5 + 0.6	15.6 to 61.3	50.0 + 0.6	30.1 to 74.6	53.9 + 0.9	33.8 to 83.5	74.5 + 0.8	47.4 to 104.7
LBN (number)	11.8 + 0.1	5.9 to 17.3	13.6 + 0.1	9.7 to 18.7	15.9 + 0.2	10.6 to 29.9	18.4 + 0.2	13.3 to 22.7
RWC (%)	81.8 + 0.1	78.0 to 86.4	84.8 + 0.1	80.5 to 87.3	na	na	81.8 + 0.4	53.2 to 93.0
δ13C (‰)	-26.8 + 0.1	-27.6 to -25.3	-27.9 + 0.1	-28.8 to -26.9	na	na	-27.7 + 0.1	-29.1 to -25.5
Yield (g)	6.3 + 0.7	0.1 to 22.1	14.6 + 0.6	0.2 to 40.9	1.3 + 0.35	0.1 to 10	1.8 + 0.2	0.56 to 10.0
TSW (g)	2.7 + 0.1	2.1 to 3.9	2.8 + 0.1	2.5 to 4.3	2.4 + 0.1	1.7 to 3.5	2.6 + 0.1	1.6 to 3.9
FB (kg)	0.3 +0.1	0.2 to 1.9	0.6 +0.1	0.3 to 1.1	0.65 + 0.1	0.1 to 2.4	1.2 + 0.8	0.2 to 3.2

DTF, days to flowering; PHT, plant height; LRN, lateral root numbers; TSW, thousand seed weight; RPF, root pulling force; LBN, lateral branch numbers; Yield seed yield per plant; FB fresh biomass; RWC, Leaf relative water content,
Bold indicates a significantly higher (P<0.05) mean value of the trait compare to the other treatment of the same year.
Na, no data available; N, number of genotypes

Table 3.3: Parental values of 10 traits measured in the DHYB canola population in 2011 and 2012 under two moisture treatments in Fort Collins, CO. (N = 444)

	2011				2012			
	Dry		Wet		Dry		Wet	
Traits	YN01-429	DH12075	YN01-429	DH12075	YN01-429	DH12075	YN01-429	DH12075
DTF	67.3	70.0	65.3	67.7	65.0	66.9	68.5	68.4
PHT	64.0	66.8	85.7	103.0	69.9	86.1	94.0	107.8
LRN	14.7	11.9	17.0	16.7	19.3	16.2	19.4	20.6
RPF	23.1	33.7	40.2	48.6	57.4	55.6	79.8	69.0
LBN	13.3	11.3	12.3	11.3	14.2	15.7	21.0	18.0
RWC	80.2	78.0	85.1	83.7	na	na	81.9	83.4
δ13C	-26.5	-26.8	-28.5	-27.9	na	na	-27.1	-28.4
Yield	4.8	4.2	31.2	9.2	1.5	0.4	1.0	0.9
TSW	2.3	2.2	2.7	3.3	0.7	0.5	3.5	2.9
FB	0.1	0.2	0.4	0.6	0.4	0.8	0.8	1.5

DTF, days to flowering; PHT, plant height; LRN, lateral root numbers; TSW, thousand seed weight; RPF, root pulling force; LBN, lateral branch numbers; Yield seed yield per plant; FB fresh biomass; RWC, Leaf relative water content; N, number of genotypes

Table 3.4: Broad sense heritability estimates for 10 traits measured in the DHYB population in 2011 and 2012 under two moisture treatments in Fort Collins, CO. (N = 444)

	2011		2012	
Traits	Dry	Wet	Dry	Wet
Days to flowering	0.43	0.63	0.45	0.56
Plant height	0.53	0.66	0.41	0.55
Lateral branch number	0.20	0.19	0.11	0.17
Root pulling force	0.48	0.47	0.22	0.34
Lateral root numbers	0.16	0.55	0.02	0.07
Leaf relative water content	0.17	0.22	na	0.11
δ13C	0 .56	0.35	na	0.51
Seed yield per plant	0.44	0.50	0.03	0.25
Thousand seed weight	0.11	0.70	0.01	0.29
Fresh biomass	0.15	0.38	0.13	0.17

na, data not available; N, number of genotypes

3.2.2 Root pulling force: In both years, the mean root pulling force value in the dry treatment was about 20 kg less than in the wet treatment. The difference of values for root pulling force between the wet and dry treatments was 17.5 in 2011 and 20 kg in 2012, with a higher root puling force recorded

in 2012 (Table 3.2). The lowest force required to pull a plant from the ground in the wet treatment was twice as high as that required in the dry treatment in both years. This indicates a better root establishment under the wet treatment. In 2011, the DH12075 parent had a higher root pulling force value in both treatments, but in 2012, a drier year, YN01-429 had higher root pulling force values in the wet treatment. Transgressive segregants were detected for root pulling force in both years and treatments (Figure 3.2). In addition to having higher root pulling force values in the wet treatment, the heritability for root pulling force was also higher in the wet than in the dry treatments in 2012, but values were about the same for the two treatments in 2011 (Table 3.4). Root pulling force was positively correlated with plant height, root numbers and fresh biomass (P<0.001) in both years and treatments (Table 3.5 and 3.6). In both years, phenotypic correlations between root pulling force and fresh biomass were higher in the dry treatment (2011: r = 0.58, P<0.001; 2012: r = 0.33, P<0.001) than in the wet treatment (2011: r = 0.47, P<0.001; 2012: r = 0.17, P<0.05). Yield and root pulling force were positively correlated in the wet treatment of 2012 (r = 0.29, P<0.001), but negatively correlated in the wet treatment of 2011 (r = -0.16, P<0.05).

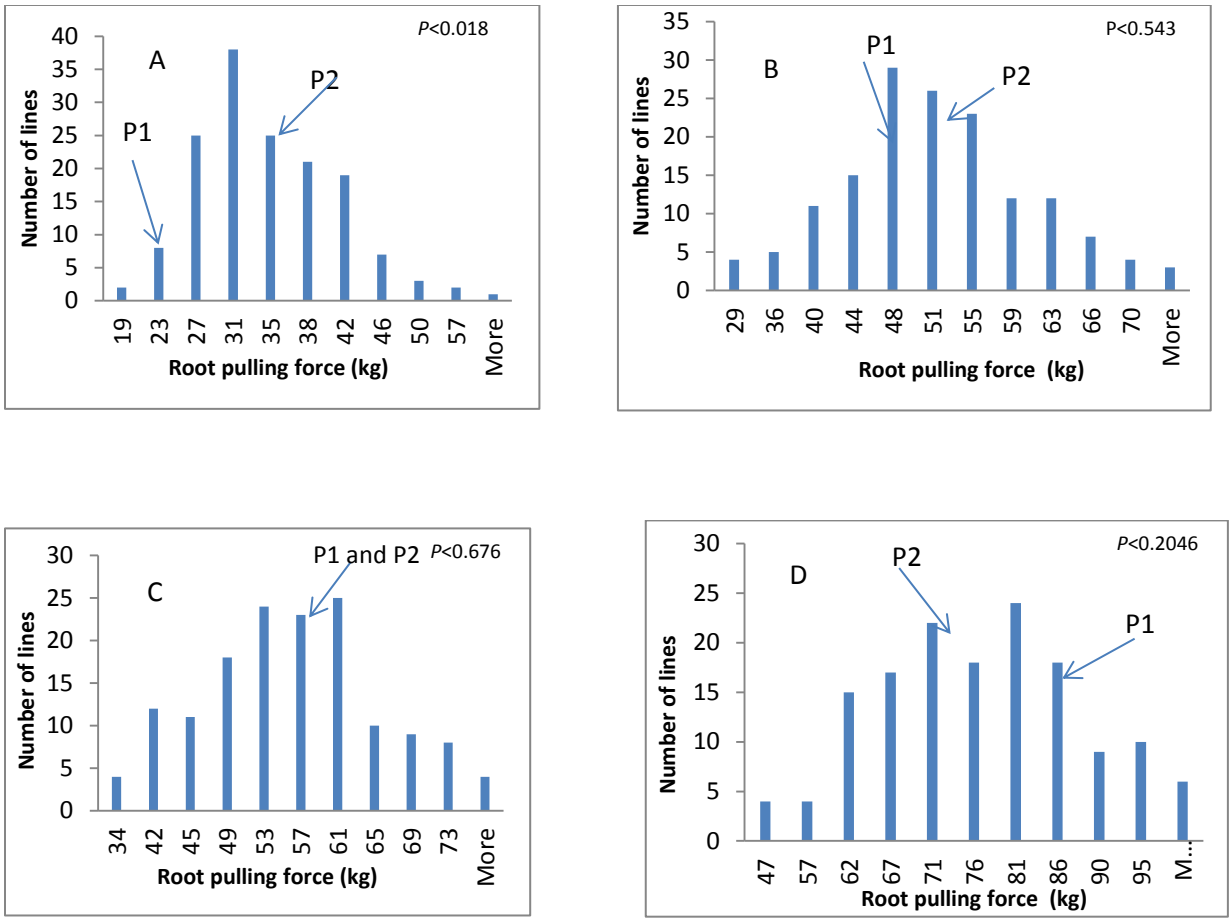


Figure 3.2: Frequency distribution of root pulling force in the DHYB canola population in Fort Collins, CO. A, dry treatment 2011; B, wet treatment, 2011; C, dry treatment, 2012 and D, wet treatment, 2012 P1, YN01-429; P2, DH012075.The *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)

3.2.3 Plant height: The population mean for plant height was more than 20 cm lower in the dry than the wet treatment in both years, a reduction of about 21% in each case (Table 3.2). The population mean for plant height was higher in 2012 than the 2011 growing season. Being a shorter plant as observed in the dry treatment may be associated with drought avoidance mechanisms of reducing surface area to minimize water loss. The shortest plants recorded were in the dry treatment at 43 cm in both 2011 and 2012. The tallest plants were in the wet treatment, with 119 and 129 cm in 2011 and 2012, respectively. The range of plant height was larger in the wet than the dry treatment in both years, indicating that plants in well-watered conditions could express their full plant height potential better than their counterparts in moisture-stressed conditions. The heritability for plant height was also higher in the wet than in the dry field treatments in both years (Table 3.4). Plant height showed a significant positive correlation with days to flowering in the wet treatment of 2011 (r = 0.45, P<0.001) and 2012 (r = 0.20, P<0.05), but no correlation was detected in the dry treatment of either year. The 2011 wet treatment studies showed a significant negative correlation between plant height and seed yield per plant (r = -0.30, P<0.001) and with thousand seed weight (r = -0.18, P<0.05). However, the 2012 wet treatment resulted in positive correlations between plant height and seed yield per plant (r = 0.26, and r = 0.28, P<0.001) in both wet and dry treatments and no significant correlation with thousand seed weight. In general, the black seeded parent, DH12075 was taller than the yellow seeded parent (YN01-429) in both years and treatments (Table 3.3). Transgressive segregants for plant height were detected in all experiments (Appendix Figure 1A).

Table 3.5: Pearson correlation coefficients among 10 traits measured in the DHYB population in 2011 under the wet treatment (above the diagonal) and dry treatment (below the diagonal) in Fort Collins, CO. (N = 444).

	DTF	PHT	LBN	RPF	LRN	FB	TSW	Yield	RWC	δ13C
DTF		0.45**	0.11	0.19*	-0.19*	0.05	-0.47**	-0.44**	0.31	0.31**
PHT	-0.13		0.05	0.54**	0.14	0.46**	-0.18*	-0.30**	-0.16	0.23*
LBN	-0.25**	0.34**		0.20*	0.17**	0.33**	-0.14	0.10	-0.20*	-0.31**
RPF	0.03	0.43**	0.20*		0.29**	0.47**	-0.11	-0.16*	-0.16*	-0.17
LRN	-0.27**	0.40**	0.24**	0.42**		0.29**	0.06	0.05	-0.29**	0.09
FB	0.01	0.42**	0.29**	0.58**	0.05		-0.10	0.12	-0.18*	0.01
TSW	-0.25**	0.21	0.23**	0.14	0.28**	0.08		0.33**	-0.04	-0.17*
Yield	-0.56**	-0.16	0.05	-0.03	0.10	-0.05	0.63*		0.05	-0.19*
RWC	-0.03	-0.07	-0.04	-0.04	-0.11	0.18*	-0.12	0.01		-0.28**
$\delta^{13}C$	0.41**	-0.09	0.02	-0.07	-0.09	0.64**	0.19*	0.10	-0.34 **	

*,** Significant at *P*<0.05 and *P*<0.001, respectively
DTF, days to flowering; PHT, plant height; LBN, lateral branch numbers; RPF, root pulling force; LRN, lateral root numbers; FB, fresh biomass; TSW, thousand seed weight; Yield, seed yield per plant; RWC, Leaf relative water content; ABO, Proportion of silique abortion; na, no data available; N, number of genotypes

Table 3.6: Pearson correlation coefficients among 11 traits measured in the DHYB population in 2012 under the wet treatment (above the diagonal) and dry treatment (below the diagonal) in Fort Collins, CO (N = 444).

	DTF	PHT	LBN	RPF	LRN	FB	TSW	Yield	ABO	RWC	$\delta^{13}C$
DTF		0.20*	-0.23*	0.15	-0.14	0.20*	-0.18*	0.04	-0.46**	0.95	0.58**
PHT	-0.04		0.18*	0.34**	0.13	0.26**	0.06	0.26**	-0.27**	0.51	0.03
LBN	-0.18*	0.44**		0.13	0.27**	0.03	0.18*	0.15	0.04	-0.07	-0.13
RPF	-0.01	0.32**	0.24**		0.31**	0.17*	0.03	0.29**	-0.11	0.05	0.08
LRN	-0.13	0.13	0.07	0.21*		0.12	0.01	0.02	0.13	-0.03	-0.15
FB	-0.07	0.40**	0.45**	0.33**	-0.04		0.07	0.01	0.04	0.07	0.01
TSW	-0.11*	0.01	0.06	-0.05	0.12	0.22*		0.05	0.23*	0.11	-0.11
Yield	0.05	0.28**	0.16	0.04	-0.04	0.22*	0.24		-0.09	0.09	0.09
ABO	na	na	na	na	na	na	na	na		0.10	-0.17
RWC	na	na	na	na	na	na	na	na	na		-0.09
$\delta^{13}C$	na	na	na	na	na	na	na	na	na	0.05	

*,** Significant at *P*<0.05 and *P*<0.001, respectively
DTF, days to flowering; PHT, plant height; LBN, lateral branch numbers; RPF, root pulling force; LRN, lateral root numbers; FB, fresh biomass; TSW, thousand seed weight; Yield, seed yield per plant; RWC, Leaf relative water content; ABO, Proportion of silique abortion; na, no data available; N, number of genotypes

3.2.4 Yield components**:** In 2011, the population mean for seed yield per plant in the wet treatment was more than two times greater than in the dry treatment (Table 3.2). In 2012, seed yields were low and the difference between the two treatments was not significant at $P<0.05$, although the population mean remained numerically higher under the wet treatment. The range for seed yield per plant was larger in the wet than in the dry treatment in 2011, but this trend was opposite in 2012. The heritability of seed yield per plant was higher for the wet than the dry treatment in both years and was much higher in 2011 than 2012 (Table 3.4). There was a positive correlation between seed yield per plant and thousand seed weight in wet ($P<0.001$) and dry ($P<0.05$) treatments in 2011 but significance was not detected in either treatment in 2012 (Table 3.5 and 3.6). The regression of seed yield on thousand seed weight indicates that variation for thousand seed weight explained around 40% of variation for seed yield under dry conditions and about 10% of the variation under the wet treatment in 2011 (Figure 3.3). The regression plot of seed yield on thousand seed weight for 2012 had a very low R2 (coefficient of determination) and was not informative; hence it is not presented here. As already stated, higher thousand seed weight was recorded in the wet treatment in both years (Table 3.3). Mean yield per plant showed a reduction of 88% and 80% in 2012 in wet and dry treatments, respectively, as compared to the 2011 growing season. In contrast, there was a 50% and a 53% increase in fresh biomass in wet and dry treatments, respectively, in 2012 compared to the 2011 growing season. This result indicates a decline in a ratio of seed yield to biomass (harvest index), which is one of the most important agronomic traits in breeding for a higher seed yield.

