DISSERTATION

AN INTEGRATED APPROACH TO LOCAL BASED BIOFUEL DEVELOPMENT

Submitted by

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ABSTRACT

AN INTEGRATED APPROACH TO LOCAL BASED BIOFUEL DEVELOPMENT

Oilseed crops have potential to replace a portion of the on-farm energy demand currently satisfied by fossil fuel. This dissertation allies mechanical engineering, field testing, and molecular breeding research into an integrated approach to solve problems associated with straight vegetable oil (SVO) production and use on Colorado farms. Four related topics of investigation and activity are reported. To identify an adapted, short-season oilseed crop that could fit into the current High Plains dryland cropping system, a genetic diversity study was conducted on three potential oilseed species: *Brassica juncea*, *Brassica carinata*, and *Camelina sativa*. To illuminate the genetic basis of camelina response to drought stress, a two-year study of quantitative trait loci (QTLs) was implemented under dry and irrigated conditions using 181 recombinant inbred lines (RILS) developed from European cultivars. To understand and eventually manage camelina production, a multi-environmental regional trial of camelina seed yield, oil content, and oil quality was conducted with eight American and European varieties. Extension activities introduced SVO information and technology to farmers in Colorado.

Camelina sativa showed better adaptation to semi-arid environments than *B. juncea* and *B. carinata*, outyielding them due to camelina's shorter stature, higher harvest index, and greater resistance to flea beetle. Camelina yield, oil content, and linolenic fatty acid (FA) content were higher in cool, wet environments than in warm, dry environments.

Linolenic FA content and seed size were negatively correlated ($p<0.05$) with early flowering time. Earlier flowering was associated with increased seed yield ($p<0.01$) under dry and hot environments, but with decreased seed yield under irrigation. Environment was a larger source of variation than genotype for all the traits measured in this study. Twenty-nine QTLs were found in camelina for seed yield, oil quality, and drought-tolerance-related traits such as leaf water content and leaf nitrogen content, which could lead to breeding for improvement of camelina performance in semi-arid environments. Some QTLs were shared by multiple traits, suggesting either pleiotropic effects or proximity of genes. The cumulative effect of stable, favorable alleles for seed yield was a 16% increase in yield. Trait responses to moisture varied widely, both in the multi-environmental regional trial using cultivars and in the single-location trial using RILs. The range of trait response reflects variation in plasticity in camelina germplasm. Two analysis methods, namely, additive main effects and multiplicative interaction (AMMI) and the moisture difference value method, were used to detect false positive QTLs and to predict QTL effect in specific environments. AMMI was used successfully to delineate mega-environments within the study region and to identify the best-adapted varieties for these mega-environments. With the QTL data developed in this study, marker-assisted selection could be used to identify camelina varieties adapted to specific environments or to a broad range of environments.

Five lines possessing three favorable yield QTLs expressed under drought conditions are undergoing seed increase and additional multi-locational testing for potential release. Oilseed-for-biofuel workshops, crusher demonstrations, and oilseed field days were conducted to demonstrate the feasibility of potential advantages of SVO

for farmers wanting an alternative energy source to reduce their use of fossil fuel. A limited number of early adopters are beginning to integrate camelina into their crop rotation. Three small oilseed crushing and processing facilities have been established from collaboration with farmers and other agencies, and another is in the design stage. Two extension fact sheets will be published on camelina production and on biofuel production at a farm scale.

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INTRODUCTION

Interest in reducing the consumption of fossil fuels has led to efforts to satisfy the demand for energy by using wind power, solar power, and biofuels, including cellulose-derived ethanol, starch-derived ethanol, and biodiesel. However, scaling these sources of energy to fit demand in a centralized system of distribution has faced numerous challenges. Alternative fuels generally have lower density, greater intermittency and uneven distribution compared to petroleum-based fuel (Cho, 2010). The processes of converting cellulose and starch to ethanol, or converting plant- and animal-derived oils and fats to diesel fuel, require specialized facilities at a central location, so biofuels must be transported to end users via a distribution system similar to the transportation infrastructure currently used for fossil fuels. The difficulty of distribution could be avoided by developing locally-produced biofuel that could be used directly without specialized processing. One such biofuel is straight vegetable oil (SVO), extracted from oilseed crops by cold crushing, then filtered without solvents or chemical modifications.

Colorado's family farms, with available land for growing a biofuel crop and machinery adapted for diesel fuel, could satisfy a portion of their on-farm fuel consumption by growing their own biofuels. Fuel costs are

- 0.14 to 0.83 \$ liter^{-1} for SVO and
- 0.40 to 1.13 \$ liter^{-1} for biodiesel, compared to
- 0.43 to 0.97 \$ liter^{-1} for diesel fuel,

according to current estimates (Fore et al., 2011; NASS, 2010). The average Colorado family farm uses approximately 76,000 liters of fuel per year, with larger farms using up to 380,000 liters, so substantial savings would be possible as a result of SVO use.

An oilseed species suitable for use as a Colorado-grown biofuel must be drought-tolerant to yield a crop under dryland production in the semi-arid western Great Plains of the United States. Semi-arid environments are characterized by annual precipitation ranging from 250 mm to 550 mm, a short growing season (Peterson et al., 2006), and year-to-year variability. In addition to these constraints, the targeted environment, the U.S. Great Plains, faces both a cold winter and early heat in the summer, which can impact plants during flowering (Ji et al., 2010). Under these conditions, cold-adapted species with good seedling establishment and high shoot vigor are needed if the plants are to use the entire growing season and generate a profitable crop for the farmer (Richards et al., 2010). *Brassica juncea* (L.) Czern, *Brassica carinata* A. Braun, and *Camelina sativa* (L.) Crantz have shown potential as oilseed crops under semi-arid climates. They tolerate dry, cold, and hot conditions better than other energy crops (Wright et al., 1995; Alemayehu and Becker, 2001; Fernandez-Martinez et al., 2001; Francis and Campbell, 2004; Oram et al., 2005; Gan et al., 2007).

Oilseeds could fit well into the dryland, wheat-based cropping system that covers more than 4 million acres in eastern Colorado. Spring oilseeds fit into the rotation after a summer crop (corn, sorghum, or proso millet). They are typically planted in early spring and harvested in July, and can be followed by winter wheat in September. In this way, a third crop can be added to the three-year rotation schedule without displacing a food crop.

This addition potentially increases the overall precipitation use efficiency of the system (Peterson and Westfall, 2004).

In water-limited areas, a better understanding of drought-tolerance mechanisms is important for maximizing the use of precipitation. Since traits underlying drought adaptation are quantitative and are influenced by environment, an approach based on quantitative trait loci (QTLs) and marker-assisted selection (MAS) is needed to improve the breeding process. Gehringer et al. (2006) constructed a genetic map of European camelina using amplified fragment length polymorphisms (AFLPs) and simple sequence repeats (SSRs). Data from field trials were used to locate QTLs for seed yield, oil content, thousand-seed weight, and plant height. However, little research has been done specifically on drought-tolerance QTLs in *C. sativa* cultivars in Great Plains climates, which vary greatly in altitude, precipitation, and temperature regime. Genotype-by-environment interactions (GEI) are expected.

Variability in oil quantity and quality greatly affect engine performance and economic feasibility when SVO is used in farm machinery. Thus, engine testing is required to establish the optimum vegetable oil quality for proper combustion, low emissions, and acceptable engine durability. Engine research was conducted in a parallel study, but is not reported here.

New production systems require pro-active public information campaigns to speed adoption in appropriate regions. Effective extension efforts targeting the major potential oilseed production regions in Colorado include workshops, field days, demonstration units, variety trials, and fact sheets.

Full development of new energy crops requires investigation into several related areas. This study used an integrated, multi-disciplinary approach with four main objectives: (1) identify adapted species that could fit the specific environments and the regional cropping system, and evaluate their genetic diversity for improvement potential; (2) in the best-adapted species, identify QTLs for drought tolerance, seed yield, oil yield, oil quality, and specific combinations of traits involved in drought adaptation; (3) test diverse cultivars of the best-adapted species in a wide range of environments to determine the effect of environment, genotype, and GEI on yield, oil content, and linolenic fatty acid content; (4) for the best-adapted species, facilitate appropriate adoption and implementation of crop production and on-farm fuel processing.

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CHAPTER ONE:

BRASSICACEAE GERMPLASM DIVERSITY FOR MORPHOLOGICAL AND SEED QUALITY TRAITS UNDER DROUGHT

ABSTRACT

Brassica juncea, *Brassica carinata* and *Camelina sativa* have previously shown potential as oilseed crops in semi-arid climates. However, there is limited information on variation of agriculturally important traits under water stress. This study screened 94 accessions of *B. juncea*, 30 of *B. carinata* and 81 of *C. sativa* under dryland and fully irrigated conditions in eastern Colorado to evaluate responses to drought stress among and within species.

Large phenotypic variability was observed among and within species. *B. carinata* had the highest phenotypic variability for all traits studied. It had the largest average thousand-seed- weight (TSW), 3.6 grams (g) (mean of both moisture treatments) vs. 2.5g for *B. juncea* and 0.99g for *C. sativa*. On the other hand, with a 10-day longer life cycle than the two other species, *B. carinata* suffered the most from summer heat stress during seed filling. Earlier-flowering accessions among the three species were more adapted to dryland conditions. *B. carinata* and *B. juncea* were both twice as tall as *C. sativa* and produced significantly higher biomass while yielding less under dryland conditions, 795 kg ha^{-1} for *B. carinata*, 973 kg ha^{-1} for *B. juncea* and 1,383 kg ha^{-1} for *C. sativa*. *C. sativa*

showed better adaption to semi-arid environments, out-yielding *B. juncea* and *B. carinata* thanks to its shorter stature, more efficient desaturation pathway, higher harvest index, and resistance to flea beetle. Seed-oil fatty acid profiles were affected by water stress as evidenced by the decrease in linolenic acid content under dryland conditions ($p<.001$). *C. sativa* had the highest levels of linolenic acid with an average of 29.5%. *B. carinata* had the highest levels of erucic acid with 42% on average. *B. juncea* accessions showed a large range of oil profiles related to their geographical origin. Oil profile characteristics, such as high linolenic acid, were related to fitness traits such as height and biomass. Oil profile traits could play an important future role in plant adaption especially under stress environments. This work highlights traits where breeding efforts can utilize existing germplasm variation such as flowering time, yield components, oil quality, and flea beetle susceptibility to improve cultivar adaptability to semi-arid environments.

INTRODUCTION

Brassicaceae oilseed crops offer the potential of producing sustainable biofuels in arid and semi-arid regions. Unlike some of the new bioenergy grass crops such as switchgrass (*Panicum virgatum* L.), Brassicaceae oilseeds fit well into semi-arid rotations, such as the dryland winter wheat-based cropping system. Ideally these new oilseed cultivars will combine positive growth and development characteristics (e.g., nutrient efficiency, pest tolerance, early maturity and heat- and water- stress tolerance) with diverse fuel, feed and food applications (Gehringer et al., 2006). Thus, genetics knowledge and breeding efforts are needed in order to optimize oilseed crops for

sustainable oil production in diversified crop rotations. This study examined genetic diversity in traits relevant to adaptation to semi-arid environments. In particular, three species, *Brassica juncea* (L.) Czern, *Brassica carinata* A. Braun and *Camelina sativa* (L.) Crantz, are crops that could be integrated into the dryland winter wheat rotations dominant in the High Plains.

B. juncea, which originated in India, can be more productive than *B. napus* in hot regions with unreliable rainfall while producing similar yields in well-watered conditions (Wright et al., 1995; Oram et al., 2005; Gan et al., 2007). These results suggest the feasibility of developing high-yielding cultivars for both dryland and irrigated conditions. *B. carinata*, an Ethiopian native, appears better adapted and more productive than *B. napus* in clay and sandy soils in semi-arid temperate climates (Alemayehu and Becker, 2001; Fernandez-Martinez et al., 2001). *C. sativa* originated in Northern Europe and Central Asia (Putnam et al., 1993). It is considered a promising oilseed crop for dryland environments, with low input requirements (Zubr, 1997) and favorable oil properties (Bernardo et al., 2003). *C. sativa* has shown higher yield performance under semi-arid conditions than *B. napus* (Francis and Campbell, 2004).

Variation in the levels of the particular fatty acids between and within species is well documented and has been exploited in *Brassica* breeding (Velasco et al. 1998; Ghamkhar et al. 2010). Not surprisingly, variation in the profiles of fatty acids (FA) in plant oils can have large impacts on fuel quality (Mullen et al., unpublished). Thus, even if biofuels derived from these crops have been shown, on average, to be similar to American Society for Testing and Materials (ASTM) certified biodiesel (Alemayehu and

Becker, 2002), genetic variation in FA profiles will lead to differences in engine performance of the oil extracted from diverse cultivars (Nettles-Anderson et al., 2009, Mullen et al., unpublished). In addition, because seed-oil composition is correlated with FA profile in leaves (Wang et al., 1989; Lemieux et al., 1990; Shaw et al., 1997), genetic variation in the FA profiles likely plays a crucial role in plant adaptation under stress environments (Mene-Saffrane et al, 2009). Lipids have a number of essential roles in plants related to stomatal closure, pathogen invasion and water loss (Slabas et al., 2001). Selection based on oil profiles may have a direct impact on drought tolerance.

Phenotypic variation in biomass production, flowering period, seed oil content, and seed oil composition are essential for improvement of these three species through plant breeding. The objective of this study was to evaluate phenotypic variation for these traits within and among the three species under dryland and irrigated conditions. In addition, specific accessions were evaluated for adaptability to Colorado dryland agricultural production.

MATERIALS AND METHODS

Plant material and environmental conditions

A total of 81 accessions of *C. sativa*, 30 accessions of *B. carinata*, and 94 accessions of *B. juncea* were evaluated for plant and seed quality traits. The *C. sativa* accessions originated in 16 countries, *B. carinata* in three countries and *B. juncea* in five countries. *B. carinata* and *C. sativa* accessions were sourced from the Institute of Plant Genetics and Crop Plant Research (IPK) at Gatersleben, Germany. *B. juncea* accessions

were received from the USDA North Central Regional Plant Introduction Station (NCRPIS) germplasm collections. Accessions were grown at the Colorado State University Agriculture Research Development and Education Center (ARDEC) in Fort Collins in the 2008 crop season. ARDEC is located at latitude 40.65 N and longitude 105.00 W, at an elevation of 1557 meters (m). The average annual precipitation is 356 mm. The climate is considered semi-arid, and the soil type is Nunn clay loam. In 2009 and 2010, three accessions per species, chosen for their yield performance and seed size, were planted at Iliff, CO (elevation 1165 m, latitude 40.7678 N, longitude 103.045 W) under both irrigated and dryland conditions.

Field experiments

In 2008, the experimental design included the three species described above, dryland and irrigated treatments, and two replicates for each accession within a treatment. The study area was divided into two 11 m by 7.3 m blocks. A ditch was dug between the blocks to enable flood irrigation. Glyphosate was used to eradicate alfalfa plants remaining from the previous year. Then, the seedbed was prepared by disking. Seeds were sown by hand on May 12, 2008, in single-row plots of 1-m length with 0.3-m row spacing between plots. Density of planting was based on 6 kg ha^{-1} for *C. sativa* and 8 kg ha^{-1} for both *Brassica* species, rates typically used in the High Plains. Both blocks were flood-irrigated until full emergence in all accessions. Then, one block became a dryland-only treatment, while the other continued to be irrigated. The irrigated treatment was flood-irrigated four times: planting, pre-flowering, post-flowering and filling period. No fertilizer was applied to the field. During the growing season, weeds were controlled

manually. Flea beetles (*Phyllotreata cruciferae*) were controlled as needed from emergence to the flowering stage with applications of Ortho Max (Ortho Group, Marysville, OH), following manufacturer instructions. The Iliff trial design was a randomized complete block design (RCBD) with three replicates. The plot size was 1.5 m wide by 4.6 m long, with seven rows, 25 cm apart. The seeding rate was 8 kg ha^{-1}. In 2009, only a dryland treatment was conducted at Iliff due to high precipitation. In 2010, the Iliff trial had two randomly assigned treatments, dryland and limited irrigation. The limited irrigation treatment received 25 mm of water at pre-flowering stage and 25 mm during seed filling stage.

The following data were taken for the Fort Collins trial in 2008. Flea beetle infestation was scored from one to three (no infestation, 50% infested, 100% infested). Flowering time was recorded in each plot as the date when 50% of the plants had reached flowering. Plant height was determined for five randomly selected plants per plot by measuring the distance from the soil surface to the highest point on the plant at the time of pod maturity. Five pods were taken randomly from each plot at full maturity to estimate seed number per pod and 1000-seed-weight (TSW). At the same time, number of pods on the primary branch, primary branch length, pod density and number of branches were counted on five randomly selected plants. The stand density was determined by counting the number of plants per row. Five plants per plot were randomly selected and cut with a sickle at soil level, for plant biomass measurement. These samples were dried at 40°C for 48 hours. Seeds were separated from the vegetative tissue after the biomass of the whole plant was measured. Single-row plots were harvested at full

maturity, between August 1 and August 10, 2008, at Fort Collins, CO. In 2009 and 2010 at Iliff, CO, plant height, flowering time and seed yield were recorded as described above. Trials were harvested between July 16 and August 3, 2009, and between July 20 and August 6, 2010.

The FA profile analysis was carried out by gas chromatography-mass spectrometry (GC-MS). A one-gram seed sample per plot was used to run through the GC-MS. Because FA contents are inter-correlated (Velasco et al., 1997), ratios were calculated to compare FA pathways (Velasco et al., 1998). The elongation ratio (ER) estimates the relative weight of the elongation pathway from oleic acid (C18:1) to eicosenoic (C20:1) and erucic acid (C22:1). Desaturation ratio (DR) estimates the relative weight of the desaturation pathway from oleic acid to linoleic (C18:2) and linolenic acid (C18:3) within the overall fatty acid biosynthetic system. Within the desaturation pathway, oleic desaturation ratio (ODR) and linoleic desaturation ratio (LDR) estimate the efficiency of the desaturation from oleic to linolenic (ODR) and from linoleic to linolenic acid (LDR) (Pleines and Friedt, 1988). These FA content ratios were calculated following Velasco et al. (1998):

$$\mathrm{ER} = \frac{\%C20{:}1 + \%C22{:}1}{\%C18{:}1 + \%C18{:}2 + \%C18{:}3 + \%C20{:}1 + \%C22{:}1}$$

$$\mathrm{DR} = \frac{\%C18{:}2 + \%C18{:}3}{\%C18{:}1 + \%C18{:}2 + \%C18{:}3 + \%C20{:}1 + \%C22{:}1}$$

$$\mathrm{ODR} = \frac{\%C18{:}2 + \%C18{:}3}{\%C18{:}1 + \%C18{:}2 + \%C18{:}3}$$

$$\mathrm{LDR} = \frac{\%C18{:}3}{\%C18{:}2 + \%C18{:}3}$$

Data analysis

For each of the three species, accessions were considered as samples from a large gene pool representing the range of variations that is available to breeders. The population means were estimated with this set of materials. Data were analyzed using the statistical analysis software SAS version 9.2 (SAS Institute, Inc., Cary, NC). A mixed model was used with irrigation and species effects as fixed, accessions as random effects nested within species using the PROC MIXED procedure. Pearson correlation coefficients among different traits and treatments were generated by the PROC CORR procedure based on LS mean. In order to assess variation in overall seed oil profiles, a principal components analysis (PCA) was performed using proportions of individual FA components, and the sum of the saturated FAs. Principal component analysis was performed on the FA profile using the PROC PRINCOMP procedure. Then, the study determined if there was a geographic correlation with oil profile for each species through PCA.

Broad-sense heritability for mean values over environments was calculated from components of variance with REML iteration in SAS using PROC VARCOMP, according to the following formulas:

(1) Smith and Dhondt (1980): $h^2 = V_g/(V_g + V_e + V_r)$, where V_g, V_e and V_r, represent the respective variance components for genotype (g), environment (e) and (r) residual.

(2) Hill et al. (1998), $h^2=V_g/[(V_g + (V_r/E*R)]$, where V_g, and V_r represent the respective variance components for genotype (g), and residual variance (r), and E and R are the number of environments and replicates, respectively.

RESULTS

For most measured growth and development and oil quality traits, significant differences (p=0.1 to p<0.0001) were found between moisture treatments and accessions within species (Table 1.1).

Variation in growth and development among species

The date of flowering in *B. carinata* was significantly later (p<0.0001) than in the two other species regardless of treatment and years, occurring more than 10 days after *B. juncea* and *C. sativa* in 2008. *B. juncea* flowered an average of 1.2 days later than *C. sativa* in 2009. Heritability for flowering time was 0.92 and 0.91 respectively for *B. juncea* and *B. carinata* (Table 1.1). Heritability in *C. sativa,* 0.28, was perhaps caused by a soil crusting issue that retarded emergence in the lower section of the field where *C. sativa* was grown. All three species' flowering times increased, on average, one day from the dryland to the irrigated treatment (Table 1.2). However, within species, flowering time of some accessions decreased from irrigated to dryland treatment (BRA-2464, Ji-019, Cam-143) while for other accessions, the inverse effect was observed (BRA-1151, jb-012, Cam-194) (Fig. 1). Consistent flowering time results were found in 2009 and 2010 at Iliff, CO. *B. juncea* and *C. sativa* flowering times were not significantly different,

while *B. carinata* flowered on average seven days later than the other two species (Table 1.3).

In 2008, the biomass per plant of *C. sativa* in the irrigated treatment was less than 48% of the *Brassica* species biomass. *C. sativa* was less sensitive to changes in water availability, such that in the dryland treatment it had similar biomass to *B. juncea* (Table 1.2). In all three species, accessions producing higher biomass under irrigation tended to have poor biomass production under dryland conditions (BRA-1155, Ji-005, and Cam-1156). In 2008, plant height and main branch length of the two *Brassica* species were greater than *C. sativa* under both treatments (Table 1.2). In 2009 and 2010, *Brassica* species were on the average twice as tall as *C. sativa* (Table 1.3).