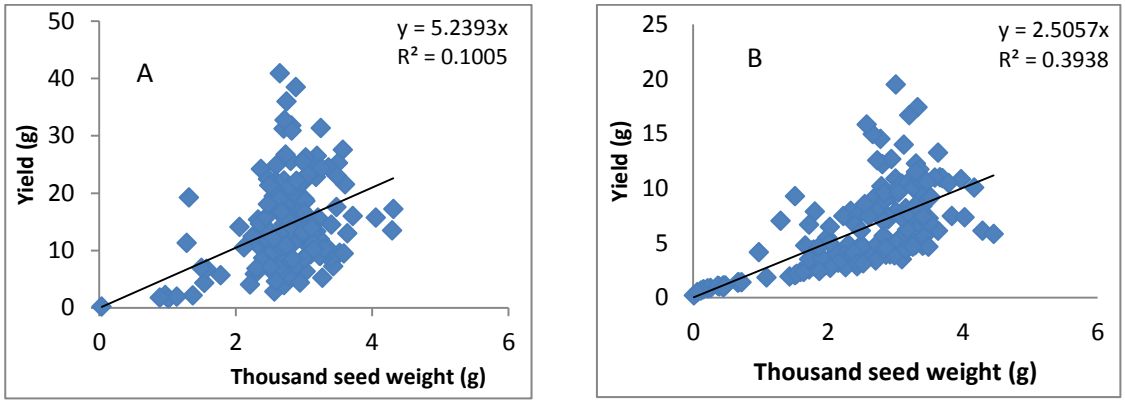


Figure 3.3: Regression of TSW on yield in 2011; A, wet and B, dry treatment in the DHYB canola population in Fort Collins, CO.

3.2.4 Silique and flower abortion**:** High temperatures and dry weather are normal in Colorado during June when our experimental genotypes flowered (Appendix Table A1). This is possibly, why we observed severe silique abortion, especially during the 2012 growing season. The high level of silique abortion significantly affected the total yield and thousand seed weight in the harvest of 2012. Proportion of aborted siliques was negatively correlated (P<0.001) with days to flowering (r = -0.46) and with plant height (r = -0.30) in the wet treatment of 2012. The proportion of aborted siliques was positively correlated with thousand seed weight in the wet treatment (r = 0.23, P<0.05). There was no detectable correlation with seed yield per plant.

3.2.5 Water Use Efficiency ($\delta^{13}C$)**:** $\delta^{13}C$ was significantly different among genotypes (P<0.001) (Table 3.1) in all experiments where leaf tissue was collected and analyzed. Transgressive segregants for $\delta^{13}C$ were detected in all experiments (Figure 3.4). $\delta^{13}C$ values obtained from the greenhouse experiment in 2010 were unusually low, suggesting that the genotypes were inefficient in their water use during their life cycle in the greenhouse. Another possible explanation is that $\delta^{13}C$ was depleted in the ambient greenhouse air. Plants grown in the dry treatment in 2011 showed higher efficiencies in utilizing the available moisture as evidenced by the higher $\delta^{13}C$ value as compared to the same lines in the wet treatment. This is shown in the reaction norm graph (Figure 3.5). The heritability of $\delta^{13}C$ was

higher under dry treatment than wet in 2011. $\delta^{13}C$ was significantly positively correlated (P<0.001) with days to flowering in all the experiments where $\delta^{13}C$ was analyzed. $\delta^{13}C$ was negatively correlated in the wet treatment with seed yield per plant and thousand seed weight in 2011 and proportion of aborted siliques in 2012 (P<0.001).

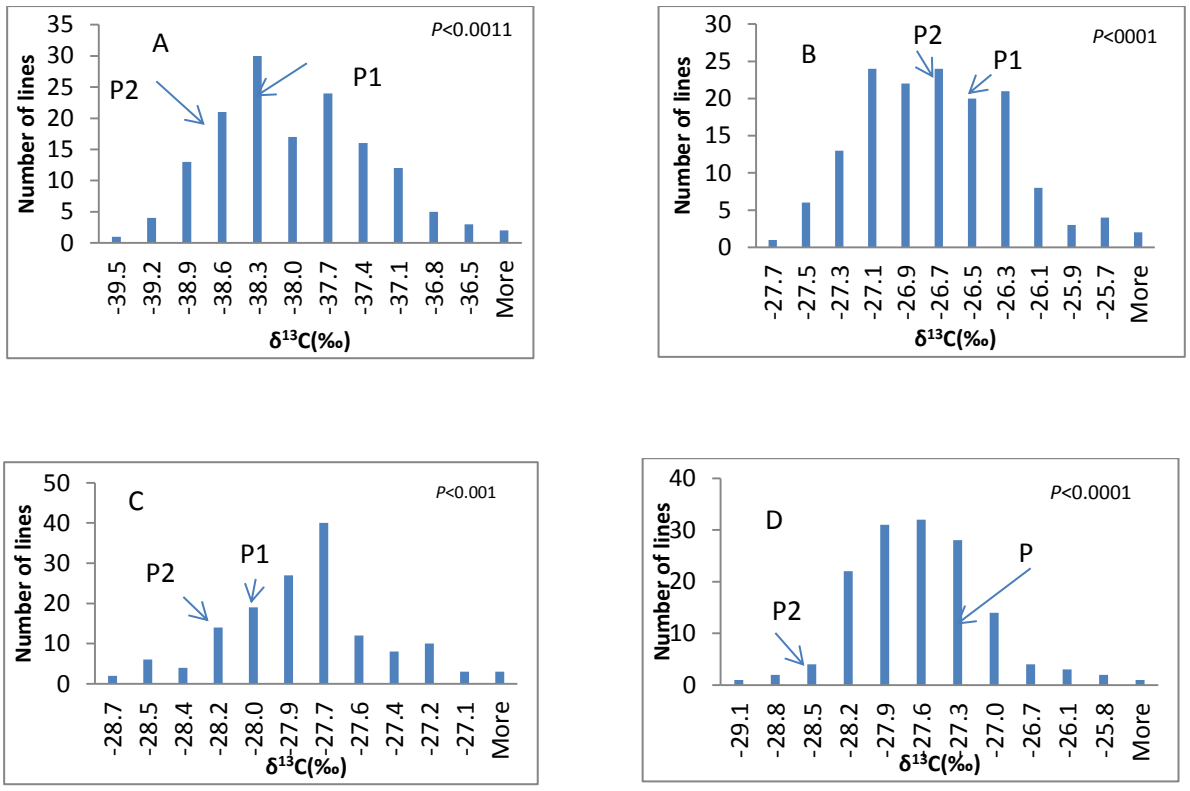


Figure 3.4: Frequency distribution of $\delta^{13}C$ in the DHYB canola population in Fort Collins, CO. A, greenhouse, 2010; B, dry treatment, 2011; C, wet treatment, 2011 and D, wet treatment, 2012 P1, YN01-429; P2, DH012075. The *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)

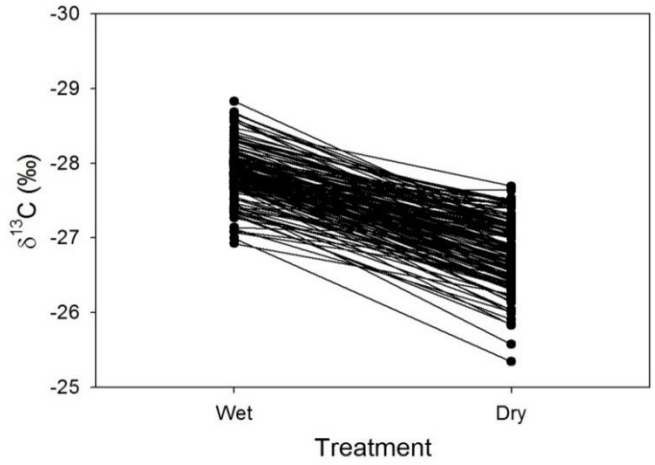


Figure 3.5: Reaction norm for $\delta^{13}C$ (‰) between wet and dry treatments on the DHYP population in 2011 in Fort Collins, CO.

3.3 QTL analysis

Using JoinMap software, Dr. Jack Mullen developed a linkage map that included 321 SSR markers and covered 87.1% of the genome. QTL were detected for all traits, but not in every environment (Table 3.7). A QTL for days to flowering in the greenhouse experiment of 2010 was detected at 1.3 cM on LG 2, with the closest marker being R12095Ia. No significant QTL were detected for days to flowering under wet and dry field treatments in 2011, but by using environment (wet and dry) as a covariate, QTL were detected for days to flowering on LG 1, 2, 5, 12T, 16, and the interaction between LG 1 and 16. In the wet treatment of 2012, two QTL were detected, one on LG 2 at position 4.6 cM with the closest marker being sR9864, and the other on LG 18 at 67.4 cM position with BSNP2410 as the nearest marker. The dry treatment also revealed two QTL, one on LG 2 at 8 cM with sR9864 as the closest marker and the other on LG 11 at 4 cM position with sN0248Ia as the closest marker. A single QTL for proportion of silique abortion under the wet treatment in 2012 was found to co-localize with the QTL for days to flowering located on LG 2 at 4 cM position with sR9864 as the nearest marker.

We were unable to detect QTL for root pulling force under either treatment in either year. However, by using environment as a covariate in our analyses, we were able to detect several QTL. In 2011, QTL for root pulling force were detected on LG 3, 5, 11, 14T, and 18 and an interaction was detected between QTL on linkage groups 3 and 18. The QTL for root pulling force co-localized with a QTL for fresh biomass on linkage group 11 at position 0.00, and with a QTL for plant height on LG 14T at position 39 cM (Figure 3.6). In 2012, individual QTL were detected on linkage groups 11 and 18; the QTL were consistent with the previous 2011 finding. However, in 2012 no QTL interaction was significant.

QTL were detected for plant height on LG 14T in both years and in both treatments. In the wet treatment of 2011, three major QTL for plant height were detected on LG 2, 10 and 14T at positions of 8, 5 and 40 cM, respectively. The closest marker (sN0881Fa) for the QTL on LG 14T was also common to the QTL detected in the dry treatment on LG 14T at a position of 39 cM. In 2012, the QTL for plant height was detected on LG at 83 cM with the nearest marker being sN0412a under the wet treatment and at 87.5 cM with the nearest marker being sN11516a under the dry treatment.

Two QTL for thousand seed weight were detected in the wet treatment of 2011 on LG 7 and 9. The allele contributions for the higher seed weight at these loci came from the black seeded (DH12075) parent and the yellow seeded (YN01-429) parent, respectively. The QTL on LG 7 was located at 16 cM with sN112940b as the closest marker. The QTL for thousand seed weight on LG 9 was closest to marker sN1988b and was 32 cM from the distal end of the short arm of the chromosome. QTL were also detected for fresh biomass in 2011 on LG 11 at 0.0 cM with the nearest marker being sORC20. In 2011, two QTLs for lateral root numbers were detected in the dry treatment on LG 2 and 17. However, only one QTL on LG 17 was detected in the wet treatment. In 2012, no QTL for lateral root numbers was detected in either treatment. QTL were detected in 2011 for branching number in the wet treatment including those on linkage group 5, 13, and 15. However only two QTL were detected on LG 5 and LG 2 in the dry treatment. Similarly, in 2012, a QTL located on LG 17 was detected only when treatment was

used as a covariate. Two QTL were detected for proportion of aborted siliques in 2012. One was on LG 2 at 4 cM and the other was located on LG 19 at a position of 26 cM with the nearest marker being BSNP2073.

In the 2010 greenhouse experiment, a QTL for $\delta^{13}C$ was found on LG 19 at a position of 42 cM with the closest marker being sR7024x. In 2011 dry, QTL for $\delta^{13}C$ were detected on LG 18 at a position of 66 cM and on LG 9 at 49 cM; the alleles contributing to the high value at these QTL are from both parents. A QTL for $\delta^{13}C$ under the wet treatment in 2011 was located on LG 18 at 61 cM. No epistatic interactions for the QTL were identified in 2011 and 2012 for this trait, suggesting strong additive gene action for $\delta^{13}C$, although the presence of dominant gene action cannot be tested in a DH population. In 2012, a QTL for $\delta^{13}C$ was detected on LG 2. Only in 2011 a QTL for leaf relative water content was detected under dry conditions on LG 7 at 67 cM and under wet on LG 8 at 65 cM.