In 2008, *B. carinata* produced the largest seed, TSW of 3.6 grams, averaged across both moisture treatments, while *C. sativa* had the smallest seed, TSW of 0.99 gram (Table 1.2). *B. carinata* and *C. sativa* seed size did not differ significantly between moisture treatments, whereas *B. juncea* had significantly larger seed under irrigation. Within the three species, a wide range of TSW treatment responses were expressed (Fig. 1.1). In some *B. carinata* accessions (BRA-2126 and BRA-1173), TSW increased from dryland to irrigation, while in others it decreased (BRA-1182 and BRA-1030). *B. juncea* and *B. carinata* seed size was positively correlated ($p<0.05$) with biomass (Table 1.4). Seed size heritability was high for all three species, 0.88, 0.64, and 0.86, respectively, for B. *juncea*, *B. carinata* and *C. sativa* (Table 1.1). In the dryland treatment, there were accessions of *C. sativa* (Cam-7, Cam-10, Cam-111) having seed as large as some *B. juncea* accessions.

B. juncea, *B. carinata* and *C. sativa* had similar seed number per pod. While *B. juncea* showed no effect of moisture treatment on number of seeds per pod, the number of seeds per pod decreased significantly ($p<0.05$) in the dry treatment for *B. carinata* and *C. sativa*. In *B. juncea* and *C. sativa,* seeds per pod under irrigation was positively correlated ($p<0.05$) with flowering time but negatively correlated to TSW (Table 1.4).

In 2009 and 2010, *B. juncea* and *B. carinata* were taller than *C. sativa* under both moisture treatments but yielded significantly ($p<0.01$) less (Table 1.3). The two-year seed yield average of *C. sativa* was 1,383 kg ha^{-1} compared to 795 kg ha^{-1} and 973 kg ha^{-1} for *B. carinata* and *B. juncea,* respectively. Hence, in 2009 and 2010, *C. sativa* plants significantly out-yielded both *Brassica* species by 36%, but were only half their size. Thus, *C. sativa* had a higher harvest index than the *Brassica* species.

Insect damage evaluation

No flea beetle damage was observed on *C. sativa* under either water treatment. *B. carinata* showed high variability in susceptibility. *B. juncea* and *B. carinata* accessions were all very susceptible to flea beetles.

Seed oil fatty acid profiles

Based on the principal component analysis run on the 2008 dataset, each species had a characteristic oil profile (Fig. 1.2A). The first component extracted, PC1, accounted for 90.7% of the variance, and was correlated with erucic fatty acid content. Principal component 2 (PC2), which accounted for 8.1% of the variance, was correlated with linolenic acid content.

The study found a geographic pattern for *B. juncea*, with accessions from Bangladesh, India, and Pakistan clustering together, while accessions from China and Russia were clustered together in a group separate from the Bangladesh-India-Pakistan group but showed larger variation in fatty acid profiles (Fig. 1.2B). The accessions from Bangladesh, India, and Pakistan tended to have higher levels of erucic acid, whereas the Chinese and Russian accessions had higher levels of oleic and linoleic acid. *B. carinata* and *C. sativa* did not express a geographic pattern. In all three species, some accessions increased their LDR between dryland and irrigated treatments e.g., BRA-2569, Ji-019, Cam-502, while in others LDR decreased e.g., BRA-2124, jb-012, Cam-655.

Considering the FA components contributing the most to the first two principal components, it appeared that much of the observed variation was related to specific FA biochemical pathways. The three species showed significant differences in the efficiency of FA pathways (Fig. 1.3), as represented by elongation and desaturation ratios (Velasco et al. 1997, 1998). Both *B. carinata* and *B. juncea* had high elongation ratios, 0.55 and 0.49, respectively, compared to *C. sativa* at 0.19. However, a few *B. juncea* accessions had very low ER (Jr-00, Jc-024 and Jr-013). Eicosenoic acid (C20:1) showed significant positive correlation ($p<0.01$) to erucic acid (C22:1) content within *C. sativa* species whereas *B. carinata* and *B. juncea* showed significant negative correlation between those FAs in both treatments. ER was negatively correlated with DR for *B. juncea* and *B. carinata* (Fig. 1.4). Thus, accessions favoring long-chain FA synthesis had lower polyunsaturated FAs.

DR decreased slightly from the irrigated to dryland treatment for *B. juncea* and *C. sativa*, while it increased for *B. carinata* (Fig. 1.4). Both *Brassica* species showed significant positive correlations ($p<0.01$) between LDR and ODR, following the same trend. *C. sativa* did not show a significant correlation between the two ratios in part due to a strong negative correlation between C18:2 and C18:3, which was not significant for the other species. *C. sativa* had the highest LDR at 0.59, compared to 0.35 and 0.48, respectively, for *B. juncea* and *B. carinata* (Fig. 1.4). Hence, *C. sativa* had a very active pathway converting C18:2 to C18:3 by the enzyme linoleate desaturase. Because of this *C. sativa* had the highest levels of linolenic acid in both treatments (Fig. 1.4). With regard to linolenic acid levels, *C. sativa* was also the least sensitive to water availability. In 2009 and 2010, FA profiles were consistent with 2008 (data not shown).

B. juncea's DR had high positive significant ($p<0.001$) correlation to flowering time, biomass, plant height and pod density under both moisture treatments (Table 1.4). *C. sativa*'s DR was positively correlated ($p<0.1$) to biomass under dryland conditions while it was negatively correlated ($p<0.05$) under irrigation. *B. carinata*'s LDR was positively correlated ($p<0.1$) to biomass and flowering time under dryland conditions.

DISCUSSION

The relative performance of these three Brassicaceae species for agronomic and seed oil traits under water stress conditions has not been extensively documented. Emergence, biomass, flowering time and oil profile were characteristics of interest and showed significant differences in this study. Within species, wide ranges of phenotypic

responses, or plasticity, were observed between dryland and irrigated treatments, a condition offering breeding opportunities for semi-arid environments. Plasticity is becoming of interest to breeding programs attempting to develop varieties better adapted to specific environments (Bradshaw, 2006; Chapman, 2008; Forde, 2009; Sadras et al., 2009).

In semi-arid climates, emergence remains a major issue (Richards, 2010) for small-seeded species due to wind, plant residue and soil crusting issues. The earlier the seed germinates, the faster the seedlings can reach deeper stored moisture. Earlier seedling establishment means that the crop flowers earlier and avoids higher temperatures later in the season that negatively impact fertilization. Seed size, known to be positively correlated to faster emergence, showed large differences in the three species and accessions within species. *B. carinata* had the largest seed and *C. sativa* the smallest. *B. carinata* demonstrated very good emergence under both moisture treatments. Seed size heritability was high among all three species, indicating potential to improve TSW in all three species. However, TSW was negatively correlated to yield components in *C. sativa*, complicating breeding. In addition to seed size, fatty acid profiles could be related to better emergence under cold soil temperatures. Indeed, northern oilseed species, such as flax (*Linum usitatissimum*) and *Brassica* species, have higher levels of polyunsaturated fatty acids than Southern oilseed species, resulting in more efficient desaturation ratios (Linder, 2000). Higher levels of poly-unsaturated FAs increase cell membrane oil fluidity under cold temperatures, improve seedling establishment, and result in higher shoot vigor (Linder, 2000). A prior study found that *C. sativa* had the fastest emergence

compared to *B. napus* and *B. juncea* at low temperatures, allowing earlier planting (Enjalbert et al., unpublished). Shorter germination time could lead to earlier flowering, a desirable characteristic under dryland conditions.

Flowering time within and among species, as well as between moisture treatments, demonstrated wide variation. For *B. juncea* and *B. carinata*, late-flowering accessions produced more biomass under both moisture treatments than early-flowering accessions. Earlier-flowering *C. sativa* accessions showed increased pod density and plant height under dryland conditions. They escaped the heat, and part of the drought, resulting in higher seed yield. Both *Brassica* species produced large amount of biomass early in the season, which impacted them negatively later on when water became scarce under dryland conditions. Earlier flowering time has been shown to increase yield under dry conditions. Plants are the most susceptible to water and heat stress at the reproductive stage. By escaping drought, plants can increase seed production (Siddique et al., 1990). The two-year average seed yield of *C. sativa* was significantly higher than that of the two *Brassica* species. However, to evaluate more precisely drought tolerance within species, it would have been more appropriate to take into account flowering time when planting so the drought event would occur when all plants are at the same developmental stage. Indeed, physiological traits such as flowering express fundamental correlation with structural traits such as biomass production and oil quality (Reich et al., 1997). Yue et al. (2006) mentioned that the flowering time variation could make the phenotyping of drought tolerance less accurate because drought at the reproductive stage drastically impacts fitness.

Major differences in fatty acid profiles were found among the three species. *C. sativa* had a distinct profile with a high DR, 0.59, and low ER, 0.20, whereas *B. carinata* and *B. juncea* had very high ER, 0.52 and 0.49, respectively. Thus, the two *Brassica* species had a very efficient ER pathway, involving two FA elongases, FAE (e1) elongating C18:1 to C20:1 and FAE (e2) elongating C20:1 to C22:1 (Barker et al., 2007). FAE (e2) was very active within *B. carinata* and *B. juncea*, creating high erucic acid levels, whereas in *C. sativa*, FAE (e1) was predominant within the elongation pathway resulting in higher levels of eicosenoic acid than erucic acid (Fig. 1). *B. carinata* accession BRA-1154 had an erucic acid content of 47.9% and BRA-2135 had 30.7% erucic acid content, implying wide genetic diversity. Alemayehu and Becker (2001) found even greater diversity for erucic acid content in *B. carinata* germplasm. However, its erucic content remains high compared to Environmental Protection Agency limits of 2% due to potential negative impact in feed (McCutcheon et al., 1976).

The *C. sativa* oil profile was most strongly influenced by the desaturation pathway. The two major enzymes involved are fatty acid desaturases two (FAD2) and three (FAD3), desaturating C18:1 to C18:2 and C18:2 to C18:3, respectively. FAD3 was more efficient in *C. sativa* than in the *Brassica* species, resulting in high levels of linolenic acid (C18:3) in *C. sativa*. *B. juncea* and *B. carinata* showed a broader genetic diversity for oil profile than *C. sativa*. Thus, oil profile selection for the two *Brassica* species can be partially achieved by choosing the appropriate combinations of alleles present in the germplasm. *C. sativa* showed less diversity within the sampled germplasm collection, making progress through selection more difficult. However, Ghamkhar et al.

(2010) recently found wide genetic diversity in *C. sativa* oil profiles. QTLs involved in the lipid biosynthesis pathway have been discovered in all three species, providing information for improved selection (Jourdren et al., 1996; Tanhuanpaa et al., 1998; Lionneton et al., 2002; Sharma et al., 2002; Gehringer et al., 2006; Qiu et al., 2006).

In *B. juncea* and *C. sativa*, linolenic acid content decreased from the irrigated to the dryland treatment, implying that water stress impacts FA content (Fig. 1.1). Previously, water deficit has been shown to impact oil composition, reducing linolenic acid content in coconut and rapeseed leaves (Canvin, 1965; Williams et al., 1992). In addition to the influence of water stress, linolenic acid synthesis is temperature sensitive with the enzyme FAD3 being down-regulated as temperature increases (Baux et al., 2008).