Table 3.7: QTL detected in 2010 greenhouse experiment, 2011 and 2012 field season for traits measured in the DHYB canola population under wet and dry treatment in Fort Collins, CO

Traits	Environment	Linkage Group	Position (cM)	R^2 (%) QTL‡	Closest marker	R^2 (%) model¶	LOD model#	Additive effect(a) ††
Days to flowering	2010 GH	2	1.3	15.1	R12095Ia	21.8	7.9	1.33
	2011 §	5	44.9	6.6	sN12873a	34.8	15.0	-0.83
		1	7.1	5.9	sN11707b			0.61
		2	2.9	5.0	sR9526a			0.69
		12T	4.0	6.1	sR13062x			0.50
		16	61.0	7.2	sR12387a			-0.01
		1,16	7.06,61	6.8	na			†
	2012 Dry	2	8.0	6.7	sR9864	14.6	4.9	1.90
		11	4.0	6.9	sN0248Ia			-1.80
	2012 Wet	2	4.6	11.8	sR9864	20.8	7.4	2.20
		18	67.4	8.6	BSNP2410			1.80
Plant height	2011 Dry	14T	39.0	8.1	sN0881Fa	14.6	5.1	-2.57
	2011 Wet	14T	40.0	16.3	sN0881Fa	32.3	12.5	-3.37
		10	5.0	13.2	sN12271b			-1.37
		2	8.0	5.5	sR9864			2.10
	2012 Dry	14T	87.5	8.7	sN11516a	15.4	5.3	-3.10
	2012 Wet	14T	83.0	8.0	sN0412a	10.2	3.4	-3.00
Lateral branch number	2011 Dry	2	4.6	6.3	sR9864	20.0	7.2	0.51
		5	35.0	12.1	sN4276a			0.63
	2011 Wet	5	35.6	9.6	sN4276a	22.9	8.4	0.54
		13	35.0	4.1	sR6211			-0.39
		15	0.0	6.0	sORH13			-0.44
	2012 §	17	62.8	4.3	sR6439x	5.8	3.7	0.52
Root pulling force	2011 §	3	79.0	4.1	sNRA85a	14.6	5.2	0.50
		5	14.0	1.5	BSNP2223			1.30
		11	0.0	1.9	sORC20			-2.10
		14T	39.0	1.4	sS2277			-1.60
		18	68.0	1.1	sR12078x			1.80
		3,18	79,68	3.6	na			†
	2012 §	11	32.0	3.7	pp338835	5.7	3.7	-3.30
		18	87.0	1.9	sR5795b			2.20
Thousand seed weight	2011 Wet	7	16.0	7.3	sN12940b	15.3	5.3	-0.18
		9	32.1	7.1	sN1988b			0.18
Fresh biomass	2011 §	11	0.0	1.1	sORC20	1.1	2.7	-0.02
Lateral root number	2011 Wet	17	51.0	9.6	sNRB93b	15.7	5.5	-0.80
Leaf relative water content	2011 Dry	7	67.0	9.5	sN12940b	9.5		0.13
	2011 Wet	8	65.0	8	sNRA85a	8.0		0.12
Lateral root number	2011 Dry	2	4.5	10.4	sR9864	23.0	8.4	0.84
		17	49.0	7.9	sN11863a			-0.66
$\delta^{13}C$	2010 GH	19	42.0	10.1	sR7024x	10.1	3.2	0.12
	2011 Dry	9	49	9.3	sN2713x	18.0	6.4	-0.14
		18	66.0	8.3	sN12646			0.13
	2011 Wet	18	61.0	8.6	BSNP2410	8.6	2.8	0.11
	2012 Wet	2	2.0	11.7	sR9526a	11.7	6.4	0.22
Proportion of aborted siliques	2012	2	4.0	11.5	sR9864	19.6	7.03	-0.12
		19	26.0	8.0	BSNP2073			-0.01

GH greenhouse;
† Notation for interaction; ‡ R^2QTL (%) is the percent of phenotypic variance explained by the QTL in the full model; § Detected by covariate analysis; ¶ R2 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;†† average additive effect for the parent YN01-429 allele

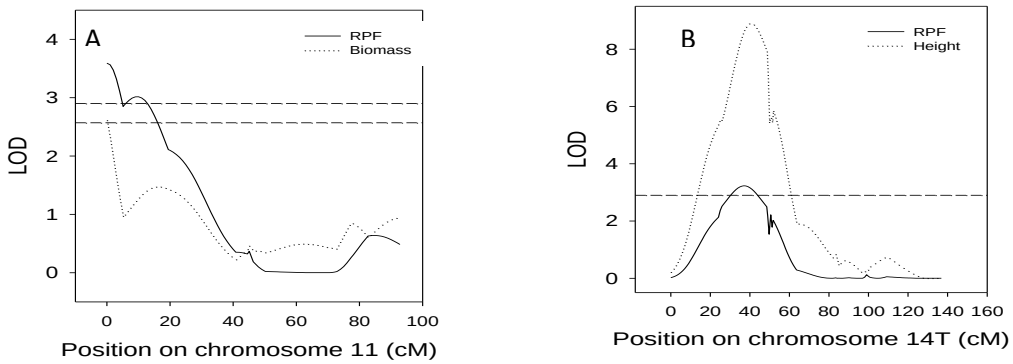


Figure 3.6: LOD profile of co-localizing QTLs. (A) Root pulling force and fresh biomass; (B) Root pulling force and plant height in 2011 in the DHYB canola population in Fort Collins, CO. The LOD thresholds for QTL were different for the two traits in figure A (2.95 for root pulling force, 2.82 for fresh biomass); B, an identical LOD of 3 hreshold was used in Figure B for both plant height and root pulling force

4. DISCUSSION

4.1 Growing conditions

The DHYB canola genotypes used in our experiment performed well in the 2011 growing season because of relatively cool temperatures and sufficient moisture during plant growth and development, as reported in the literature (Morrison et al., 2002). McGregor (1980) reported that hailstorms impact phenotypic variation depending on the stage at which damage occurs and foster secondary inflorescence development if the storm occurs at flowering, which agrees with our results. The storm during mid-June in our 2011 field experiment caused loss of leaves and flower buds. Heiliger (2012) reported that the hailstorm did not have a significant effect on traits measured in the same population in a nearby field, which is in agreement with our findings.

In the 2012 growing season, moisture and heat stress started right at seedling establishment and continued though the season. This had a negative impact on plant growth and development, which is in agreement with findings by Angadi et al. (2000) and Morrison et al. (2002). In canola, heat and moisture stress at flowering result in silique abortion, minimal seed set and reduction in yield (Angadi et al., 2000; Gan et al., 2004; Young et al., 2004; Din et al., 2011). Our experimental results concur with these reports, as higher than normal temperatures in June and July of 2012 overlapped with flowering time and resulted in yield reduction. In some situations, the indeterminate growth habit of canola can help in recovery from stress, for example late and continuous flowering may allow greater seed set if weather conditions improve (Mendham and Salisbury, 1995). However, the prolonged heat stress in the 2012 season did not allow recovery and resulted in only limited seed set. The yield loss in 2012 was mainly due to silique abortion, drop in seed number and reduced seed weight. The loss was worse under the moisture stressed (dry) treatment, possibly due to the combination of the two stresses (heat and drought) as reported in Gan et al. (2004).

Thousand seed weight, under both dry and wet treatments in 2012, negatively correlated with days to flowering, which agrees with Udall et al. (2006). Morrison et al. (2002) and Gan et al. (2004) explained the impact of drought stress on minimizing thousand seed weight and yield, which is consistent with our finding. Thousand seed weight and yield in 2012 were much lower as compared to the 2011 growing season, reflecting heat and moisture stress effects on yield and yield components. Ahmadi and Bahrani (2009) reported yield reduction in canola when moisture stress occurs during seed filling and concluded that the reduction was mainly due to loss in seed weight. Angadi et al. (2000) mention that moderately high temperature during early flowering resulted in larger seed but extreme heat stress at any stage of plant development decreased seed weight. Both these results agree with our finding of a higher thousand seed weight in the 2011 growing season than in 2012.

The contribution of thousand seed weight to seed yield was greater in the dry treatment than the wet in 2011, indicating that thousand seed weight is a better trait to estimate variation for yield in canola in a dry environment. A highly significant correlation between the two traits (thousand seed weight and yield) was reported in Marjanović-Jeromela et al. (2008), where thousand seed weight has been suggested as being an important trait to select yield indirectly in dry environments. This higher contribution of thousand seed weight to yield may be due to the generally smaller seed size, which reduces thousand seed weight when there is drought stress. A corollary to this relationship is that yield variation in wet environments is most likely more closely related to seed numbers, though we did not directly measured that trait in our study.

A significant positive correlation between days to flowering and plant height was detected under the wet treatment, while a non-significant correlation was observed under dry conditions in both years; however, Udall et al. (2006) and Mei et al. (2009) reported a positive correlation between the two traits under normal growing conditions in multiple canola populations.

The genotypes we studied showed a consistent and significant variation for $\delta^{13}C$ and a low genotype by environment interaction for that trait in 2011. This stability was indicated by the repeatability (no G x E across three environments) of results across years and treatments and a relatively higher heritability, which reinforces the potential that the trait has as a target for breeding (Richards, 1996, Rebetzke et al., 2002 and Condon et al., 2004). In both years of our experiment and under both treatments, $\delta^{13}C$ showed a strong correlation with days to flowering. The correlation of $\delta^{13}C$ with yield, fresh biomass, and thousand seed weight was inconsistent, so it was difficult to draw a conclusion about the relationship of $\delta^{13}C$ to yield components which has also been noticed in other crops (Matus et al., 1995, Condon et al., 2004). The $\delta^{13}C$ values in the 2010 greenhouse experiment were unusually low. This may be due to the fact that plants had access to plenty of water during their growth and development. The $\delta^{13}C$ values in 2012 were also smaller in value than in 2011. This is most likely due to the fact that the 2012 season was warmer than 2011, which agrees with the findings of Craufurd (1999), which indicated that warmer temperature reduces carbon isotope discrimination. A significant decline in yield between the 2011 and 2012 growing seasons may be due to the fact that heat and drought stress in 2012 increased transpiration rate, impaired photosynthetic efficiency and reduced carbon fixation (Craufurd, 1999, Bunce, 2000, Condon et al., 2004). Higher evapotranspiration and higher stomatal conductance are known to be cooling mechanisms in plants, especially during a higher temperature stress, as plant water loss depends on a water gradient between the leaf and atmosphere (Radin, et al., 1994, Condon et al., 2004).

4.2 Root pulling force and yield components

Studies on root related traits in canola are severely lacking. Yadav et al. (1994) indicated the potential of root pulling force for indirect selection for drought tolerance in *Brassica* spp. In our study a wider range of values and variation was observed for root pulling force under the dry treatment than the wet in both years. This supports the suggestion that root pulling force can be a potential trait of

interest to use in breeding and selection under dry environments in canola. Root pulling force has been used as a tool for selection for drought adaptation in rice (Ekanayake et al., 1985). Generally, in our study more force was required to pull plants in the wet treatment than in the dry treatments, signifying a well-established root system under moist soil conditions. Similar to our findings, genotypes that were tolerant to drought stress required a higher root pulling force in rice (O'Toole, 1981; Ekanayake et al., 1985).

A significant positive correlation of root pulling force with fresh biomass, lateral root numbers, and plant height suggests its strong relationship with yield and related components through directional or non-directional correlation. A similar finding was reported in rice (O'Toole, 1981; Ekanayake, et al., 1985). This indicates root pulling force could be used to select genotypes with a higher biomass under dry conditions, which could help in breeding for higher yield and biomass for dry environments. Root pulling force showed relatively better correlation (r = 0.24, P <0.001 under dry conditions in 2012 compared with r = 0.20, P<0.05 under both wet and dry in 2011) with branch number in 2012 than in 2011 suggesting it is more highly associated with branch number in a more stressed environment. Our study indicated that there exists a high correlation between root pulling force and lateral root numbers under both wet and dry treatments in both years. For example, in the dry treatment of 2011 (r = 0.42, P< 0.001) and under wet condition in 2012 (r = 0.31, P< 0.001); this indicates the importance of lateral root numbers to a higher yield and biomass. Ekanayake et al. (1985) reported a similar finding.

4.3 QTL Detection

The QTL for days to flowering explained 2.9% of the phenotypic variation (on linkage group 2 in 2011) to 15% of the phenotypic variation (on the same linkage group 2) in the greenhouse experiment in 2010. The same region had QTL for days to flowering in both treatments in 2012. Heiliger (2012) reported this QTL in a separate study on the same DHYB canola population. Our result indicates QTL for days to flowering detected on LG 2 is consistent across years and treatments and appears to be a major

QTL. Chen et al. (2010), Delourme et al. (2006), and Schranz et al. (2002) also reported QTL for days to flowering on LG 2 across different environments and mapping populations; however, we could not determine the exact position of those QTL relative to ours due to the different set of markers. The consistency of this QTL across populations and environments suggests it has lesser QTL X E as well as QTL X G (genetic background) interactions. Therefore, it can be used in marker-assisted selection, and also can be a target for cloning genes associated with days to flowering. Smaller effect or less consistent QTL were detected for days to flowering on LG 1, 5, 16, 18 and were not reported by Heiliger (2012). The QTL for days to flowering co-localized with QTL associated with lateral branch numbers in 2011 and with proportion of aborted siliques in 2012, which is a yield associated trait in canola. This association shows the importance of flowering time in selection and breeding for higher yield in canola in a drought prone environment.

QTL for root pulling force were detected in both the 2011 and 2012 growing seasons. The detection of similar QTL across years and treatments implies reliability of the QTL and reduced QTL X E interaction. QTL with minor effects were detected on linkage groups 11, 14T, 18, 5 and 3 contributing to the phenotypic variation of the trait from 1.1% on linkage group 18 to 4.1 % on linkage group 3. In 2012 QTL for root pulling force were detected on LG 11 and 18, the same as in 2011. These QTL contributed 3.7 and 1.9 % to the phenotypic variation, respectively. QTL on LG 11, 14T, 18 were also co-located with QTL for fresh biomass, plant height, and lateral branch numbers. The co-localization was not a surprise due to the strong phenotypic correlation of these traits with root pulling force.

The detection of QTL associated with root pulling force was possible when treatments were used as a covariate which helps in finding constituent QTL between the two treatments due to increased statistical power of QTL detection, as the number of observations for genotypes and markers doubled. However, the QTL detected have a small effect on the phenotype. It may be the similarity in the growth habit of both parents of the population that reduced genetic diversity in the DH lines for the trait.

In 2010 greenhouse experiment a QTL for $\delta^{13}C$ was closer to the QTL for proportion of aborted siliques on LG 19. In 2011, a QTL for $\delta^{13}C$ was detected on LG 18, which was consistent between treatments (wet and dry) also located in a similar region on the map. A QTL for $\delta^{13}C$ located on LG 2 co-localized with a QTL for days to flowering and a QTL for proportion of aborted siliques; this finding is supplemental to the strong correlation among these traits. This finding informs us that $\delta^{13}C$ has a very strong relationship with days to flowering at the gene(s) or QTL level. The correlation of days to flowering and $\delta^{13}C$ and consistent co-localization of QTL for the traits has been reported in *Arabidopsis* (McKay et al., 2003, Juenger et al., 2005).

QTL for plant height were detected on LG 14T in both years but in different locations. In 2011 QTL in the wet and dry treatment were at 39 cM, but in 2012 the QTL for both treatment was located at 83.5 and 87.5 cM in the wet and dry treatment, respectively. We assume these QTL detected in different years are two different QTL that each affect the phenotype under different sets of conditions. Chen et al. (2007) also reported QTL associated with plant height on LG 14. These authors also identified QTL for branch number on LG 8 and 17 in canola, which concurs with our results that detected QTL for lateral branch number on LG 8 and 17.

5. CONCLUSION

This study provided distinct environments, defined by irrigation treatment and temperature regime, for evaluating the DHYB population. Root pulling force had strong phenotypic correlations in both wet and dry environments with yield and biomass and had a relatively high heritability. This indicates the importance of root traits to yield across moisture environments, and suggests that root pulling force could be a useful selection criterion. However, the QTL detected for root pulling force had relatively small contributions to the phenotypic variation of the trait, although some of the QTL were consistent across years. Additional studies of root pulling force would improve understanding of the trait and QTL associated with it, for there is limited information on studies related to canola roots. Days to flowering correlated with most traits in our study, including seed yield and thousand seed weight, which were negatively correlated with days to flowering. Thousand seed weight and root pulling force can be good predictors of higher yield in drought environments. $\delta^{13}C$ showed a consistent variation and was correlated with days to flowering and relative water content.

A major QTL for days to flowering detected on linkage group 2 was consistent and co-localized with QTL for $\delta^{13}C$, proportion of aborted siliques, and lateral branch number. QTL were associated with traits such as plant height, root pulling force and $\delta^{13}C$ in the DHYB canola population in both treatments and years. The QTL detected have a range of effect size for the phenotypic variation of traits and both parents contributed high value alleles. A QTL for $\delta^{13}C$ on LG 18 was repeatable between treatments. A QTL for plant height was mapped on LG 14T in both treatments and years.

This study provides insights about root pulling force, $\delta^{13}C$ and their relationships with yield, and yield related traits in canola. In order to utilize these traits in breeding for drought tolerance and marker assisted breeding further research on the relationship among these traits is imperative.

LITERATURE CITED

Ahmadi, M., & Bahrani, M. J. (2009). Yield and yield components of rapeseed as influenced by water stress at different growth stages and nitrogen levels. American-Eurasian Journal of Agricultural and Environmental Sciences, 5(6), 755-761.