C. sativa appears to be the most drought-tolerant among the three species, as defined by the least reduction in biomass production under dry conditions and the highest seed yield. One key difference between *C. sativa* and the *Brassica* species was the high linolenic acid content in *C. sativa* seeds, around 30% compared to approximately 5% for *B. juncea* and *B. carinata*. High linolenic acid in the seed has been reported to be correlated to high levels of this acid in the leaf (Lemieux et al., 1990; Miquel and Browse, 1994). We did not measure FA levels in the leaf, but numerous studies in Arabidopsis (McConn et al., 1994; Matsuda et al., 2005; Mene-Saffrane et al., 2009), canola (Merrien et al., 2007; Triboi-Blondel and Renard, 1999), sunflower (Izquierdo et al., 2002), tobacco (Kodama et al., 1994; Murakami et al., 2000), and coconut (Repellin et al., 1997) show that an increase in leaf trienoic fatty acids, such as linolenic acid,

improved cold and drought tolerance. Therefore, we suggest that linolenic acid levels are likely to contribute to superior drought-tolerance performance in *C. sativa*.

Finally, *C. sativa* did not have flea beetle damage in either moisture treatment, which provides an agronomic advantage in the High Plains where flea beetle pressure is very important from April to the end of May. *B.carinata* and *B. juncea* accessions were very susceptible.

CONCLUSION

This research was unique in that three cruciferous species were analyzed side by side under dryland and fully irrigated conditions in a semi-arid climate. The results indicated that large variations exist for morphological and oil profile traits both within and among the three species. The study pointed out issues and advantages for the three species. *B. carinata* had large seed size, but was susceptible to flea beetle, flowered later, and yielded poorly, especially under dryland conditions. *B. juncea* flowered early and had wide variation for seed size, biomass production, and FAs, but was susceptible to flea beetle, and produced low seed yield under dryland conditions. *C. sativa* out-yielded *B. juncea* and *B. carinata* in 2009 and 2010 under both dry and wet conditions. *C. sativa* had a shorter stature, higher harvest index, more efficient desaturation pathway and resistance to flea beetle, although seed size was small. *C. sativa* flowered as early as *B. juncea* and had low erucic and high linolenic acid, two traits that are valuable for use as meal and for oil quality for fuel and food. Oil profile characteristics, such as high linolenic acid, could play an important role in plant adaption especially under stress

environments. Further work is needed within species, controlling more precisely the timing of drought relative to flowering time and measuring more specific drought-tolerant traits.

Table 1.1: ANOVA table of multiple traits of three oilseed species grown in Fort Collins, CO, in 2008.

Species	Effect	Days To Flower	Biomass/Plant	Main Branch Length	Plant Height	Pod Density	Yield/ Plant	Seed/ Pod	Seed Size	Sats	PUFA	ER	DR	ODR	LDR
		p	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*	*p*
Camelina	Treatment	***	***	***	***	***	***	***	ns	***	***	***	***	***	***
n=81	Genotype	0.78	ns	***	ns	***	ns	***	***	***	***	***	***	***	***
	CV	1.87	72.00	22.70	14.40	15.60	112.00	16.70	9.80	3.10	2.30	4.20	2.45	2.55	3.56
	R	0.45	0.31	0.64	0.72	0.59	0.35	0.47	0.72	0.73	0.67	0.58	0.66	0.59	0.73
	Heritability[1]	0.28	0.04	0.54	0.03	0.50	0.11	0.57	0.86	0.88	0.81	0.81	0.81	0.73	0.85
	Heritability[2]	0.09	0.01	0.23	0.01	0.20	0.03	0.25	0.62	0.66	0.52	0.51	0.52	0.42	0.62
B.juncea	Treatment	***	***	***	***	***	***	ns	***	***	***	*	***	***	***
n=94	Genotype	***	***	***	***	***	***	***	***	***	***	***	***	***	***
	CV	4.40	61.40	19.20	15.10	20.70	70.60	15.10	15.20	4.72	5.90	7.32	6.09	4.02	6.75
	R	0.85	0.58	0.68	0.80	0.56	0.58	0.55	0.81	0.74	0.82	0.89	0.82	0.84	0.81
	Heritability[1]	0.92	0.22	0.51	0.75	0.73	0.22	0.60	0.88	0.82	0.92	0.96	0.92	0.89	0.89
	Heritability[2]	0.78	0.08	0.25	0.54	0.44	0.08	0.27	0.65	0.53	0.75	0.85	0.74	0.72	0.69
B.carinata	Treatment	ns	***	***	***	§	***	*	***	ns	***	***	***	ns	ns
n=30	Genotype	***	***	***	***	ns	**	***	***	ns	ns	*	ns	ns	ns
	CV	3.36	36.00	26.00	13.90	22.10	44.60	17.10	15.10	6.90	5.60	3.90	3.20	3.20	7.90
	R	0.82	0.75	0.67	0.88	0.25	0.75	0.43	0.58	0.30	0.51	0.51	0.30	0.30	0.35
	Heritability[1]	0.91	0.60	0.61	0.44	0.00	0.07	0.19	0.64	0.00	0.35	0.46	0.32	0.00	0.18
	Heritability[2]	0.75	0.37	0.30	0.22	0.00	0.03	0.06	0.36	0.00	0.12	0.17	0.10	0.00	0.05

[1] $h^2 = V_g/[(V_g+(V_{ge}/E)+(V_r/E*R)]$

[2] $h^2 = V_g/(V_g + V_{ge} + V_r)$

"ns": non significant

§ Significant at the 0.10 probability level.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

Table 1.2: Summary of yield components and oil quality trait under irrigation and dryland conditions for three oilseed species at Fort Collins, CO, 2008.

Environment		Dryland			Irrigated		
Trait	Variables	*B.carinata*	*C.sativa*	*B.Juncea*	*B.carinata*	*C.sativa*	*B.Juncea*
Flowering	mean	59.88	49.69	50.56	60.13	50.67	52.24
days	min.	53.00	47.00	40.24	54.00	48.00	31.00
	max.	67.50	50.24	62.50	66.00	51.00	64.23
Biomass	mean	2.68	2.09	1.89	5.43	2.82	5.48
gram	min.	0.60	0.96	0.50	2.40	0.90	0.34
	max.	5.40	3.95	5.99	13.20	14.11	21.03
MainBranch	mean	19.41	8.94	28.17	32.14	13.59	41.09
cm	min.	12.20	5.72	15.17	15.20	8.84	19.17
	max.	26.70	13.34	41.51	51.20	23.31	61.50
Height	mean	53.23	38.04	65.54	101.26	55.19	92.39
cm	min.	38.50	27.80	32.70	75.80	41.40	35.40
	max.	72.00	46.60	90.30	125.50	69.90	142.80
Poddensity	mean	2.46	2.00	0.59	2.64	1.61	0.55
no.	min.	1.70	1.14	0.33	2.10	1.12	0.31
	max.	3.80	2.66	0.91	3.30	2.05	0.88
TSW	mean	3.76	0.98	2.08	3.45	0.99	2.85
gram	min.	2.70	0.59	1.09	2.40	0.69	1.47
	max.	5.00	1.38	3.20	5.60	1.36	4.22
Seedpod	mean	13.90	11.24	13.74	15.06	12.20	14.07
no.	min.	8.40	6.40	9.30	11.10	8.40	8.10
	max.	18.60	16.75	19.90	20.00	15.90	19.55
ER	mean	0.52	0.20	0.49	0.58	0.19	0.49
ratio	min.	0.50	0.17	0.03	0.50	0.17	0.06
	max.	0.60	0.22	0.60	0.60	0.21	0.60
DR	mean	0.38	0.59	0.30	0.32	0.60	0.32
ratio	min.	0.30	0.52	0.26	0.30	0.54	0.27
	max.	0.40	0.63	0.50	0.40	0.64	0.44
ODR	mean	0.78	0.73	0.59	0.79	0.74	0.64
ratio	min.	0.70	0.65	0.45	0.70	0.68	0.46
	max.	0.80	0.80	0.69	0.80	0.79	0.72
LDR	mean	0.49	0.58	0.33	0.48	0.60	0.37
ratio	min.	0.40	0.49	0.23	0.40	0.52	0.26
	max.	0.60	0.66	0.42	0.50	0.65	0.46

Table 1.3: Irrigated and dryland performance trials of three oilseed species growing in Iliff, CO, in 2009 and 2010.

			2009			2010								
Species	Origin	**line**	Dryland			Dryland			Irrigated			3-environments average		
Trait			height	flowering	yield	height	flowering	yield	height	flowering	yield	height	flowering	yield
Unit			cm	day	kg ha^{-1}	cm	day	kg ha^{-1}	cm	day	kg ha^{-1}	cm	day	kg ha^{-1}
B. carinata	Zambia	BC-1029	125	60	1395	111	72	298	122	72	857	120	66	986
	Ethiopia	BC-1151	116	58	1218	104	72	114	112	72	801	111	65	838
	Ethiopia	BC-2135	88	56	528	122	72	339	151	72	850	112	64	561
B.juncea	Russia	DZJ	134	51	708	138	66	834	140	57	1021	137	57	818
	China	Jc-015	142	57	1172	136	62	546	117	60	890	134	59	945
	Russia	Jr-006	152	53	1390	151	63	772	142	60	1067	150	57	1155
C. sativa	Poland	IPK33	61	53	1485	61	61	1175	60	60	1314	61	58	1325
	Germany	IPK739	55	55	1486	52	59	1190	64	59	1366	57	58	1348
	Italy	IPK851	61	55	1652	62	59	1245	53	59	1535	58	58	1477
LSD, 0.05			11.51	2.79	473	21.6	6.9	267	38	2.7	593	18.2	3.4	481
CV, %			6.4	2.92	24.3	9	4.6	15.7	15.4	1.84	23.5	14.6	4.75	24.2
R-square			0.98	0.83	0.78	0.96	0.87	0.96	0.91	0.98	0.72	0.87	0.85	0.56

Table 1.4: Pearson correlation of the measured traits under irrigation and dryland of *B. carinata* (n = 30), B. juncea (n=96), and *Camelina sativa* (n=81) at Fort Collins in 2008

B.carinata		Irrigated									
Dryland	Flowering	0.27 ns	-0.11 ns	0.67 ***	-0.15 ns	0.10 ns	-0.44 *	0.08 ns	0.20 ns	0.38 .	0.20 ns
	0.24 ns	Biomass	-0.03 ns	0.21 ns	0.00 ns	0.32 .	-0.21 ns	-0.09 ns	0.25 ns	0.33 .	0.37 *
	0.04 ns	0.27 ns	Mainbranch	0.41 *	-0.10 ns	-0.09 ns	-0.24 ns	0.20 ns	-0.21 ns	0.04 ns	0.13 ns
	0.25 ns	0.28 ns	0.49 *	Height	-0.28 ns	0.03 ns	-0.45 *	0.06 ns	0.12 ns	0.31 .	0.13 ns
	0.35 .	0.09 ns	-0.08 ns	0.46 *	Poddensity	0.10 ns	0.12 ns	-0.10 ns	0.03 ns	-0.10 ns	0.33 .
	0.46 *	0.46 *	-0.15 ns	0.19 ns	0.38 *	TSW	-0.21 ns	-0.09 ns	0.03 ns	-0.08 ns	0.08 ns
	-0.21 ns	0.02 ns	0.11 ns	-0.05 ns	-0.32 .	-0.21 ns	Seedpods	0.25 ns	-0.30 .	-0.02 ns	-0.13 ns
	0.31 .	0.08 ns	-0.06 ns	0.07 ns	0.23 ns	0.06 ns	0.21 ns	ER	-0.79 ***	0.25 ns	0.06 .
	-0.27 ns	0.13 ns	0.03 ns	0.05 ns	-0.11 ns	0.07 ns	-0.10 ns	-0.86 ***	DR	0.34 .	-0.12 ns
	0.03 ns	0.30 ns	-0.04 ns	0.24 ns	0.17 ns	0.18 ns	0.13 ns	-0.09 ns	0.57 **	ODR	0.02 ns
	0.31 .	0.32 .	-0.02 ns	0.27 ns	0.27 ns	0.26 ns	-0.17 ns	-0.05 ns	0.36 *	0.69 ***	LDR

B.juncea		Irrigated									
Dryland	Flowering	0.32 **	0.50 ***	0.60 ***	0.37 ***	0.05 ns	0.20 *	-0.15 ns	0.12 ns	-0.19 .	-0.06 ns
	0.33 **	Biomass	0.34 **	0.35 **	0.12 ns	0.09 ns	0.12 ns	0.01 ns	-0.07 ns	-0.08 ns	0.03 ns
	0.10 ns	0.44 ***	Mainbranch	0.70 ***	0.19 .	0.09 ns	0.17 ns	-0.21 *	0.08 ns	-0.34 **	-0.15 ns
	0.34 **	0.44 ***	0.39 ***	Height	0.53 ***	0.15 ns	0.31 **	-0.48 ***	0.31 **	-0.62 ***	-0.35 **
	0.44 ***	0.09 ns	-0.29 **	0.35 **	Poddensity	-0.26 0.01	0.26 *	-0.48 ***	0.42 ***	-0.49 ***	-0.20 .
	0.11 ns	0.37 **	0.12 ns	0.42 ***	0.00 ns	TSW	-0.07 ns	-0.10 ns	0.03 ns	-0.18 .	-0.19 .
	-0.02 ns	-0.18 .	0.10 ns	0.15 .	0.00 ns	-0.37 **	Seedpods	-0.17 ns	0.05 ns	-0.27 *	-0.12 ns
	-0.22 *	-0.14 ns	0.06 ns	-0.44 ***	-0.38 **	-0.17 ns	-0.01 ns	ER	-0.90 ***	0.85 ***	0.58 ***
	0.23 *	0.26 *	-0.04 ns	0.40 ***	0.39 ***	0.12 ns	-0.04 ns	-0.92 ***	DR	-0.56 ***	-0.41 ***
	-0.16 ns	0.11 ns	0.08 ns	-0.41 ***	-0.24 *	-0.20 .	-0.13 ns	0.74 ***	-0.45 ***	ODR	0.64 ***
	-0.07 ns	0.19 .	0.18 .	-0.29 *	-0.28 *	-0.11 ns	-0.08 ns	0.53 ***	-0.37 **	0.65 ***	LDR

The table is continued on the next page.

C.sativa					Irrigated						
	Flowering	-0.07 ns	-0.13 ns	0.12 ns	-0.12 ns	-0.06 ns	0.32 **	0.11 ns	0.05 ns	0.09 ns	-0.09 ns
	0.14 ns	Biomass	0.17 ns	0.26 *	-0.08 ns	0.07 ns	-0.09 ns	-0.02 ns	-0.26 *	-0.27 *	-0.27 *
	-0.49 ***	0.01 ns	Mainbranch	0.47 ***	0.02 ns	-0.39 **	-0.02 ns	-0.04 ns	-0.06 ns	-0.08 ns	0.24 *
	-0.26 *	0.23 *	0.52 ***	Height	0.05 ns	-0.29 *	0.08 ns	0.01 ns	-0.20 .	-0.22 *	0.05 ns
	-0.18 .	-0.0018 ns	0.07 ns	0.17 ns	Poddensity	-0.48 ***	0.17 ns	-0.01 ns	-0.30 **	-0.32 **	0.07 ns
Dryland	0.12 ns	0.2192 *	-0.45 ***	-0.24 *	-0.40 **	TSW	-0.25 *	0.25 *	0.15 ns	0.24 *	-0.21 *
	0.08 ns	0.0376 ns	0.07 ns	0.20 .	0.21 *	-0.19 .	Seedpods	0.12 ns	-0.13 ns	-0.02 ns	-0.13 ns
	0.07 ns	0.0096 ns	-0.07 ns	0.07 ns	-0.03 ns	0.17 ns	-0.08 ns	ER	-0.28 *	0.01 ns	0.15 ns
	0.08 ns	0.1985 .	-0.09 ns	-0.06 ns	-0.12 ns	0.32 *	-0.07 ns	-0.22 *	DR	0.93 ***	0.11 ns
	0.04 ns	0.1777 .	-0.09 ns	-0.06 ns	-0.11 ns	0.36 **	-0.08 ns	0.05 ns	0.93 ***	ODR	0.16 ns
	0.11 ns	-0.16 ns	0.25 *	0.06 ns	0.18 .	-0.42 ***	-0.09 ns	0.01 ns	-0.05 ns	-0.02 ns	LDR

"ns" non significant

. Significant at the 0.1 probability level.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

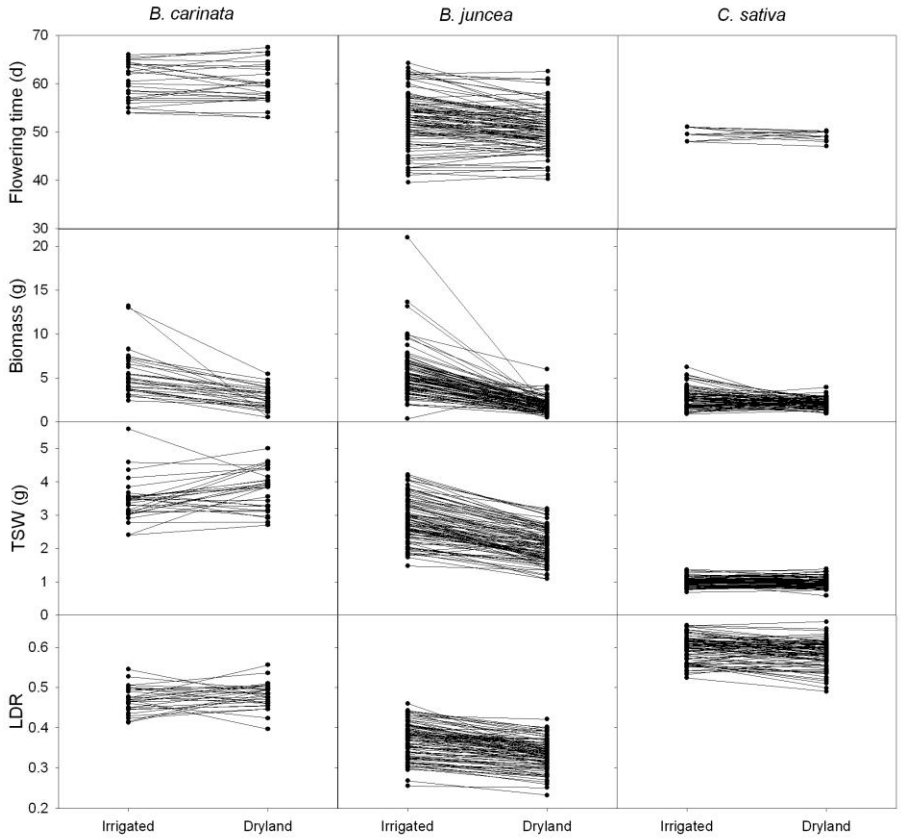

Figure 1.1: Flowering time, biomass, TSW and LDR reaction norms between irrigation and dryland of three oilseed species at Fort Collins, CO, 2008.

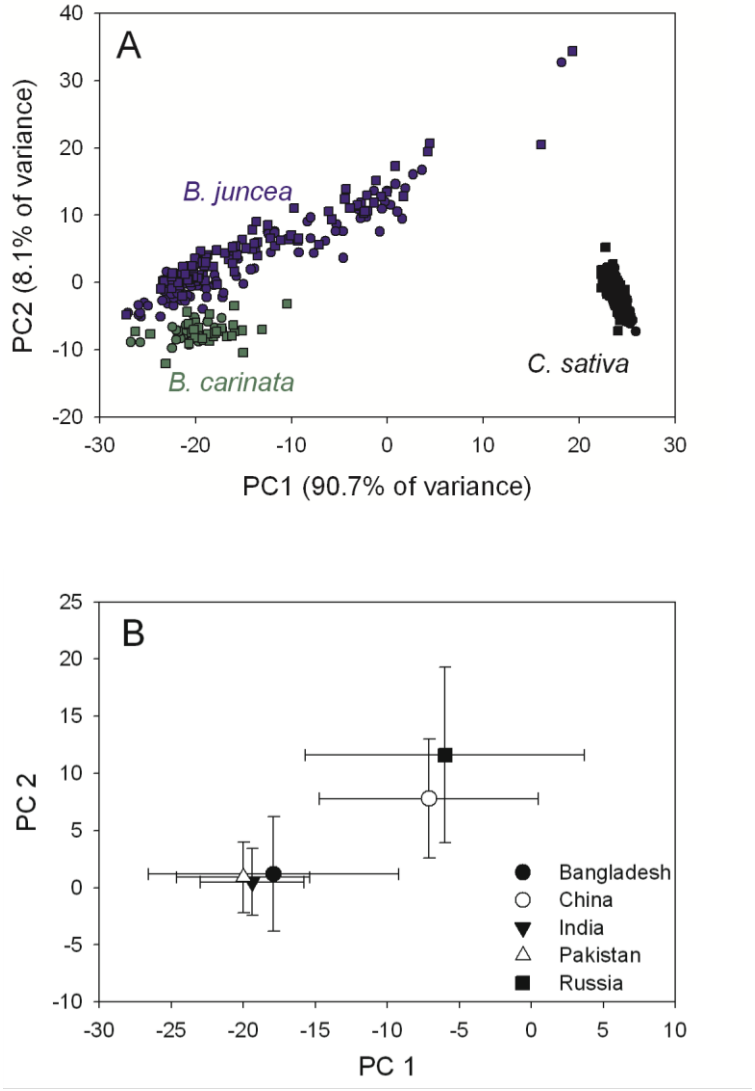

Figure 1.2: Principal component analysis of seed-oil fatty acid profiles; (A) Oil profiles for *B. carinata* (green), *B. juncea* (blue), and *C. sativa* (black). Circles, irrigated treatment; squares, dryland treatment; (B) Oil profiles for accessions of *B. juncea* based upon country of origin.