Angadi, S. V., Cutforth, H. W., Miller, P. R., McConkey, B. G., Entz, M. H., Brandt, S. A., & Volkmar, K. M. (2000). Response of three *Brassica* species to high temperature stress during reproductive growth. Canadian Journal of Plant Science, 80(4), 693-701.

Bahrani, M. A. a. M. J. (2009). Yield and Yield Components of Rapeseed as Influenced by Water Stress at Different Growth Stages and Nitrogen Levels. American-Eurasian J. Agric. & Environ. Sci., 5(6), 6.

Barbetti, M. J., & Khangura, R. (2000). Fungal diseases of canola in Western Australia. Agriculture Western Australia.

Bernardo, R. (2008). Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Science, 48(5), 1649-1664.

Bernier, J., Serraj, R., Kumar, A., Venuprasad, R., Impa, S., RP, V. G., ... & Atlin, G. (2009). The large-effect drought-resistance QTL increases water uptake in upland rice. Field Crops Research, 110(2), 139-146.

Blum, A. (2005). Drought resistance, water-use efficiency, and yield potential—are they compatible, dissonant, or mutually exclusive?. Crop and Pasture Science, 56(11), 1159-1168.

Blum, A. (2011). Plant breeding for water-limited environments. Springer.

Borrell, A. K., & Hammer, G. L. (2000). Nitrogen dynamics and the physiological basis of stay-green in sorghum. Crop science, 40(5), 1295-1307.

Broman, K. W., & Sen, S. (2009). A guide to QTL mapping with R-qtl. Springer.

Broman, K. W., Wu, H., Sen, Ś., & Churchill, G. A. (2003). R/qtl: QTL mapping in experimental crosses. Bioinformatics, 19(7), 889-890.

Brooks, N. (2009). US food import patterns, 1998-2007. DIANE Publishing.

Brummer, E. C., Barber, W. T., Collier, S. M., Cox, T. S., Johnson, R., Murray, S. C., ... & Thro, A. M. (2011). Plant breeding for harmony between agriculture and the environment. Frontiers in Ecology and the Environment, 9(10), 561-568.

Bunce, J. A. (2000). Responses of stomatal conductance to light, humidity and temperature in winter wheat and barley grown at three concentrations of carbon dioxide in the field. Global Change Biology, 6(4), 371-382.

Cattivelli, L., Rizza, F., Badeck, F. W., Mazzucotelli, E., Mastrangelo, A. M., Francia, E., ... & Stanca, A. M. (2008). Drought tolerance improvement in crop plants: an integrated view from breeding to genomics. Field Crops Research, 105(1), 1-14.

Champoux, M. C., Wang, G., Sarkarung, S., Mackill, D. J., O'Toole, J. C., Huang, N., & McCouch, S. R. (1995). Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theoretical and Applied Genetics, 90(7-8), 969-981.

Cheema, K. L., & Sadaqat, H. A. (2004). Potential and genetic basis of drought tolerance in canola (*Brassica napus*) II. heterosis manifestation in some morpho-physiological traits in canola. Inter. J. Agric. Bio., 6, 82-85.

Chen, C., Jackson, G., Neill, K., Wichman, D., Johnson, G., & Johnson, D. (2005). Determining the feasibility of early seeding canola in the northern Great Plains. Agronomy Journal, 97(4), 1252-1262.

Chen, G., Geng, J., Rahman, M., Liu, X., Tu, J., Fu, T., ... & Tahir, M. (2010). Identification of QTL for oil content, seed yield, and flowering time in oilseed rape (*Brassica napus*). Euphytica, 175(2), 161-174.

Chen, W., Zhang, Y., Liu, X., Chen, B., Tu, J., & Tingdong, F. (2007). Detection of QTL for six yield-related traits in oilseed rape (*Brassica napus*) using DH and immortalized F2 populations. Theoretical and Applied Genetics, 115(6), 849-858.

Collard, B. C., Jahufer, M. Z., Brouwer, J. B., & Pang, E. C. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142(1), 169-196.

Condon, A. G., & Richards, R. A. (1992). Broad Sense Heritability and Genotype X Environment Interaction for Carbon Isotope Discrimination in Field-Grown Wheat. Australian Journal of Agricultural Research, 43(5), 921-934.

Condon, A. G., Richards, R. A., Rebetzke, G. J., & Farquhar, G. D. (2002). Improving Intrinsic Water-Use Efficiency and Crop Yield. Crop Sci, 42(1), 122-131.

Condon, A. G., Richards, R. A., Rebetzke, G. J., & Farquhar, G. D. (2004). Breeding for high water-use efficiency. [Review]. Journal of Experimental Botany, 55(407), 2447-2460.

Craufurd, P. Q., Wheeler, T. R., Ellis, R. H., Summerfield, R. J., & Williams, J. H. (1999). Effect of temperature and water deficit on water-use efficiency, carbon isotope discrimination, and specific leaf area in peanut. Crop Science, 39(1), 136-142.

Crosses, E. (2001). Review of statistical methods for QTL mapping in experimental crosses. Lab animal, 30(7).

de Dorlodot, S., Forster, B., Pages, L., Price, A., Tuberosa, R., & Draye, X. (2007). Root system architecture: opportunities and constraints for genetic improvement of crops. Trends in Plant Science, 12(10), 474-481.

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

Diamond, J. (2002). Evolution, consequences and future of plant and animal domestication. Nature, 418(6898), 700-707.

Din, J., Khan, S. U., Ali, I., & Gurmani, A. R. (2011). Physiological and agronomic response of Canola varieties to drought stress. J. Anim. Plant Sci, 21, 78-82.

Doust, A. N., Devos, K. M., Gadberry, M. D., Gale, M. D., & Kellogg, E. A. (2004). Genetic control of branching in foxtail millet. [Comparative Study Research Support, U.S. Gov't, Non-P.H.S.]. Proc Natl Acad Sci U S A, 101(24), 9045-9050.

Dubcovsky, J. (2004). Marker-assisted selection in public breeding programs: the wheat experience.

Ekanayake, I. J., Garrity, D. P., Masajo, T. M., & Otoole, J. C. (1985). Root Pulling Resistance in Rice - Inheritance and Association with Drought Tolerance. Euphytica, 34(3), 905-913.

Ekanayake, I. J., O'toole, J. C., Garrity, D. P., & Masajo, T. M. (1985). Inheritance of root characters and their relations to drought resistance in rice. Crop Science, 25(6), 927-933.

Falconer, D. S., & Mackay, T. F. C. 1996. Introduction to quantitative genetics, 4.

FAO, 2007 http://fasostat.fao.org

Farquhar, G. D., & Richards, R. A. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Functional Plant Biology, 11(6), 539-552.

Farquhar, G. D., Ehleringer, J. R., & Hubick, K. T. (1989). Carbon isotope discrimination and photosynthesis. Annual review of plant biology, 40(1), 503-537.

Farquhar, G. D., O'leary, M. H., & Berry, J. A. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Functional Plant Biology, 9(2), 121-137.

Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. Crop and Pasture Science, 29(5), 897-912.

Frederick, J. R., Camp, C. R., & Bauer, P. J. (2001). Drought-stress effects on branch and mainstem seed yield and yield components of determinate soybean. Crop Science, 41(3), 759-763.

Galili, G., Galili, S., Lewinsohn, E., & Tadmor, Y. (2002). Genetic, molecular, and genomic approaches to improve the value of plant foods and feeds. Critical Reviews in Plant Sciences, 21(3), 167-204.

Gan, Y., Angadi, S. V., Cutforth, H., Potts, D., Angadi, V. V., & McDonald, C. L. (2004). Canola and mustard response to short periods of temperature and water stress at different developmental stages. Canadian journal of plant science, 84(3), 697-704.

Gómez-Campo, C. (Ed.). (1999). Biology of Brassica coenospecies (Vol. 4). Elsevier Science.

Gowda, V. R. P., Henry, A., Yamauchi, A., Shashidhar, H. E., & Serraj, R. (2011). Root biology and genetic improvement for drought avoidance in rice. Field Crops Research, 122(1), 1-13.

Hall, N. M., Griffiths, H., Corlett, J. A., Jones, H. G., Lynn, J., & King, G. J. (2005). Relationships between water-use traits and photosynthesis in *Brassica oleracea* resolved by quantitative genetic analysis. Plant breeding, 124(6), 557-564.

Harris, K., Subudhi, P. K., Borrell, A., Jordan, D., Rosenow, D., Nguyen, H., ... & Mullet, J. (2007). Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. Journal of experimental botany, 58(2), 327-338.

Harvey, B. L., & Downey, R. K. (1964). The inheritance of erucic acid content in rapeseed (*Brassica napus*). Canadian Journal of Plant Science, 44(1), 104-111.

Hegedus, D., Yu, M., Baldwin, D., Gruber, M., Sharpe, A., Parkin, I., ... & Lydiate, D. (2003). Molecular characterization of *Brassica napus* NAC domain transcriptional activators induced in response to biotic and abiotic stress. Plant molecular biology, 53(3), 383-397.

Heiliger, A. (2012). Quantitative trait locus mapping of yield and yield components in canola (*Brassica napus* L.) under irrigated and rain-fed treatment. M.S. Thesis, Colorado State University, Fort Collins.

Henry, A., Gowda, V. R., Torres, R. O., McNally, K. L., & Serraj, R. (2011). Variation in root system architecture and drought response in rice (*Oryza sativ*): Phenotyping of the OryzaSNP panel in rainfed lowland fields. Field Crops Research, 120(2), 205-214.

Jiang, G. H., He, Y. Q., Xu, C. G., Li, X. H., & Zhang, Q. (2004). The genetic basis of stay-green in rice analyzed in a population of doubled haploid lines derived from an indica by *japonica* cross. Theoretical and Applied Genetics, 108(4), 688-698.

Joehanes, R., & Nelson, J. C. (2008). QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics, 24(23), 2788-2789.

Johnson, J., Enjalbert, N., Schneekloth, J., Helm, A., Malhotra, R., & Coonrod, D. (2009). Development of oilseed crops for biodiesel production under Colorado limited irrigation conditions. Completion Report, Boulder, (211), 1-49.

Juenger, T. E., Mckay, J. K., Hausmann, N., Keurentjes, J. J., Sen, S., Stowe, K. A., ... & Richards, J. H. (2005). Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: $\delta^{13}C$, stomatal conductance and transpiration efficiency. Plant, Cell & Environment, 28(6), 697-708.

Kamara, A. Y., Kling, J. G., Ajala, S. O., & Menkir, A. (2002). The relationship between vertical root-pulling resistance and nitrogen uptake and utilization in maize breeding lines. Maydica, 47(2), 135-140.

Kashiwagi, J., Krishnamurthy, L., Upadhyaya, H. D., Krishna, H., Chandra, S., Vadez, V., & Serraj, R. (2005). Genetic variability of drought-avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). Euphytica, 146(3), 213-222.

Kearsey, M. J., & Farquhar, A. G. L. (1998). QTL analysis in plants; where are we now?. Heredity, 80(2), 137-142.

Kerry T. Hubick, R. s. a. G. D. F. (1988). Heritability and Genotype X Environment Interactions of Carbon Isotope Discrimination and Transpiration Efficiency in Peanut (*Arachis hypogaea* L.). Aust. J. Plant Physiol, 15, 799-813.

Landi, P., Giuliani, S., Salvi, S., Ferri, M., Tuberosa, R., & Sanguineti, M. C. (2010). Characterization of root-yield-1.06, a major constitutive QTL for root and agronomic traits in maize across water regimes. Journal of Experimental Botany, 61(13), 3553-3562.

Lawlor, D. W., & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant, Cell & Environment, 25(2), 275-294.

Lawrence B. Flanagan, K. H. J. (1995). Genetic variation in carbon isotope discrimination and its relationship to growth under field conditions in full-sib families of *Piceamariana*. Canadian Journal of Forest Research, 25:((1)), 39-47.

Le Roux, X., Bariac, T., Sinoquet, H., Genty, B., Piel, C., Mariotti, A., ... & Richard, P. (2001). Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. Plant, Cell & Environment, 24(10), 1021-1032.

Li, H., Barbetti, M. J., & Sivasithamparam, K. (2005). Hazard from reliance on cruciferous hosts as sources of major gene-based resistance for managing blackleg (*Leptosphaeria maculans*) disease. Field Crops Research, 91(2), 185-198.

Liu, B. H. (1998). Statistical Genomics: Linkage Mapping, Mapping, and QTL Analysis. CRC press.

Londo, M., Lensink, S., Wakker, A., Fischer, G., Prieler, S., van Velthuizen, H., ... & Könighofer, K. (2010). The Refuel EU road map for biofuels in transport: Application of the project's tools to some short-term policy issues. Biomass and Bioenergy, 34(2), 244-250

Luo, Y., Ahmed, I., Kubátová, A., Šťávová, J., Aulich, T., Sadrameli, S. M., & Seames, W. S. (2010). The thermal cracking of soybean/canola oils and their methyl esters. Fuel Processing Technology, 91(6), 613-617.

Maccaferri, M., Sanguineti, M. C., Corneti, S., Ortega, J. L., Salem, M. B., Bort, J., Tuberosa, R. (2008). Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. Genetics, 178(1), 489-511. doi: 10.

MacMillan, K., Emrich, K., Piepho, H. P., Mullins, C. E., & Price, A. H. (2006). Assessing the importance of genotype× environment interaction for root traits in rice using a mapping population II: conventional QTL analysis. Theoretical and Applied Genetics, 113(5), 953-964.

Manichaikul, A., Moon, J. Y., Sen, Ś., Yandell, B. S., & Broman, K. W. (2009). A model selection approach for the identification of quantitative trait loci in experimental crosses, allowing epistasis. Genetics, 181(3), 1077-1086.

Marjanović-Jeromela, A., Marinković, R., Mijić, A., Zdunić, Z., Ivanovska, S., & Jankulovska, M. (2008). Correlation and path analysis of quantitative traits in winter rapeseed (*Brassica napus* L.). Agriculturae Conspectus Scientificus (ACS), 73(1), 13-18.

Martin, B., & Thorstenson, Y. R. (1988). Stable Carbon Isotope Composition (δ13CC), Water Use Efficiency, and Biomass Productivity of Lycopersicon esculentum, *Lycopersicon pennellii*, and the F(1) Hybrid. Plant Physiol, 88(1), 213-217.

Matus, A., Slinkard, A., & van Kessel, C. (1995). Carbon isotope discrimination: potential for indirect selection for seed yield in canola. Crop science, 35(5), 1267-1271.

McGregor, D. I. (1980). The nature of hail injury to rapeseed. Canadian Journal of Plant Science, 60(4), 1441-1449.

McKay, J. K., Richards, J. H., & Mitchell-Olds, T. (2003). Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. Molecular Ecology, 12(5), 1137-1151.

Mei, D. S., Wang, H. Z., Hu, Q., Li, Y. D., Xu, Y. S., & Li, Y. C. (2009). QTL analysis on plant height and flowering time in *Brassica napus*. Plant Breeding, 128(5), 458-465.

Mendham, N. J., & Salisbury, P. A. (1995). Physiology: crop development, growth and yield.

Mohan, M., Nair, S., Bhagwat, A., Krishna, T. G., Yano, M., Bhatia, C. R., & Sasaki, T. (1997). Genome mapping, molecular markers and marker-assisted selection in crop plants. Molecular Breeding, 3(2), 87-103.