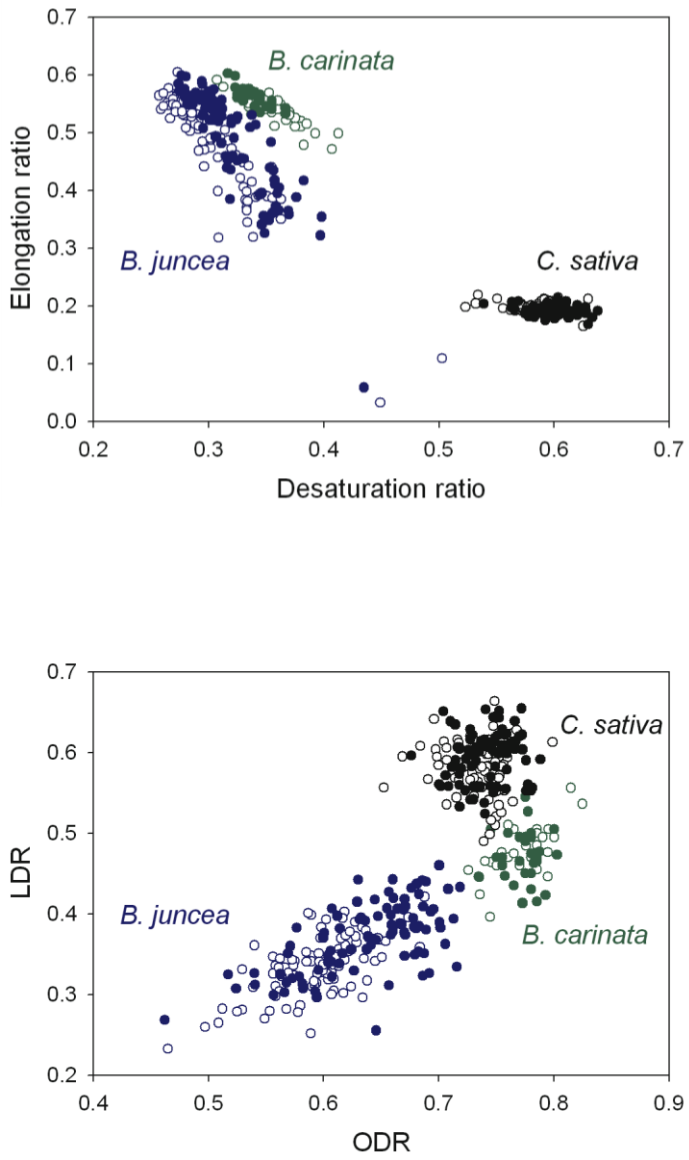

Figure 1.3: Correlations between (A) ER and DR and (B) ODR and LDR among accessions of *B. carinata*, *B. juncea*, and *C. sativa*. Solid circles, irrigated treatment; open circles, dryland treatment.

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CHAPTER TWO:

MULTI-ENVIRONMENT QTLS FOR DROUGHT ADAPTATION IN *CAMELINA SATIVA*

ABSTRACT

Camelina (*Camelina sativa* (L.) Crantz) has been grown with success under semi-arid conditions. Yet, there is a lack of understanding of camelina's response to drought stress and the location of quantitative trait loci (QTL) corresponding to drought tolerance mechanisms. This study was conducted over a two-year period under two treatments, dry and irrigated, and three replicates on a recombinant-inbred-lines (RILs) population in Fort Collins, Colorado. As fitness in dry environments can be achieved through very different mechanisms, a wide range of traits was recorded: carbon isotope ratio, leaf water and nitrogen content, flowering, seed yield, seed size and oil quality traits. Reaction norms and phenotypic differences between moisture treatments were used for a better understanding of drought mechanisms and plasticity.

High trait plasticity was observed between moisture treatments among the recombinant inbred lines. In dry and hot environments, earlier flowering improved fitness (p<0.01) while decreasing it under optimal conditions. Linolenic fatty acid and seed size were negatively correlated with flowering time (p<0.01), which could be explained by their impact on seedling establishment and shoot vigor. The positive correlation between

water use efficiency (WUE) ($\delta^{13}C$) and leaf nitrogen content (LNC) ($p<0.1$) suggests that higher LNC may delay leaf senescence and allow further uptake of soil water and nitrogen, resulting in higher seed yield. A total of 29 significant QTLs were found. Flowering, seed yield, oil content and linolenic acid content revealed very stable QTLs, some of which coincided with a previous QTL study. A seed yield increase up to 16% was achieved by combining specific stable QTLs. In addition, significant flowering, seed yield, oil content and linolenic acid QTLs for the phenotypic differences between dry and irrigated conditions were found. The use of such QTLs could improve stability and drought adaptation.

INTRODUCTION

Because water availability is essential to all plants, the increased frequency of drought events characterized by higher temperatures and less precipitation (Allison et al., 2010) limits agriculture production and yield progress (Richards et al., 2010). Semi-arid environments are characterized by a range of precipitation between 250 mm and 550 mm, with a short growing season (Peterson et al., 2006) and high year-to-year precipitation variability. In semi-arid regions, precipitation is never sufficient to allow production of the highest-yielding crops, but some Brassicaceae species may perform well due to their short growing seasons, and cold and drought tolerance (Wright et al., 1995; Alemayehu and Becker, 2002; Fernandez-Martinez et al., 2001; Francis and Campbell, 2004; Oram et al., 2005; Gan et al., 2007). The large reported genetic diversity within the Brassicaceae species (Velasco at al., 1998; Ghamkhar et al., 2010) allows for selection for many

specific traits, particularly in the case of selecting species most favorable for large-scale target environments and cultivars less sensitive to year-to-year variation. Year-to-year rainfall variation increases genotype by environment interaction (GEI) and reduces heritability for yield, thereby limiting breeding for yield increase (Richards et al., 2010). By better understanding drought adaptation and GEI, breeding could optimize yield in these environments.

Fitness in dry environments is achieved through very different mechanisms. Strong selective pressure has been imposed by natural water deficit environment on species, maintaining genetic diversity across different environments (Bohnert et al., 1995; Bray, 1997). A large range of mechanisms are involved in plant adaptation to limited water availability such as developmental, morphological, molecular and physiological traits (McKay et al., 2003; Yue et al., 2006). Three main mechanisms are related to water deficit tolerance: dehydration tolerance, drought avoidance and drought escape. Dehydration tolerance is characterized by plant survival despite an internal water deficit (Scott, 2000). Leaf water content reflects internal water deficit and is an indirect measure of osmotic adjustment (Barrs and Weatherley, 1962). Dehydration avoidance is characterized by maintenance of adequate internal water levels in spite of low moisture levels in the environment. Architectural and physiological traits such as hairy leaves, short stature, quick stomatal closure, and small specific leaf area can minimize water losses. Stomatal closure limits water loss, while limiting CO_2 uptake, which could increase water use efficiency (WUE) but decrease plant growth (Schulze et al., 1987). WUE is the efficiency of CO_2 uptake over water loss. Carbon isotope discrimination has

been shown to be an accurate method for measuring WUE. Studies have confirmed the positive correlation between carbon isotope composition ($\delta^{13}C$) of C3 plant tissues and WUE (Farquhar et al., 1989; Ehleringer and Monson, 1993; Araus et al., 2002; McKay et al., 2003). Finally, drought escape is the avoidance of water deficit through shorter life cycle or timing of life cycle (White, 1993; Araus et al., 2002; McKay et al., 2008). Shorter life cycle can be induced by higher linolenic fatty acid content, which has been shown to improve seedling establishment, shoot vigor and early flowering in *Arabidopsis thaliana*, *Brassica napus* and *Camelina sativa* (Miquel, 1994; Linder and Schmitt, 1995; Thompson and Li, 1997; Linder, 1998; Linder, 2000; Enjalbert et al., unpublished). However, all the described mechanisms can be expressed with a different magnitude by a single genotype across different environments, which is called plasticity (Bradshaw, 1984; Schlichting and Levin, 1986). The added complexity of plant response to the environment makes breeding more difficult.

Because plasticity is becoming of greater interest in breeding programs (Forde, 2009; Sadras et al., 2009), numerous methods and statistical models have been developed to analyze its effect and establish mega-environments to predict genotype fitness within similar environments (Chenu et al., 2009). Since timing of drought is important for some traits to be expressed and large plasticity associated with these traits is expected (Manneh et al., 2007), it is crucial to understand the targeted environments. Reaction norms between moisture treatments underline phenotypic plasticity by showing the change in phenotype across environments by a slope (Nicotra and Davidson, 2010). Moreover, QTL scans of the phenotype difference between irrigated and dryland treatments have been

shown to improve drought-related QTL detection (Mathews et al., 2008). For this reason, both reaction norm and phenotype differences between dry and irrigated conditions were used in the study.

Camelina sativa was chosen for this study because it has been grown with success under semi-arid conditions (Angelini et al., 1997). Because traits underlying such adaptation are quantitative and influenced by environment (Richards et al., 2002; Cattivelli et al., 2008), QTL analysis is needed for a better understanding of the genetic basis of camelina's drought response and to improve camelina fitness under semi-arid environments. QTL analyses are widely used in oilseed breeding programs to detect marker-trait associations, which may improve selection. However, few molecular genetic resources are currently available for *Camelina sativa*. Gehringer et al. (2006) conducted a quantitative study on camelina and found QTLs for seed yield components and oil quality traits in multiple environments. However, lack of understanding remains as to specific QTLs corresponding to drought mechanisms. Given the limited information on QTLs corresponding to drought mechanisms in *Camelina sativa*, this study was developed to address this research need using a recombinant-inbred-line population. Three objectives were targeted. First, the research looked for phenotypic variability, trait correlation and associated QTLs of drought-related traits. Second, the study focused on year-to-year QTL stability. Third, the research looked for QTLs associated with drought adaptation and plasticity within the population.

MATERIALS AND METHODS

Plant material

A total of 181 recombinant inbred lines (RILs) were evaluated for plant and seed quality traits. The RIL population was created from a cross between the registered German camelina cultivars ‘Lindo’ and ‘Licalla’, using single-seed descent (SSD) to the F6 generation. The parents used to produce the inbred lines showed high variation in seed yield, thousand seed weight (TSW), plant height and seed-oil content (Gehringer et al., 2006). Along with the 181 RILs, both parents as well as three released varieties were grown as checks. The genetic map was constructed by Gehringer et al. (2006). The map consisted of 20 linkage groups, corresponding to 2*n*=6x=20 (Hutcheon et al., 2010), with 157 amplified fragment length polymorphism (AFLP) markers and three *Brassica* simple sequence repeat (SSR) markers. The map covers a total of 1385.6 centiMorgans (cM) with an average marker interval of 8.6 cM. At each locus, allele "a" corresponded to the Lindo allele and "b" to the Licalla allele.

Environmental conditions

Accessions were grown at the Colorado State University Agricultural Research Development and Education Center (ARDEC) in Fort Collins in the 2009 and 2010 crop seasons. ARDEC is located north-east of Fort Collins, Colorado (latitude: 40.65; longitude: 105.00). The elevation is 1557 m. The average annual precipitation is 356 mm. The climate is considered semi-arid. The soil type is Nunn clay loam. Soil samples were taken prior to each growing season and analyzed at Olsen’s Agricultural Laboratory, Inc., in McCook, Nebraska.

Sonalan, ethalfluralin, was used as a pre-planting herbicide at a rate of 2.5 L ha^{-1}. Then the seedbed was disked. The field was divided into six blocks of 18.3 m by 7.6 m, three drip-irrigated, and three under dryland treatment, randomly assigned. Hence, all accessions were sown in three replications for each moisture treatment. The set of accessions was hand planted in two-row plots of 1.5-m length and 0.3 m row spacing in between plots on April 16, 2009, and April 18, 2010. Seeding rate was based on a seeding rate typical for the region of 8 kg ha^{-1}. No fertilizer was applied on the field. During the growing season, weeds were controlled manually. Each double row-plot was harvested at the full maturity stage of the experiment between July 25 and August 1 in 2009 and 2010.

Data collected

Weather data were analyzed specifically for the flowering period (June 5 to June 19), early grain filling (June 20 to July 4) and late filling (July 5 to July 19). Precipitation, average maximum temperatures and days above 32°C were recorded for both years.

Days to flowering were noted for each plot when 50% of the plants had flowered. Plant height (measured from the soil surface to highest point on the plant) was determined by measuring two randomly selected plants per plot at time of maturity.

Data on $\delta^{13}C$ were collected as follows. Ten fully expanded leaves were randomly harvested per plot at the end of the seed filling period and put in paper envelops. The samples were dried in an oven at 60°C (Farquhar et al., 1989), placed in 1.5-ml tubes with bee-bees and ground to a fine powder with a paint shaker. Then, between 1.2 and 1.8 mg of each sample was transferred to a tin combustion cup for isotope analysis. Samples were analyzed for $\delta^{13}C$ at the Stable Isotope Facility at Colorado State University. Data

are presented as carbon isotope ratios relative to the Pee Dee Belemnite (PDB) standard (RPDB), where $\delta^{13}C$ (‰) = (RS/ RPDB − 1) * 1000 (Hubick et al., 1986). Percent mass nitrogen (%N) in the leaf tissue, which may reflect differences in biochemical photosynthetic capacity, was measured through mass spectrometry performed on the same leaf sample as for $\delta^{13}C$. Leaf water content was measured by randomly collecting five green leaves per plot during filling period. Leaves were rapidly put into a pre-weighed 1.5 ml tube, in a portable refrigerator, before the fresh weight was determined in the lab. Leaf samples were then freeze-dried. The percentage moisture was calculated by difference (Turner, 1981).

After total seed weight per plot and thousand-seed-weight (TSW) were measured, two seed sub-samples were taken to conduct oil content and oil profile analysis. Seed samples were dried in an oven at 50°C. Then, the fatty acid profile analysis was carried out by gas chromatography-mass spectroscopy. Following the procedure outlined by Hammond (1991), oil content was determined on single 12-gram samples using a Newport MKIIIA Nuclear Magnetic Resonance (NMR) Analyzer (Oxford Instruments Inc., Concord, MA). The NMR was calibrated with a single reference sample of known oil content and the sample analysis carried out as described by Howard and Daun (1991). This procedure, and that of the Canadian Grain Commission, is based on the International Organization for Standardization procedure ISO 5511-1984 (Oilseeds - Determination of oil content - Low resolution nuclear magnetic resonance spectrometric method).

Statistical analysis

The experimental design was a randomized complete block design (RCBD) with three replicates and two moisture treatments over two years. Data were analyzed using the statistical analysis software SAS version 9.2 (SAS Institute, Inc., Cary, NC). A mixed model was used with treatment as fixed, accessions as random effects nested within treatment using PROC MIXED of SAS. Pearson correlation coefficients among different traits and treatments were generated by PROC CORR of SAS based on LSMEAN.

Broad-sense heritability for mean values over environments was calculated from components of variance with REML iteration in SAS using PROC VARCOMP, according to:

(1) Smith and Dhondt (1980): $h^2 = V_g/(V_g + V_e + V_r)$, where V_g, V_e and V_r, represent the respective variance components for genotype (g), environment (e) and (r) residual.

(2) Hill et al. (1998): $h^2 = V_g/[(V_g + (V_r/E*R)]$, where V_g, and V_r represent the respective variance components for genotype (g), and residual variance (r), and E and R are the number of environments and replicates, respectively.

Quantitative traits loci (QTL) analysis was done through R/qtl, an add-on package for the freely available statistical software R, using the LSMEAN of the traits generated by SAS version 9.2 (SAS Institute Inc., Cary, NC). In accordance with Broman et al. (2003) and Manichaikul et al. (2009), this study followed a penalized likelihood approach with penalties on QTL and pairwise interactions chosen to control false positive rates. The LOD thresholds were determined by 1000 random permutations. The LOD threshold at a

5% genome-wise type I error rate ranged from 2.35 to 2.6. The results of both years across both treatments are presented for QTLs with significant LOD score.

RESULTS

Understanding the environmental variables is critical for better interpretation of the phenotypic data, trait correlation, plasticity, QTL and QEI. Therefore, environmental conditions are described first, followed by trait variability, correlation, and QTL analysis.

Environmental variables

During the overall growing season, from April to July, the 2009 crop received 230 mm of rain with an average maximum temperature of 26.1°C whereas the 2010 crop had 180 mm of rain with an average maximum temperature of 28.6°C. Maximum temperatures were significantly higher in 2010 during flowering and early filling (Fig. 2.1). Moreover, in 2010, maximum temperatures exceeded 32°C nine times, and in 2009, six times. Drought started early in the seed filling stage in 2009 while occurring later in the seed filling stage in 2010. Heat started at flowering in 2010 but later in 2009. The upper 40 cm of soil had the equivalent of 78 kg ha^{-1} of nitrogen in 2009 and 60 kg ha^{-1} in 2010. Thus, year by location was interpreted as one environment considering the wide difference between years.

Phenotypic data

Analysis of variance (ANOVA) over four environments revealed highly significant differences between environments and genotypes for flowering, seed yield,

linolenic and eicosenoic fatty acids. The ANOVA, the means, coefficient of variation (CV), and heritability are reported in Table 2.1.

Transgressive segregation was observed in the RIL population for all the investigated traits. Licalla flowered later than Lindo under irrigation in both years, 75.6 vs. 74.7 days. Lindo had higher seed yield than Licalla under dryland treatment, 83.4 vs. 61.5 grams, and irrigated treatment, 158 vs. 131.8 grams (Table 2.2). The difference was greater in 2010, when heat and drought were greater under dryland, 60 vs. 36.7 grams, and irrigated treatment, 158.2 vs. 115 grams (Table 2.2). Lindo had higher oil content than Licalla under irrigation in both years, 33.6 vs. 32.6%. Licalla had higher linolenic fatty acid than Lindo under dryland, 30.3% vs. 27.8%, and irrigated treatment, 33% vs. 30.5% (Table 2.2).

Variations in growth and development traits

Plant height environmental variability was higher than genetic variability, with 16.5% of the total sums of squares versus 5%. Plant height heritability was from 0.03 to 0.003 (Table 2.1).

Flowering time environmental variability was much higher than genetic variability, with 84.7% of the total sums of squares versus 6.4%. However, flowering time heritability was from 0.39 to 0.86 (Table 2.1). Even though flowering time environmental variability was very high, moisture treatment was not significant (Fig. 2.2A). Some RILs had earlier flowering under dryland than under irrigation while others tended to have equal or later flowering. In 2010, a drier and hotter year, RILs flowered an average nine days earlier than in 2009 (Table 2.2).

In 2010, leaf water content, at the seed filling stage, increased from dry, 0.78, to irrigated, 0.86, treatment (Table 2.2). However, some RILs were more stable than others (Fig. 2.2B). Carbon isotope ratio, $\delta^{13}C$, was lower under irrigation, -29.08, expressing higher water efficiency under dryland, -28.10. Once again, some RILs were much more sensitive than others (Fig. 2.2C). Carbon isotope ratio between irrigated and dryland had strong positive correlation ($p<0.001$), expressing potential high heritability. In 2009, leaf nitrogen content was significantly ($p<0.05$) higher under irrigation than dryland, 2.11% and 2.66 %, respectively, with a broad range among RILs.

Seed quantity and quality traits

Seed yield environmental variability was much higher than genetic variability, with 49% of the total sums of squares versus 8.1%. Seed yield between treatments had high positive ($p<0.001$, r=0.3) correlation (Table 2.4). Yield heritability was from 0.1 to 0.50 (Table 2.1). In 2010, dryland seed yield was lower than 2009, 50.5 vs. 96.5 grams (Table 2.2) due to higher level of drought stress in 2010. Thousand seed weight genetic variability was high, with 46% of the sums of squares and heritability from 0.36 to 0.87.

Oil content genetic sum of squares was high, accounting for 37% of the total sums of squares. Its heritability was from 0.42 to 0.87 (Table 2.1). In the 2010 dryland treatment, the RILs had a lower oil content average than the 2009 dryland treatment, 29.2 vs. 30.4% (Table 2.2). The oil content range of the RILs was higher under irrigation, 7.8% vs. 5.5% difference between minimum and maximum values (Table 2.2).

Linolenic and eicosenoic acid content genetic sums of squares were high, with 29.2% and 56.7% respectively of the total sums of squares. Their heritability, therefore,

was high, 0.40-0.83, and 0.64-0.95, respectively (Table 2.1). Linolenic acid content significantly increased from dryland to irrigated treatment, specifically in 2010, increasing from 29.1% to 32.2% (Table 2.2). Linolenic acid content had a range of 9.3% under dryland and 6.8% under irrigation between minimum and maximum values. Eicosenoic acid did not show significant difference between the moisture treatments. Eicosenoic content ranged from 11.6% to 16% among RILs across treatments (Table 2.2).

Trait correlation

Pearson correlation coefficients of all studied traits are reported in Table 2.4. Flowering time had a positive correlation to irrigated leaf water content ($p<0.1$), dryland and irrigated leaf nitrogen content ($p<0.05$), and irrigated and dryland seed yield in 2009 ($p<0.05$). Flowering time had a significant negative correlation to dryland leaf water content ($p<0.05$), linolenic content ($p<0.05$), thousand seed weight ($p<0.0001$) and dryland and irrigated seed yield in 2010 ($p<0.001$). Carbon isotope ratio, $\delta^{13}C$, was positively correlated to leaf nitrogen content under dryland ($p<0.1$) and negatively correlated under irrigation ($p<0.001$). It was positively correlated to oil content ($p<0.1$) and eicosenoic acid content ($p<0.001$) under irrigation but not under dryland. Leaf nitrogen content was negatively correlated to 2009 dryland linolenic content ($p<0.1$) but not under irrigation. Seed yield and oil content were strongly positively correlated under both moisture treatments and years ($p<0.05$). TSW had positive correlation ($p<0.05$) to linolenic content.

QTL analysis

Details of all significant QTLs detected for drought-related traits, oil quality traits and seed yield using lsmeans data within water treatment are presented in Table 2.3. A total of 29 significant QTLs were found for the measured traits. Six QTLs were common to the Gehringer et al. (2006) study on the same RIL population: one for plant height, one for TSW, two for oil content, one for linolenic and one for eicosenoic fatty acid. Finally, six QTLs were significant for the value of the difference between dryland and irrigated phenotype.

Plant height QTLs

Two significant QTLs were detected for height. A QTL at the marker E38M62_330 on chromosome LG19 was found in this study as well as in the study of Gehringer et al. (2006). The height QTL at the marker E40M49_580 at the 37.84 cM region on chromosome LG4 was significant for seed yield in Gehringer et al. (2006).

Flowering time QTLs

Four significant QTLs were detected for flowering time. Three of them, E39M51_230 (LG1), E33M54_370 (LG4), and E43M55_243 (LG7), were detected for the overall mean across the four environments (Table 2.3). When combined, they explained 31.45% of the variation with a LOD score of 14.7 (Table 2.3). The selection of RILs having the combination of alleles “aba” at the three loci flowered 2.4 days later than the RILs with the opposite combination of alleles “bab”. The R-square of the combined QTLs for the fitted linear regression between 2009 and 2010 was 0.31 and 0.30 respectively for “aba” and “bab” allelic combination (Fig. 2.5A). A QTL was found for

the value of the phenotypic difference between moisture treatments on chromosome LG4 at 43 cM at the marker E33M54_370. RILs with the Lindo allele "a" had shorter flowering time under 2009 dryland treatment.