Moose, S. P., & Mumm, R. H. (2008). Molecular plant breeding as the foundation for 21st century crop improvement. Plant Physiology, 147(3), 969-977.

Morrison, M. J., & Stewart, D. W. (2002). Heat stress during flowering in summer *Brassica*. Crop science, 42(3), 797-803.

Nagaharu, U. (1935). Genome analysis in Brassica with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap J Bot, 7, 389-452.

Nguyen, H. T., Babu, R. C., & Blum, A. (1997). Breeding for drought resistance in rice: Physiology and molecular genetics considerations.Crop Science, 37(5), 1426-1434.

Nielsen, D. C. (1997). Water Use and Yield of Canola under Dryland. J. Prod. Agric, 10(2), 213.

Nyvall, R. F. (1979). Field crop diseases handbook. Avi Publishing Company.

O'Toole, J. C. (1981). Evaluation of a simple technique for characterizing rice root systems in relation to drought resistance. Euphytica, 30(2), 283-290.

Passioura, J. B. (1997). Drought and drought tolerance. In Drought tolerance in higher plants: genetical, physiological and molecular biological analysis (pp. 1-5). Springer Netherlands.

Phillips, R. L. (2010). Mobilizing science to break yield barriers. Crop Science, 50(Supplement_1), S-99.

Pico, A. F. a. B. (2008). Genetics of Root System Architecture Using Near-isogenic Lines of Melon. J. AMER. SOC. HORT. SCI., 133(3), 448–458.

Radin, J. W., Lu, Z., Percy, R. G., & Zeiger, E. (1994). Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation. Proceedings of the National Academy of Sciences, 91(15), 7217-7221.

Rakow, G., & Relf-Eckstein, J. (2005). YN01–429. Plant Var. Seeds, 55, 40-41.

Raymer, P. L. (2002). Canola: An emerging oilseed crop. Trends in new crops and new uses, 122-126.

Rebetzke, G. J., Condon, A. G., Richards, R. A., & Farquhar, G. D. (2002). Selection for reduced carbon isotope discrimination increases aerial biomass and grain yield of rainfed bread wheat. Crop Science, 42(3), 739-745.

Reynolds, M., & Tuberosa, R. (2008). Translational research impacting on crop productivity in drought-prone environments. Current opinion in plant biology, 11(2), 171-179.

Ribaut, J. M., & Ragot, M. (2007). Marker-assisted selection to improve drought adaptation in maize: the backcross approach, perspectives, limitations, and alternatives. Journal of Experimental Botany, 58(2), 351-360.

Richards, R. A. (1996). Defining selection criteria to improve yield under drought. Plant Growth Regulation, 20(2), 157-166.

Röbbelen, S., Downey, R. K., & Ashri, A. (1989). Oilcrops of the world: their breeding and utilization. Mcgraw-Hill Publishing Company.

Robin, S., Pathan, M. S., Courtois, B., Lafitte, R., Carandang, S., Lanceras, S., ... & Li, Z. (2003). Mapping osmotic adjustment in an advanced back-cross inbred population of rice. Theoretical and applied genetics, 107(7), 1288-1296.

Roush, R. T. (2002). GMOs in food production–the perceptions and the reality. Animal Production Australia, 24, 512-17.

Sakurai, H., & Pokorný, J. (2003). The development and application of novel vegetable oils tailor-made for specific human dietary needs. European journal of lipid science and technology, 105(12), 769-778.

SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2000-2004

Sax, K. (1923). The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics, 8(6), 552.

Schranz, M. E., Quijada, P., Sung, S. B., Lukens, L., Amasino, R., & Osborn, T. C. (2002). Characterization and effects of the replicated flowering time gene FLC in *Brassica rapa*. Genetics, 162(3), 1457-1468.

Schubert, S. D., Suarez, M. J., Pegion, P. J., Koster, R. D., & Bacmeister, J. T. (2004). Causes of long-term drought in the US Great Plains. Journal of Climate, 17(3), 485-503.

Sediqi, M. N. (2012). Adaptability of Oilseed species at high altitudes of Colorado and technology transfer to Afghanistan. (M.S. Thesis, Colorado State University).

Sen, S., & Churchill, G. A. (2001). A statistical framework for quantitative trait mapping. Genetics, 159(1), 371-387.

Shahidi, F. (1990). Rapeseed and canola: global production and distribution. Canola and Rapeseed Production, Chemistry, Nutrition and Processing Technology. Van Nostrand Reinhold, New York, 1-13.

Sharp, R. E., Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K., Bohnert, H. J., & Nguyen, H. T. (2004). Root growth maintenance during water deficits: physiology to functional genomics. Journal of Experimental Botany, 55(407), 2343-2351.

Sharp, Z. (2007). Principles of stable isotope geochemistry (p. 344). Upper Saddle River, NJ, USA: Pearson Education.

Shirani Rad, A. H., Shahsavari, N., & Jais, H. M. (2012). Effects of water shortage in late season on agronomic traits of rapeseed (*Brassica napus* L.). African Journal of Agricultural Research, 7(25), 3677-3684.

Sinaki, J. M., Heravan, E. M., Rad, A. S., Noormohammadi, G. H., & Zarei, G. H. (2007). The effects of water deficit during growth stages of canola (*Brassica napus* L.). Am. Eurasian J. Agric. Environ. Sci, 2, 417-422.

Singh, S. K., Kakani, V. G., Brand, D., Baldwin, B., & Reddy, K. R. (2008). Assessment of Cold and Heat Tolerance of Winter-grown Canola (*Brassica napus* L.) Cultivars by Pollen-based Parameters. Journal of Agronomy and Crop Science, 194(3), 225-236.

Soon, Y. K., & Clayton, G. W. (2002). Eight years of crop rotation and tillage effects on crop production and N fertilizer use. Canadian journal of soil science, 82(2), 165-172.

Steele, K. A., Price, A. H., Shashidhar, H. E., & Witcombe, J. R. (2006). Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. Theoretical and Applied Genetics, 112(2), 208-221.

Tanksley, S. D. (1993). Mapping polygenes. Annual review of genetics, 27(1), 205-233.

Teulat, B., Monneveux, P., Wery, J., Borries, C., Souyris, I., Charrier, A., & This, D. (1997). Relationships between relative water content and growth parameters under water stress in barley: a QTL study. New Phytologist, 137(1), 99-107.

Tewari, J. P., Strelkov, S. E., Orchard, D., Hartman, M., Lange, R. M., & Turkington, T. K. (2005). Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Canadian journal of plant pathology, 27(1), 143-144.

Tsunoda, S., Hinata, K., & Gomez-Campo, C. (1980). Brassica crops and wild allies. Biology and breeding. *Brassica* crops and wild allies. Biology and breeding.

Tuberosa, R., & Salvi, S. (2006). Genomics-based approaches to improve drought tolerance of crops. Trends in plant science, 11(8), 405-412.

Tuberosa, R., Salvi, S., Sanguineti, M. C., Maccaferri, M., Giuliani, S., & Landi, P. (2003). Searching for quantitative trait loci controlling root traits in maize: a critical appraisal. In Roots: The Dynamic Interface between Plants and the Earth (pp. 35-54). Springer Netherlands.

Tuberosa, R., Sanguineti, M. C., Landi, P., Michela Giuliani, M., Salvi, S., & Conti, S. (2002). Identification of QTLs for root characteristics in maize grown in hydroponics and analysis of their overlap with QTLs for grain yield in the field at two water regimes. Plant Molecular Biology, 48(5), 697-712.

Udall, J. A., Quijada, P. A., Lambert, B., & Osborn, T. C. (2006). Quantitative trait analysis of seed yield and other complex traits in hybrid spring rapeseed (*Brassica napus* L.): 2. Identification of alleles from unadapted germplasm. Theoretical and Applied Genetics, 113(4), 597-609.

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

Van Ooijen, J. W. (2006). JoinMap 4. Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, Netherlands.

Voesenek, L. A. C. J., Armstrong, W., Colmer, T. D., Bögemann, G. M., & McDonald, M. P. (1999). A lack of aerenchyma and high rates of radial oxygen loss from the root base contribute to the waterlogging intolerance of *Brassica napus*. Functional Plant Biology, 26(1), 87-93.

Wan, J. a. G., Rebecca and Ying, Jifeng and McCourt, Peter and Huang, Yafan. (2009). Development of drought-tolerant canola (*Brassica napus* L.) through genetic modulation of ABA-mediated Stomatal responses Crop Science, 49, 1539-1554.

Wang, Y., Beaith, M., Chalifoux, M., Ying, J., Uchacz, T., Sarvas, C., . . . Huang, Y. (2009). Shoot-specific down-regulation of protein farnesyltransferase (alpha-subunit) for yield protection against drought in canola. Mol Plant, 2(1), 191-200.

Wickman, F. E. (1952). Variations in the relative abundance of the carbon isotopes in plants. Geochimica et Cosmochimica Acta, 2(4), 243-254.

Wittkop, B., Snowdon, R. J., & Friedt, W. (2009). Status and perspectives of breeding for enhanced yield and quality of oilseed crops for Europe. Euphytica, 170(1), 131-140.

Xiong, L. M., Wang, R. G., Mao, G. H., & Koczan, J. M. (2006). Identification of drought tolerance determinants by genetic analysis of root re Maccaferri sponse to drought stress and abscisic acid. Plant Physiol, 142(3), 1065-1074.

Xu, Y., & Crouch, J. H. (2008). Marker-assisted selection in plant breeding: from publications to practice. Crop Science, 48(2), 391-407.

Yadav, Y., & Singh, H. (1994, November). Morpho-physiological determinants of yield under water stress condition in Indian mustard (*brassica juncea* (L.) CzernN & Coss.). In ISHS Brassica Symposium-IX Crucifer Genetics Workshop 407 (pp. 155-160).

Young, L. W., Wilen, R. W., & Bonham-Smith, P. C. (2004). High temperature stress of Brassica napus during flowering reduces micro-and megagametophyte fertility, induces fruit abortion, and disrupts seed production. Journal of Experimental Botany, 55(396), 485-495.

Yue, B., Xue, W., Xiong, L., Yu, X., Luo, L., Cui, K., ... & Zhang, Q. (2006). Genetic basis of drought resistance at reproductive stage in rice: separation of drought tolerance from drought avoidance. Genetics, 172(2), 1213-1228.

Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. Genetics, 136(4), 1457-1468.

Zhang, H., Turner, N. C., Poole, M. L., & Simpson, N. (2006). Crop production in the high rainfall zones of southern Australia—potential, constraints and opportunities. Animal Production Science, 46(8), 1035-1049.

Zhang, H., Turner, N. C., Poole, M. L., & Simpson, N. (2006). Crop production in the high rainfall zones of southern Australia—potential, constraints and opportunities. Animal Production Science, 46(8), 1035-1049.

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.

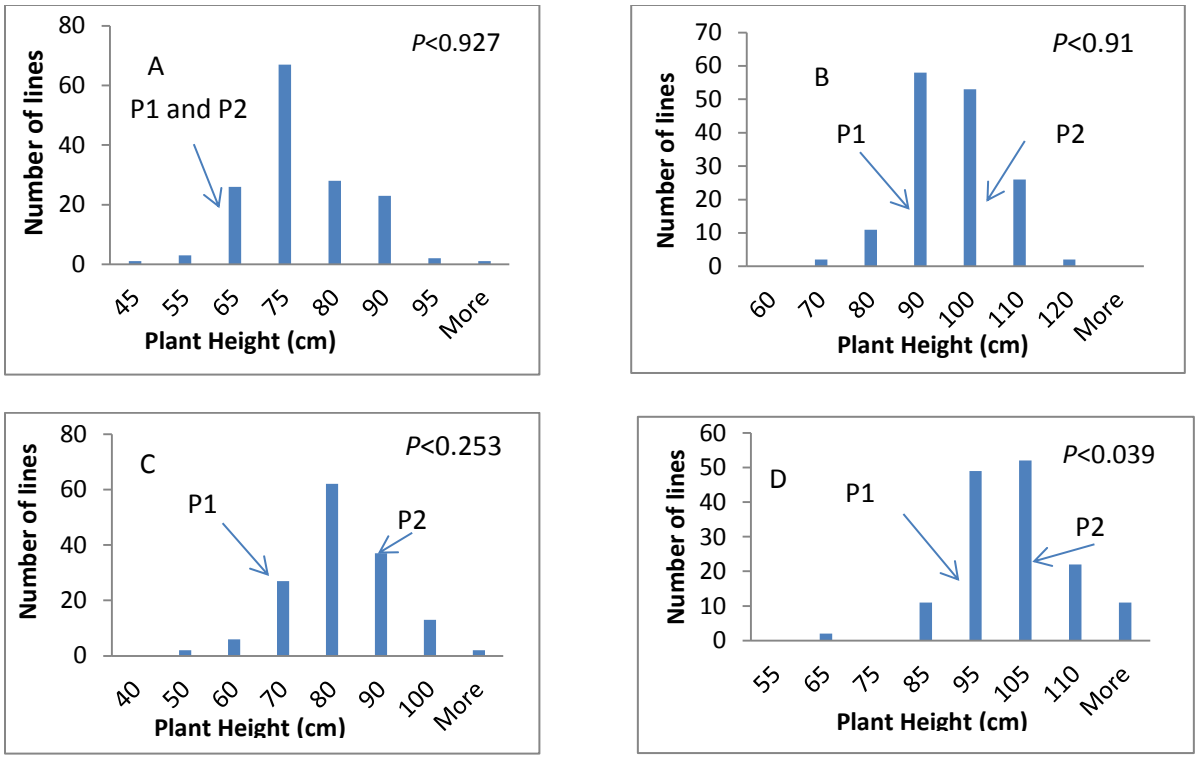
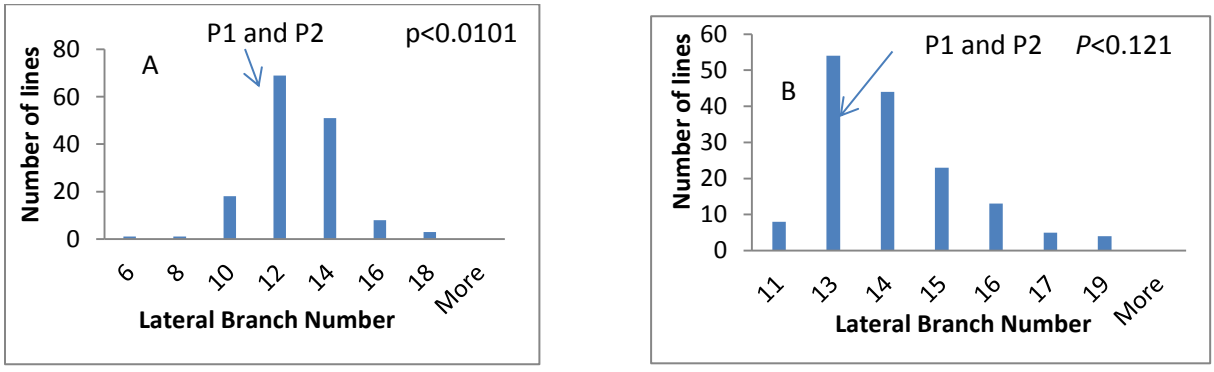
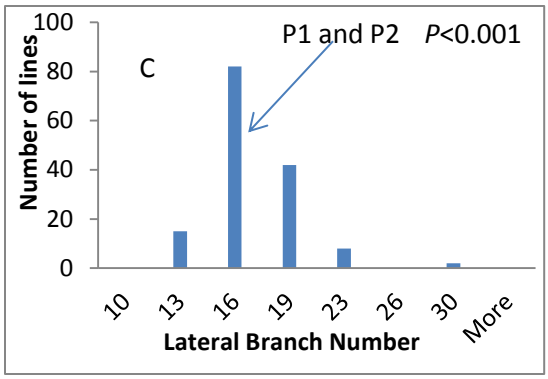


Figure A1: Frequency distribution of plant height in the DHYB canola population in Fort Collins, CO. A dry treatment 2011; B wet treatment, 2011; C dry treatment, 2012 and D wet treatment, 2012 P1, YN01-429; P2, DH012075. The *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)





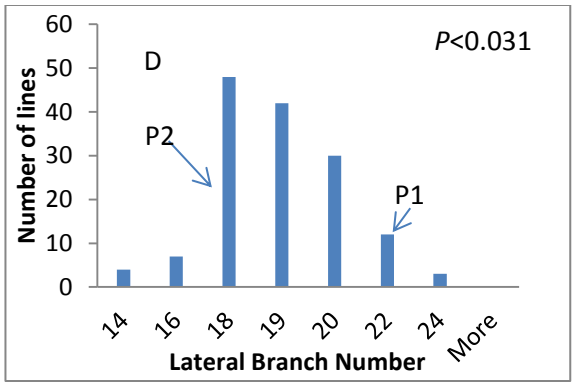
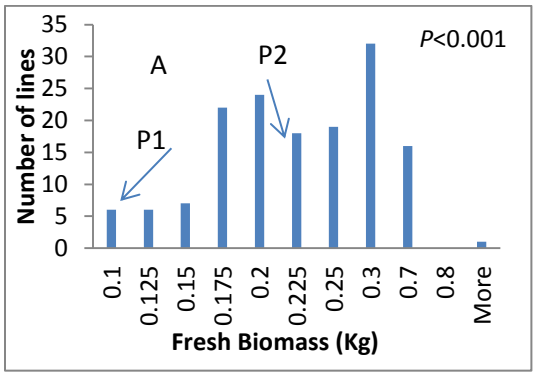
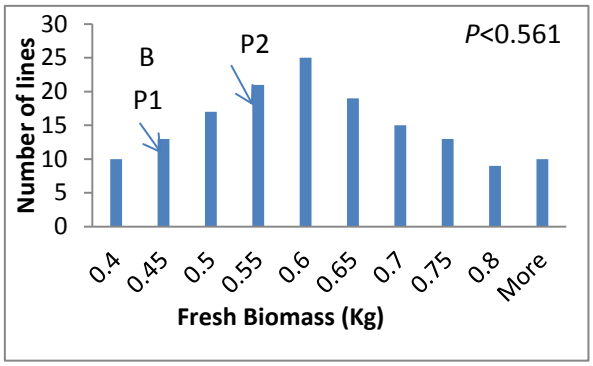
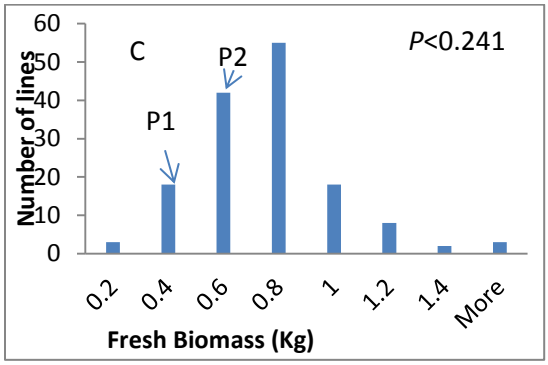


Figure A2: Frequency distribution of lateral branch number in the DHYB canola population in Fort Collins, CO. A dry treatment 2011; B wet treatment, 2011; C dry treatment, 2012 and D wet treatment, 2012; P1, YN01-429; P2, DH012075; the *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)







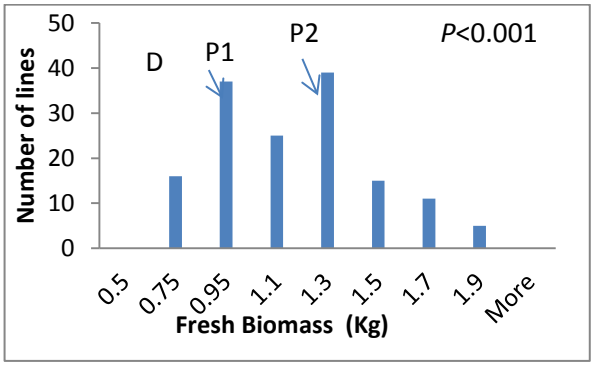


Figure A3: Frequency distribution of Fresh Biomass in the DHYB canola population in Fort Collins, CO. A dry treatment 2011; B wet treatment, 2011; C dry treatment, 2012 and D wet treatment, 2012; P1, YN01-429; P2, DH012075; the *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)

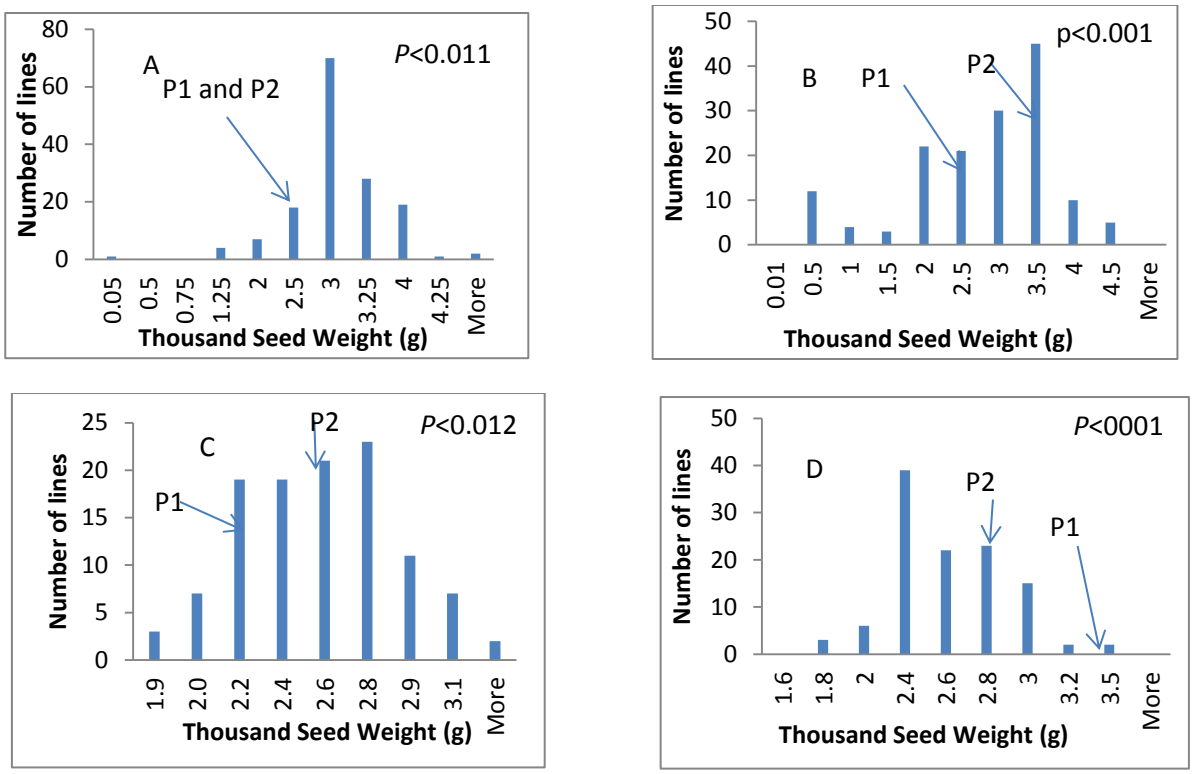
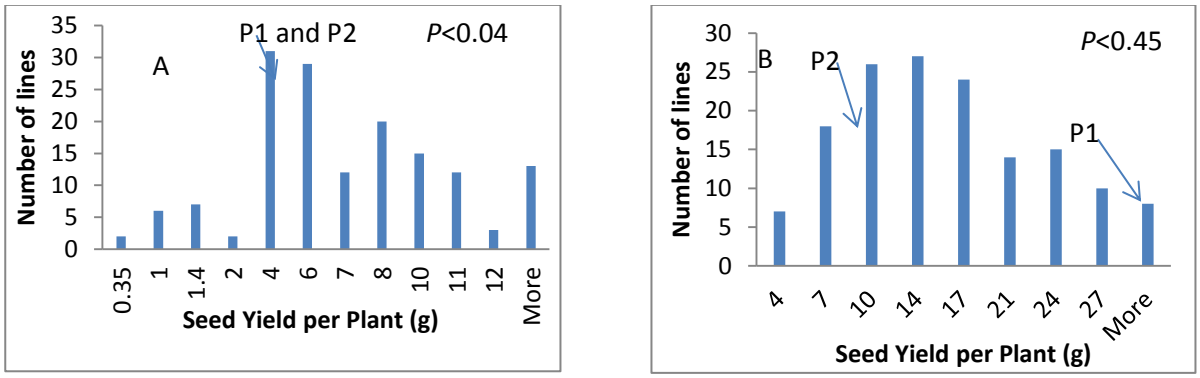


Figure A4: Frequency distribution of Thousand Seed Weight in the DHYB canola population in Fort Collins, CO. A dry treatment 2011; B wet treatment, 2011; C dry treatment, 2012 and D wet treatment, 2012; P1, YN01-429; P2, DH012075; the *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)



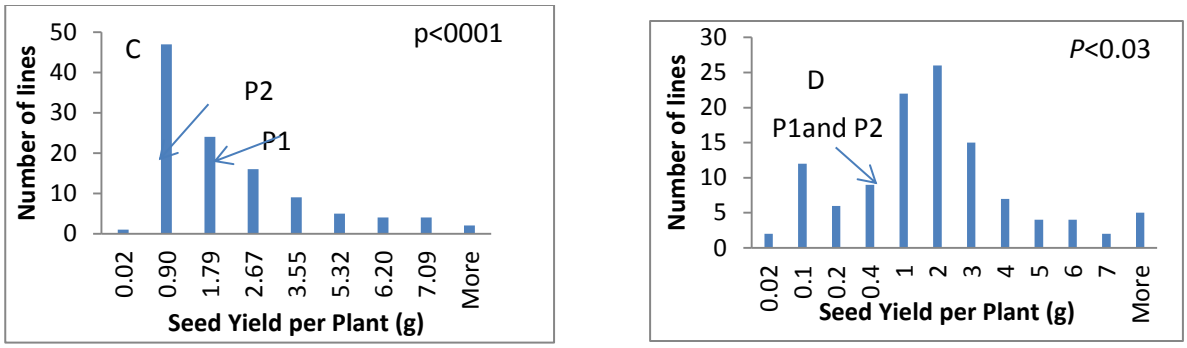


Figure A5: Frequency distribution of Thousand Seed Weight in the DHYB canola population in Fort Collins, CO. A dry treatment 2011; B wet treatment, 2011; C dry treatment, 2012 and D wet treatment, 2012; P1, YN01-429; P2, DH012075; the *P* value indicates significance of deviation from a normally distributed population (Shapiro–Wilk test)

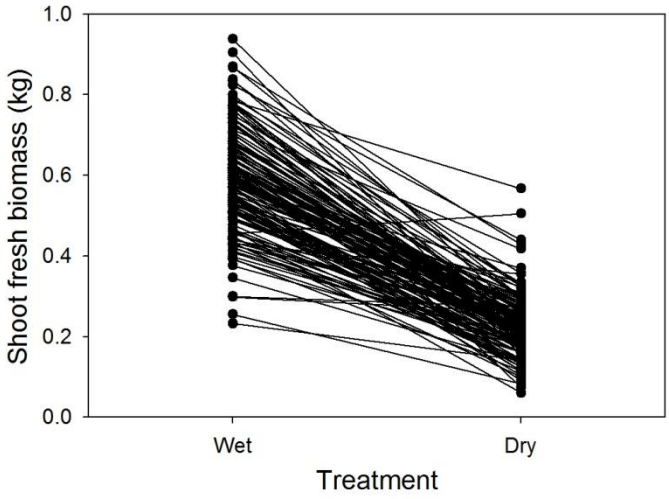


Figure A6: Reaction norm for Fresh biomass between wet and dry treatments on the DHYP population at in 2011 in Fort Colorado

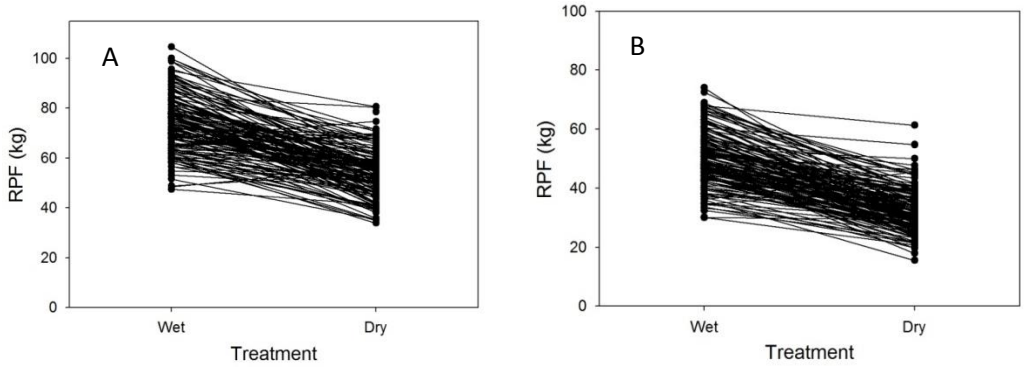
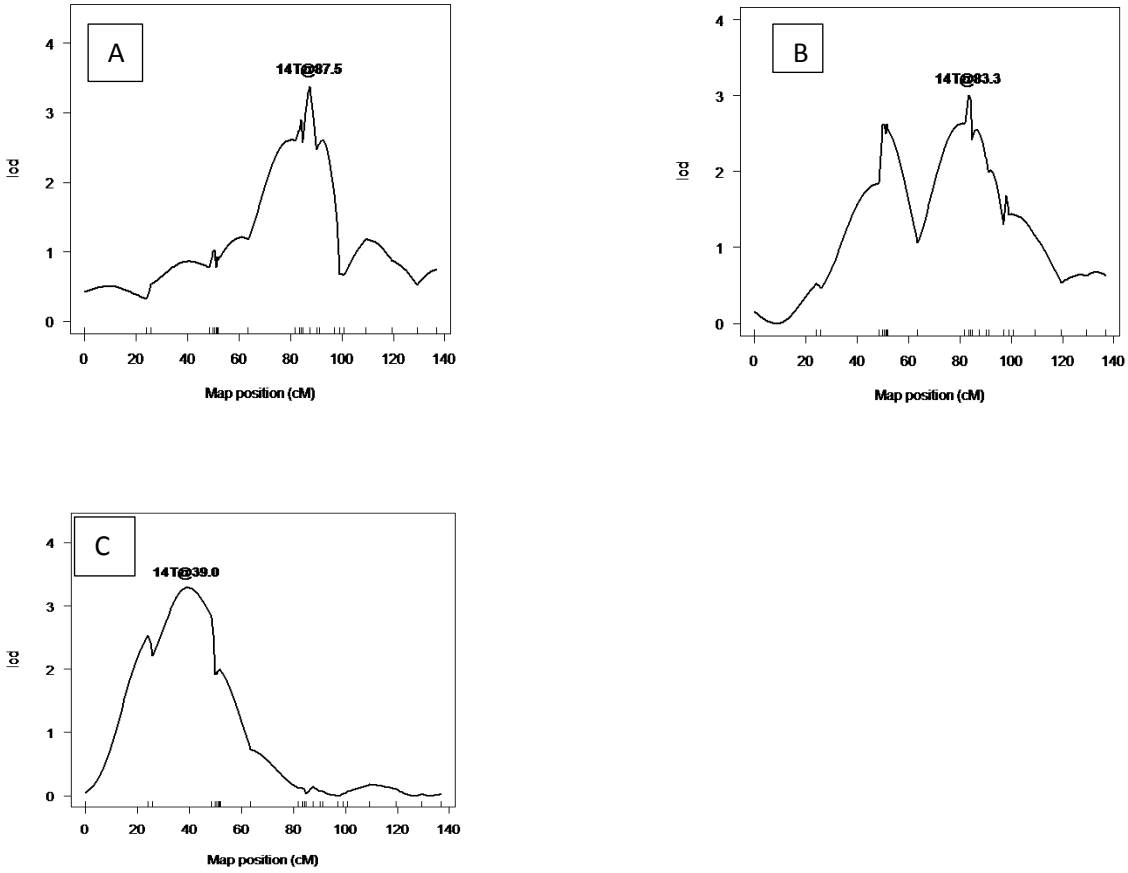


Figure A7: Reaction norm for root pulling force between wet and dry treatments on the DHYP population at in Fort Colorado; A, 2012; B, 2011



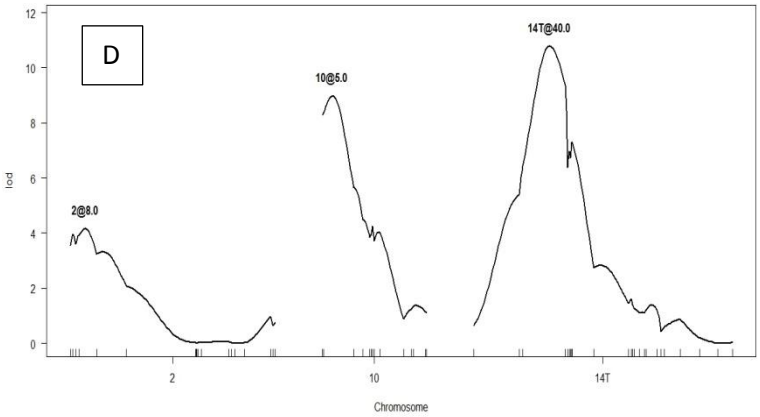


Figure A8: LOD profile of co-localizing QTL for plant height (A) Dry 2012; (B) plant height in Wet 2012 (C) Dry 2011 (D) Wet 2012 in the DHYB canola population in Fort Collins, CO

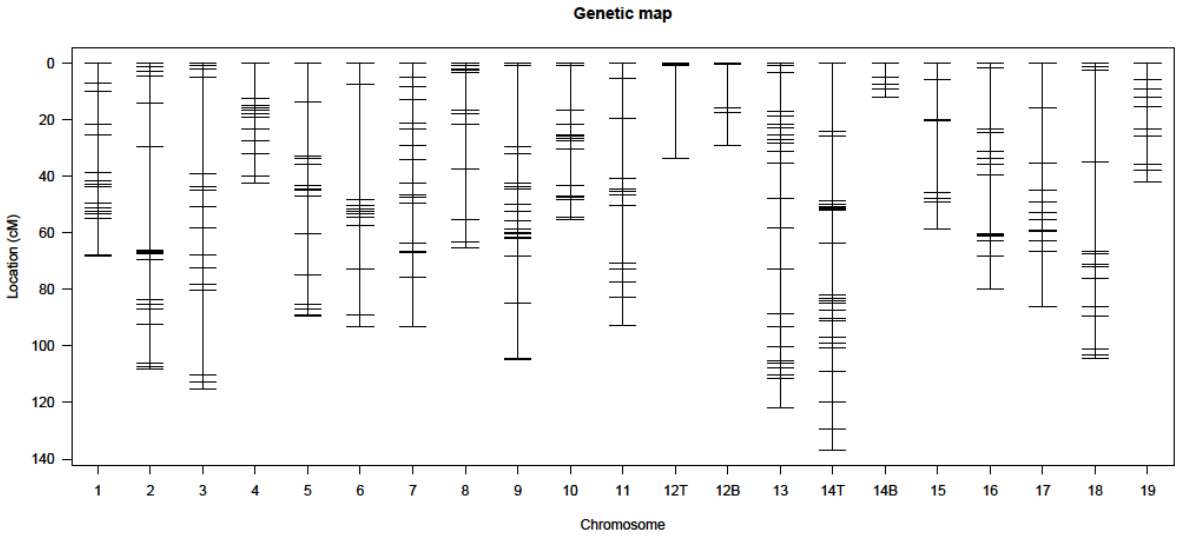
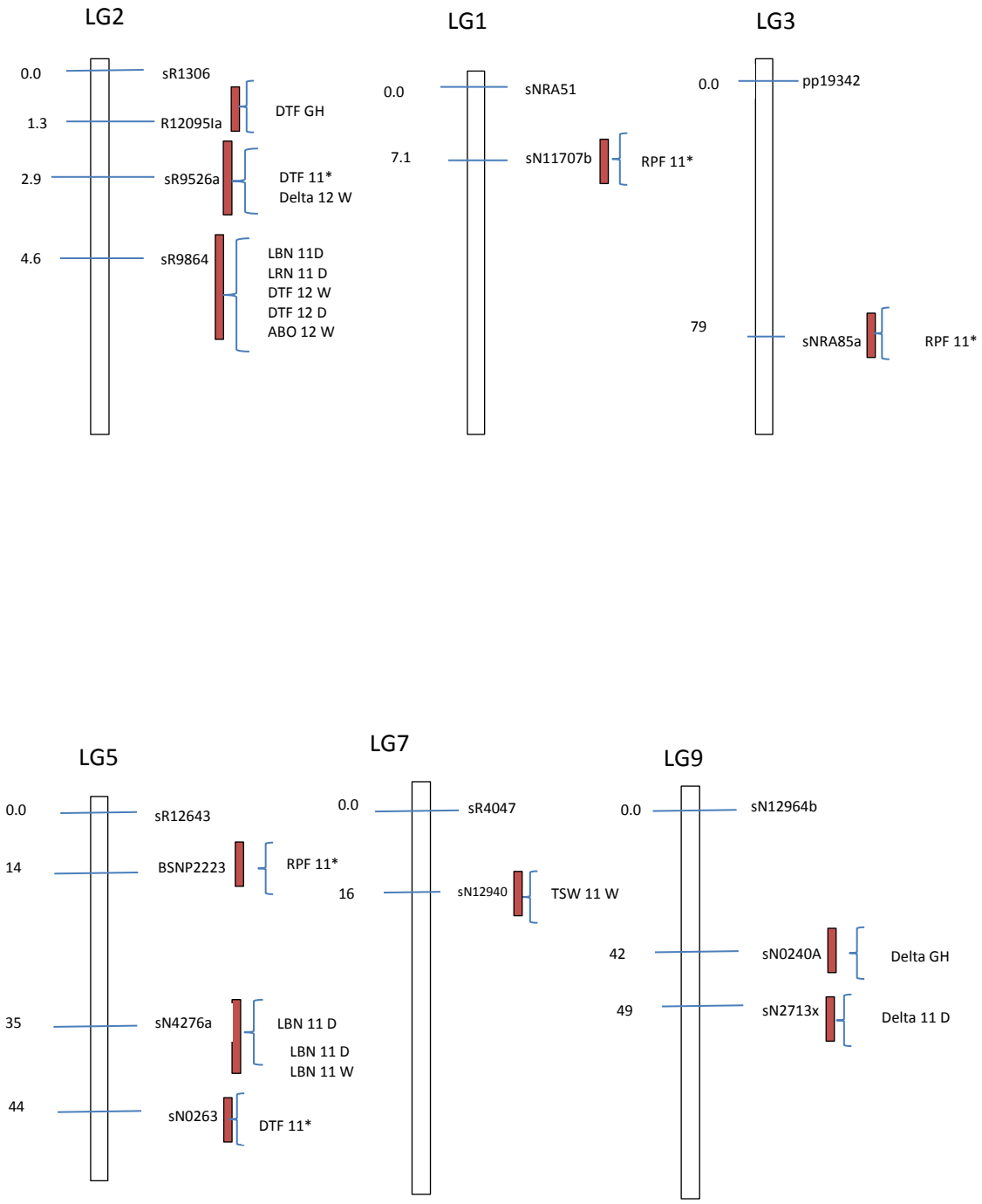
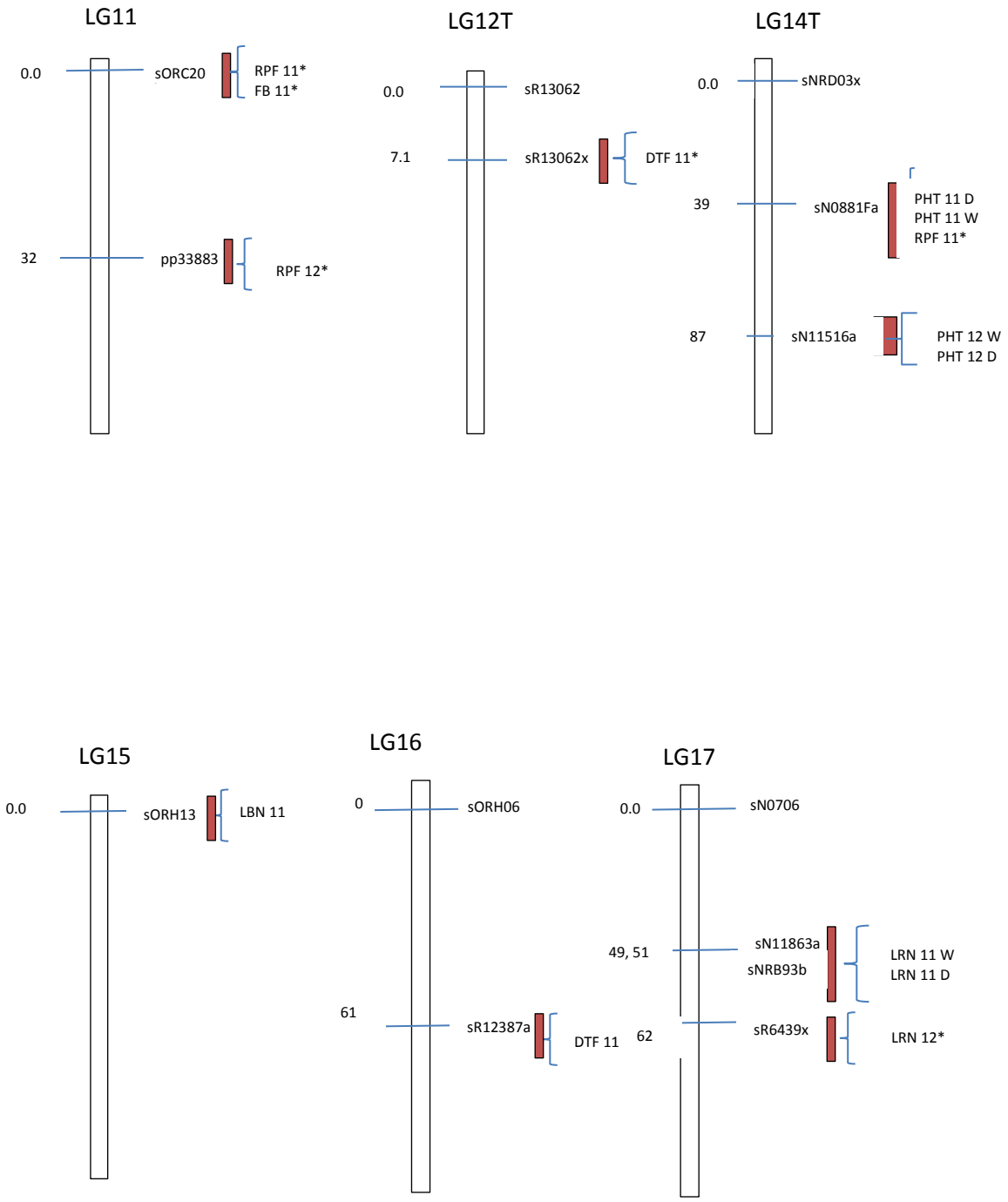


Figure A9: Genetic linkage map of the DHYB canola population used for QTL analysis. The markers cover about 87.1% of the genome.


LG2
0.0
sR1306
1.3
R12095Ia
DTF GH
2.9
sR9526a
DTF 11*
Delta 12 W
4.6
sR9864
LBN 11D
LRN 11 D
DTF 12 W
DTF 12 D
ABO 12 W
LG1
0.0
sNRA51
7.1
sN11707b
RPF 11*
LG3
0.0
pp19342
79
sNRA85a
RPF 11*
LG5
0.0
sR12643
14
BSNP2223
RPF 11*
35
sN4276a
LBN 11 D
LBN 11 D
LBN 11 W
44
sN0263
DTF 11*
LG7
0.0
sR4047
16
sN12940
TSW 11 W
LG9
0.0
sN12964b
42
sN0240A
Delta GH
49
sN2713x
Delta 11 D


LG11
0.0
sORC20
RPF 11*
FB 11*
32
pp33883
RPF 12*
LG12T
0.0
sR13062
7.1
sR13062x
DTF 11*
LG14T
0.0
sNRD03x
39
sN0881Fa
PHT 11 D
PHT 11 W
RPF 11*
87
sN11516a
PHT 12 W
PHT 12 D
LG15
0.0
sORH13
LBN 11
LG16
0
sORH06
61
sR12387a
DTF 11
LG17
0.0
sN0706
49, 51
sN11863a
sNRB93b
LRN 11 W
LRN 11 D
62
sR6439x
LRN 12*

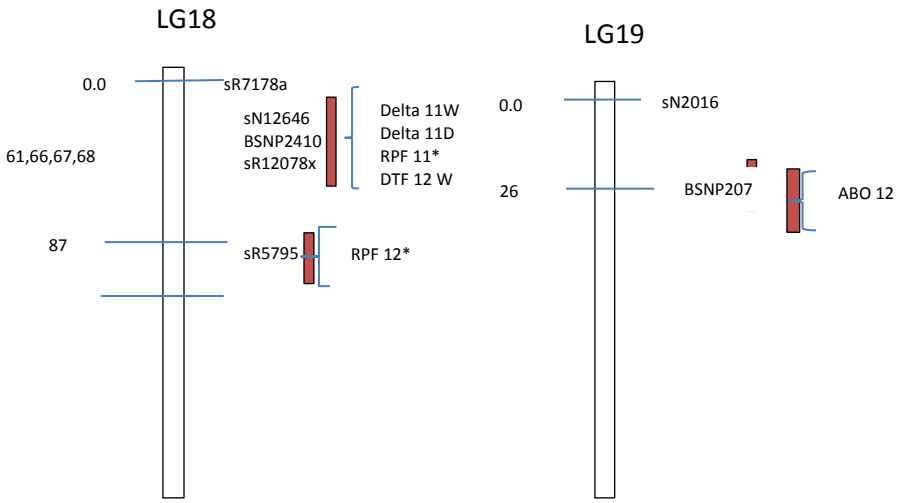


Figure A10: location of QTL on linkage groups in DHYB canola population for traits measured in wet and treatments in Fort Collins, CO.
* Detected by covariate analysis

Table A1: Monthly average high and low temperature and precipitation during the growing season in 2011 and 2012 at ARDEC, in Fort Collins, CO.

	2011			2012		
Month	Max	Min	Prec mm	Max	Min	Prec mm
April	27.7	5.0	19.3	**30.4**	11.3	**0.0**
May	29.5	12.2	89.7	**33.7**	11.5	**40.1**
June	35.5	14.3	51.1	**38.2**	18.2	**15.8**
July	34.9	17.2	46.2	**36.7**	19.4	**39.9**
August	36.6	19.4	5.8	**35.4**	17.2	**2.0**

Bold is higher temperature and minimal precipitation obtained in 2012 growing season

Table A2: Drought index for selected traits of DHYB canola population in 2011 and 2012 growing seasons in Fort Collins, CO

	2011					2012		
DH lines	**RPF**	**FB**	**RWC**	**LBN**	**Yield**	**RPF**	**FB**	**LBN**
DH29882	1.11	1.12	1.01	1.88	0.78	1.55	2.40	1.68
DH29883	0.91	0.99	0.71	1.14	1.47	1.23	0.73	1.02
DH29296	0.69	0.80	0.51	0.53	1.22	0.82	2.58	0.80
DH29297	0.35	1.07	0.75	1.28	1.19	0.96	1.12	0.69
DH29298	0.96	0.71	1.35	-0.04	1.34	1.19	0.48	0.61
DH29299	1.46	1.04	0.67	2.01	1.22	0.92	1.12	1.29
DH29370	0.79	1.06	0.93	0.62	-0.40	1.22	1.55	0.89
DH29371	0.00	-0.18	1.04	0.12	0.62	0.45	1.74	1.82
DH29374	1.00	0.76	1.16	0.26	-0.75	1.46	1.84	1.49
DH29378	0.61	0.75	0.35	0.11	1.40	1.10	1.62	1.07
DH29379	1.03	1.10	1.22	-0.72	-1.14	1.88	1.65	1.92
DH29384	0.56	1.05	1.15	0.09	0.99	1.40	1.40	1.40
DH29386	0.18	1.05	1.02	1.95	1.31	1.60	0.95	0.83
DH29394	0.81	1.13	1.36	0.62	1.59	1.07	2.20	-1.13
DH29395	1.30	1.12	0.75	1.51	1.19	0.50	1.45	0.29
DH29397	0.36	0.29	0.99	0.00	-0.09	0.45	-0.57	0.85
DH29400	0.89	0.91	0.66	1.54	1.03	na	0.21	na
DH29406	1.32	1.10	1.01	1.37	1.11	1.01	1.47	0.34
DH29409	1.52	1.16	1.20	0.54	1.40	0.15	na	1.20
DH29410	1.17	1.02	1.09	0.91	1.55	1.21	2.08	0.96
DH29412	0.90	1.11	1.43	1.40	1.19	1.16	2.03	2.25
DH29418	1.47	1.26	0.71	1.06	1.54	1.34	1.33	0.67
DH29419	1.04	1.01	0.46	0.06	1.04	0.98	1.61	-0.37
DH29420	0.50	1.05	1.23	1.01	-0.02	0.81	1.49	1.52
DH29421	1.68	1.10	0.51	1.29	0.33	1.94	1.07	1.45
DH29424	1.46	0.74	0.62	-0.30	1.05	1.10	0.92	-0.03
DH29425	1.66	0.98	1.31	1.84	-0.05	0.44	2.16	0.65
DH29478	1.34	0.86	1.02	0.51	0.01	0.19	1.05	-2.44
DH29481	1.58	1.25	1.72	2.50	0.80	1.65	3.19	-0.58
DH29483	1.12	0.48	0.95	1.05	1.57	0.44	1.26	0.43
DH29487	1.33	0.97	0.82	2.07	0.78	0.54	0.10	0.34
DH29489	0.07	0.94	0.77	2.27	1.15	0.97	1.83	0.73
DH29491	0.94	1.21	0.84	2.43	1.48	1.18	1.23	1.15
DH29492	0.91	1.20	1.09	2.46	1.37	1.67	0.30	1.53
DH29493	0.63	0.89	0.48	0.83	-0.56	1.09	1.82	1.67
DH29494	1.33	1.16	0.74	1.83	0.59	1.34	1.34	1.70
DH29495	0.97	1.10	1.16	0.32	0.74	1.39	2.75	0.12
DH29496	1.54	1.19	1.41	0.82	1.48	-0.35	2.08	-0.03

	2011					2012		
DH lines	**RPF**	**FB**	**RWC**	**LBN**	**Yield**	**RPF**	**FB**	**LBN**
DH29498	1.41	1.01	1.41	1.79	0.99	0.73	0.34	2.46
DH29499	0.95	1.04	1.18	1.03	1.06	1.09	-1.73	0.51
DH29500	0.41	0.90	0.15	1.32	1.11	0.47	1.88	0.00
DH29545	1.44	1.22	0.79	1.48	0.22	-0.60	2.17	1.52
DH29551	0.76	0.79	0.62	0.41	1.21	-0.10	1.02	na
DH29554	1.64	1.07	1.17	1.04	0.62	0.76	1.25	1.32
DH29557	1.13	0.95	1.20	0.72	1.30	1.02	na	0.86
DH29558	0.40	1.05	1.01	0.73	0.92	1.08	2.03	2.15
DH29564	0.89	0.92	0.83	2.16	0.68	0.32	-0.09	1.79
DH29566	1.02	0.87	0.54	0.78	1.11	0.12	2.39	0.13
DH29568	0.43	0.87	0.46	0.60	0.76	-0.22	1.88	1.18
DH29576	1.26	1.12	0.90	0.06	1.24	0.85	2.01	2.40
DH29577	1.27	1.03	0.47	2.55	1.28	0.87	2.46	0.99
DH29578	1.06	0.95	1.36	0.38	1.58	0.70	1.22	0.44
DH29580	0.97	1.22	1.12	0.22	1.45	0.92	1.22	0.65
DH29581	1.57	1.15	0.37	0.83	0.40	0.85	1.25	-0.06
DH29584	0.53	0.97	0.94	0.75	0.93	0.51	2.18	1.38
DH29585	0.61	0.97	1.08	1.40	1.63	0.99	2.12	0.53
DH29590	0.11	1.17	1.35	1.07	0.76	0.95	2.13	1.33
DH29592	1.36	1.03	0.81	0.85	1.04	1.49	0.09	1.75
DH29595	1.03	1.02	1.27	0.93	0.43	1.85	2.02	1.56
DH29633	0.44	0.98	0.64	1.57	0.98	0.11	1.95	0.25
DH29637	1.07	0.63	0.95	1.06	1.75	-0.09	1.89	1.74
DH29643	0.54	0.73	0.81	1.82	-0.60	-0.22	1.19	-0.45
DH29648	1.48	1.05	1.51	2.05	0.68	0.37	1.66	0.23
DH29651	1.28	0.85	0.60	0.98	0.98	1.82	-0.74	-0.41
DH29654	0.97	0.96	1.58	1.09	0.53	0.73	1.32	0.92
DH29655	1.24	1.09	1.17	1.81	1.33	0.81	0.24	0.97
DH29656	0.20	0.95	1.10	1.84	1.19	0.88	1.06	1.19
DH29657	0.60	1.40	0.64	1.28	0.48	0.40	0.36	1.13
DH29658	1.16	1.10	1.60	-1.22	0.32	1.13	0.02	1.72
DH29662	1.21	1.34	0.87	1.35	1.56	1.46	0.03	0.85
DH29676	0.28	0.90	0.96	2.05	1.35	1.33	2.49	1.03
DH29715	1.28	1.24	0.78	2.11	1.45	0.19	-1.53	1.91
DH29716	0.90	1.01	0.99	0.20	0.83	1.08	1.67	0.51
DH29719	0.63	0.95	1.06	0.34	1.24	0.50	1.53	-0.96
DH29721	0.72	0.90	1.16	1.35	0.85	0.37	1.28	0.56
DH29722	0.87	0.68	1.48	0.14	na	1.35	1.19	0.26
DH29726	0.26	0.96	0.89	1.38	0.12	1.49	0.51	1.24

	2011					2012		
DH lines	**RPF**	**FB**	**RWC**	**LBN**	**Yield**	**RPF**	**FB**	**LBN**
DH29728	1.03	1.17	0.94	0.83	0.93	0.76	2.00	0.77
DH29730	1.76	1.07	1.79	0.43	0.59	0.22	1.33	-0.13
DH29731	1.40	1.15	1.24	2.54	0.61	0.48	0.80	1.01
DH29735	0.65	0.59	0.90	0.80	0.07	1.55	0.42	0.24
DH29737	1.78	1.12	0.50	1.63	-0.14	1.52	-0.14	1.55
DH29738	1.01	1.12	0.89	0.97	1.54	1.06	1.06	0.75
DH29739	1.18	0.95	1.39	0.35	-0.54	1.78	1.62	1.41
DH29741	1.22	1.10	0.94	0.54	1.21	1.59	0.91	1.15
DH29742	0.73	1.04	0.93	1.51	0.07	0.94	1.74	1.95
DH29746	1.63	1.04	1.09	0.76	1.36	0.91	1.37	0.50
DH29747	0.54	1.24	1.39	1.38	0.49	1.19	1.35	-5.07
DH29749	0.73	0.96	1.21	-0.16	0.78	1.66	0.77	0.06
DH29753	0.80	1.21	1.46	0.90	1.38	1.41	-22.44	1.28
DH29757	0.90	0.20	0.30	1.06	1.53	0.55	1.43	0.56
DH29763	1.39	1.04	1.06	0.90	1.06	0.94	1.83	3.20
DH29764	1.21	1.35	1.34	2.00	1.59	1.48	0.63	2.05
DH29765	1.30	1.28	0.87	1.74	1.57	1.36	2.27	2.00
DH29766	1.22	0.98	-0.40	0.53	1.30	1.25	1.56	1.23
DH29812	0.87	0.45	0.95	0.77	0.73	1.46	1.42	1.70
DH29819	1.26	0.70	0.34	1.53	-1.42	1.60	2.95	2.03
DH29820	0.29	0.88	1.20	1.83	1.01	1.31	2.45	-0.26
DH29825	0.61	0.86	-0.38	0.88	-0.21	1.66	0.29	2.57
DH29826	1.23	0.84	1.22	0.93	0.40	na	1.30	na
DH29827	0.83	1.22	0.66	0.17	1.35	0.98	2.50	1.98
DH29829	1.24	1.00	0.44	0.21	-30.22	0.50	na	1.38
DH29830	1.05	0.95	1.10	1.80	0.59	1.10	1.38	0.89
DH29832	0.98	0.89	0.99	0.61	1.74	1.79	-0.33	0.12
DH29850	1.66	1.07	1.35	0.98	0.97	1.03	0.39	1.70
DH29853	1.40	1.18	0.96	1.66	0.98	1.75	0.45	2.11
DH29863	0.27	0.75	0.70	0.76	1.62	1.36	2.02	1.11
DH29865	0.59	0.99	1.53	0.42	0.94	0.99	2.76	1.82
DH29867	0.89	0.90	0.94	1.67	0.60	1.72	0.85	1.61
DH29868	1.37	0.71	0.36	0.94	0.70	1.72	1.85	1.41
DH29869	0.88	0.98	0.93	1.56	0.41	1.87	1.83	2.58
DH29880	1.06	1.09	0.84	2.09	1.44	0.91	2.10	1.33
DH30024	1.03	1.09	1.49	0.68	1.28	1.39	1.81	-0.13
DH30027	0.53	0.66	0.94	0.71	-0.39	1.62	3.21	1.50
DH30030	1.34	1.12	1.12	1.29	0.96	-0.24	1.16	0.62
DH30033	0.83	1.00	1.23	0.91	0.91	0.62	1.97	1.25

	2011					2012		
DH lines	**RPF**	**FB**	**RWC**	**LBN**	**Yield**	**RPF**	**FB**	**LBN**
DH30034	1.06	1.06	1.42	1.07	0.62	1.40	2.28	1.89
DH30037	0.97	0.67	0.26	1.14	-0.04	1.12	1.54	0.80
DH30038	0.68	0.93	0.91	0.27	1.66	0.96	0.27	0.34
DH30042	1.37	1.07	1.83	0.69	1.21	0.83	1.51	1.09
DH30045	0.97	0.81	0.50	0.45	1.08	0.69	1.23	1.31
DH30046	1.09	1.07	0.48	0.34	1.26	0.42	1.26	0.15
DH30082	0.83	1.19	1.75	2.20	1.38	0.56	1.88	0.82
DH30085	1.35	0.98	0.90	1.82	1.19	0.58	-27.25	-0.05
DH30090	1.15	1.29	0.58	0.22	0.73	1.72	1.82	1.29
DH30091	1.19	1.14	0.87	1.30	1.09	0.62	2.34	1.67
DH30092	1.01	1.06	1.22	0.32	-0.05	1.38	2.21	1.06
DH30093	-0.43	0.57	0.64	-1.49	-0.22	0.20	0.00	1.61
DH30094	1.17	0.69	1.09	-0.70	0.00	0.83	1.86	1.40
DH30095	-0.74	0.13	1.78	-1.39	1.21	1.05	0.78	0.38
DH30101	1.19	0.85	1.13	0.40	0.98	1.13	1.92	1.39
DH30102	0.78	0.79	1.48	1.59	1.05	1.35	2.31	1.92
DH30104	1.38	1.27	0.95	2.34	1.28	1.90	1.67	1.81
DH30105	1.21	0.97	1.28	0.45	1.29	-0.40	-1.22	-1.83
DH30107	0.87	0.82	0.80	0.42	1.28	1.64	1.38	0.89
DH30110	0.93	1.23	1.37	1.29	1.18	0.83	1.70	1.05
DH30169	0.66	0.98	1.37	-0.52	-2.47	na	na	na
DH30178	1.03	0.94	-0.11	1.21	0.94	1.10	1.49	0.25
DH30185	1.04	1.30	1.25	0.11	1.43	0.79	1.95	-0.28
DH30189	0.57	0.71	1.79	0.39	1.31	1.04	1.02	-2.78
DH30190	1.62	1.09	0.79	1.58	1.17	0.12	1.16	0.10
DH30192	1.36	1.07	0.85	0.44	0.74	0.63	1.35	1.40
DH30193	0.69	0.84	0.63	-0.58	1.16	0.75	1.84	0.12
DH30194	1.41	1.07	0.96	2.22	1.24	na	na	na
DH30195	1.08	0.83	1.61	0.43	0.23	1.04	0.61	1.17
DH30196	1.01	0.87	1.00	0.11	0.41	1.77	-51.81	1.66
DH30197	0.54	1.46	-0.40	4.70	1.60	1.25	2.62	2.25
DH30199	1.59	1.10	1.17	0.12	1.20	0.59	1.03	1.41

RPF, root pulling force; FB, fresh biomass; LBN, lateral branch numbers;
RWC, leaf relative water content; Yield, seed yield per plant; na, no data available