Leaf water and nitrogen content and $\delta^{13}C$ QTLs

No QTL was found for leaf nitrogen content or $\delta^{13}C$ despite significant correlation with other traits. A QTL was detected for 2010 dryland leaf water content mapped to the 69.5 cM region on chromosome LG7.

Seed yield and seed size QTLs

Five significant QTLs were detected for seed yield. Three of them, E33M54_370 (LG4), E41M50_235 (LG6), and E31M60_365 (LG7), were detected for the overall mean across the four environments (Table 2.3). When combined, they explained 21.9% of the phenotypic variations with a LOD score of 9.7 (Table 2.3). The average seed yield was 102.6 grams and 86.7 grams respectively for the RILs with allelic combination "aaa" and "bbb" or a 16% difference. The R-square of the fitted linear regression between the two years for the combined QTLs was 0.17 (p=0.03) for combination "bbb" (Fig. 2.5B). A QTL was found for the value of the phenotypic difference between moisture treatments on chromosome LG7 at 69.5 cM at the marker E31M60_365. At this marker, RILs with the Lindo allele "a" had higher yield under the 2010 irrigated treatment.

Two significant QTLs were detected for thousand seed weight (TSW). The QTL found at the marker E31M53_345 at 14.0 cM on chromosome LG9 was consistent with the findings of Gehringer et al. (2006).

Oil content QTLs

Five significant QTLs were detected for oil content. Three of them, E31M50_215 (LG1), E31M60_365 (LG7), and E31M51_332 (LG9), were detected for the overall mean across the four environments (Table 2.3). When combined, they explained 29.9% of the phenotypic variation with a LOD score of 13.9 (Table 2.3). The average oil content across environments was 31.2 and 29.4%, respectively, for the allelic combination "aaa" and "bbb", a 5.6% difference. The R-squares of the fitted linear regression between the two years for the combined QTLs were highly significant (p<.0001), 0.71 and 0.38 respectively for "aaa" and "bbb" (Fig. 2.5C). A QTL was found for the value of the phenotypic difference between moisture treatments on chromosome LG7 at the 69.5 cM region at the marker E31M60_365. At this marker, RILs with the Lindo allele "a" had higher oil content under irrigated treatment. Two of the main effect QTLs at the markers E31M50_215 (LG1) and E31M51_332 (LG9) were found in this study, as well as in a study by Gehringer et al. (2006).

Fatty acid composition QTLs

Six significant QTLs were detected for linolenic content. Four of them, E31M51_369 (LG1), E33M57_150 (LG4), E31M60_365 (LG7), and E31M53_345 (LG9), were detected for the overall mean of the four environments (Table 2.3). When combined, they explained 23.8% of the variation with a LOD score of 10.7 (Table 2.3). The average linolenic content was 31.9 and 29.9% respectively for allelic combination "aaba" and "bbab" or a 6% difference. The R-squares of the fitted linear regression between the two years for the combined QTLs were significant (p<.05), 0.71 and 0.38

respectively for the “aaba” and “bbab” combinations (Fig. 2.5D). A QTL was found for the value of the difference between moisture treatments on chromosome LG1 at the 140.7 cM region at the marker E31M51_369. At this given marker, RILs with the Lindo allele “a” had higher linolenic content under irrigated treatments. A main QTL effect at the marker E31M51_369 (LG1) was found in this study, which was consistent with the findings of Gehringer et al. (2006).

Potential pleiotropy effect

Some QTLs were shared by multiple traits suggesting pleiotropic effect or proximity of genes. Yield, flowering time and linolenic fatty acid content shared one QTL on chromosome LG4 at the marker E33M54_370 (Fig. 2.4A). Seed yield had high positive significant (p<0.01) correlation in 2009 and negative significant correlation in year 2010 with flowering time (Table 2.4). Linolenic fatty acid content had high significant negative correlation with flowering time. The “a” allele at the marker E33M54_370 negatively impacted linolenic content under the dry treatments.

Yield, oil content, leaf water content, linolenic and eicosenoic fatty acid content shared one QTL on chromosome LG7 at the marker E31M60_365 (Fig. 2.4B). The “a” allele at this marker had a positive effect on leaf water content and linolenic fatty acid while having a negative effect on yield, oil content and eicosenoic fatty acid. Indeed, eicosenoic and seed yield had significant positive (p<0.01) correlation to oil content whereas eicosenoic was negatively (p<0.05) correlated to linolenic fatty acid. Yield was positively correlated (p<0.001) to oil content across all environments. The effect of

marker E31M60_365 was stronger under irrigated treatment than dryland, translated by significant IPC and treatment difference scans (Table 2.3).

DISCUSSION

Camelina has shown superior yield performance under semi-arid conditions in comparison with other oilseed species (Angelini et al., 1997; Francis and Campbell, 2004; Enjalbert et al., unpublished). In this study, camelina revealed strong potential for seed yield improvement under semi-arid environments. Positive and negative trait correlations, high heritability, stable QTLs and high plasticity variability were found. These findings offer breeding potential to improve camelina varieties in dry and variable environments.

Trait expression varies in response to variations in water availability, maximum and minimum temperatures, photoperiod, light intensity, soil fertility and crop management (Crossa et al., 1991). Therefore, to understand phenotypic and genotypic variability in this study, it was critical to understand the environmental conditions under which the experiment was conducted. Moreover, because drought timing is important for some traits to be effective, a better understanding of the environmental stress helped to better understand the phenotypic data. In both years of data collection, the drought stress was post flowering. Thus drought impacted mainly stay-green traits such as LNC, seed yield stability and seed quality traits. The higher water stress and heat stress in 2010 characterized the year-to-year variability common within semi-arid environments. Such environmental variation impacted phenotypic variability and correlation direction.

Many trait correlations were found. Flowering was correlated to all traits except carbon isotope ratio. Correlation signs changed according to environment. Under the cooler year, 2009, and irrigated conditions, RILs that flowered later had higher leaf water content (LWC), leaf nitrogen content (LNC), and seed yield, and smaller seed size than earlier flowering ones. Those RILs retained their leaves in an active photosynthetic stage longer than the early-flowering RILs.

In contrast, under dryland conditions RILs that flowered later had lower leaf water content, corresponding to a lower osmoregulation, which induced lower seed yield in 2010, a hotter year during flowering. In such conditions, RILs flowering earlier escaped the heat and part of the drought at early seed filling and yielded better. Earlier flowering time has been shown to increase yield under dry conditions. Plants are the most susceptible to water stress and heat stress at the reproduction stage. By escaping the drought, plants can increase their seed production (Siddique et al., 1990). Furthermore, under dry treatment RILs with higher LNC expressed higher WUE ($\delta^{13}C$) while under irrigation they expressed lower WUE. It is usually reported that higher LNC is related to higher WUE. Borrel et al. (2001) associated such behavior to stay-green traits under dry conditions. In this case, high WUE is related to higher assimilation compared to water loss. Higher LNC delays leaf senescence and allows further uptake of soil water and nitrogen, resulting in higher yields (Borrell and Hammer, 2000). Thus, under irrigation, RILs with high WUE might have low stomatal conductance, limiting water loss and assimilation, resulting in lower rates of photosynthesis (Donovan et al., 2007).

RILS flowering earlier had higher linolenic acid content under both moisture regimes and better fitness. Two main explanations have been documented: cold adaptation and drought tolerance. First, higher seed linolenic acid content increases oil fluidity, and therefore plant metabolism, at lower temperature (Linder, 2000). This study found that RILs with higher TSW flowered earlier and had higher linolenic acid content. Because RILs having bigger seed and higher linolenic acid content could have higher seedling establishment and shoot vigor at lower temperature, they will flower earlier. Thus, they would be better adapted to dry environments with a short growing season by using the entire growing season for enhanced yield (Richards et al., 2010).

Second, high linolenic acid in the seed appears to be correlated to linolenic acid in the leaf (Lemieux et al., 1990; Miquel & Browse, 1994; Kang et al., 2011). And, it has been shown in Arabidopsis (McConn et al., 1994; Matsuda et al., 2005; Mene-Saffrane et al, 2009), canola (Merrien et al., 2007; Triboi-Blondel and Renard, 1999), sunflower (Izquierdo et al., 2002), tobacco (Kodama et al., 1994; Murakami et al., 2000) and coconut (Repellin et al., 1997) that an increase of leaf trienoic fatty acids such as linolenic acid in leaves improved cold and drought tolerance. Polyunsaturated lipids, primarily linolenic fatty acid, play a central role in chloroplast structure, maintaining normal photosynthetic function and regulating thermal stability of photosynthetic membranes (Hugly et al, 1989). Linolenic content was negatively correlated with LNC under dryland treatment, showing a relationship between photosynthesis and linolenic content. Furthermore, trienoic fatty acid is a precursor of oxylipin, a signaling compound that regulates final maturation processes and the release of pollen (McConn and Browse,

1996). Thus, it would appear that under dryland conditions higher linolenic acid content RILs flowered earlier and were better adapted to drought and heat resulting in higher seed and oil yield.

Eicosenoic fatty acid content might play a role in plant adaptation in addition to linolenic fatty acid. Under irrigated conditions RILs with higher eicosenoic fatty acid content had higher water use efficiency, oil content, and seed yield for both years of data. Many QTLs were reported for such traits allowing for potential future camelina improvement.

A total of 29 QTLs were found for all the studied traits. Some of those QTLs were very stable, overlapping with the Gehringer at al. (2006) study. A strong correlation for flowering, seed yield, oil content and linolenic fatty acid was found when predicting QTL response from 2009 to 2010. When combining appropriate seed yield QTLs, results showed a 16% seed yield increase. Moreover, three stable linolenic fatty acid QTLs were found allowing up to 6% increase. Such improvement could have a significant economic impact for growers. However, correlations between traits were observed that could bring challenges to breeding programs.

Pleiotropy occurs when a gene influences or controls multiple phenotypic traits. QTL analysis suggested a pleiotropic effect at two chromosome regions: on chromosome four near 24.7 cM and on chromosome seven near 69.5 cM region. The chromosome four 24.7 cM region influenced flowering, seed yield, linolenic and eicosenioc fatty acid, whereas the 69.5 cM region influenced expression of leaf water content, seed yield, oil content and linolenic and eicosenoic fatty acid. Pleiotropic effect, as seen here, reflects

potential interaction between those phenotypic traits or genes located close to each other. In that regard, Yue et al. (2006) mentioned that flowering time variation in a segregating population could make the phenotyping of drought traits less accurate because drought at the reproductive stage drastically impacts fitness. In this study, drought stress occurred post flowering, but some heat stress during the flowering period in 2010 could have impacted the results and confounded heat and drought, creating some of the pleiotropic effect. However, some traits such as $\delta^{13}C$, seed yield, thousand seed-weight, oil content and oil profile had highly significant correlation among environments, expressing high heritability. In previous studies, $\delta^{13}C$ had expressed strong heritability as well (Condon and Richards, 1992). As such, these traits appear reliable when studying drought. But others traits such as height, leaf water content (LWC) and leaf nitrogen content (LNC) were less predictable. LNC has been shown to be effective during pre-flowering drought (Borrell et al., 2001). Yet, because the specific choice of the leaf, sample timing and timing of drought are crucial for accurate results for LWC and LNC, some error might have occurred, as mentioned by Yue et al. (2006).

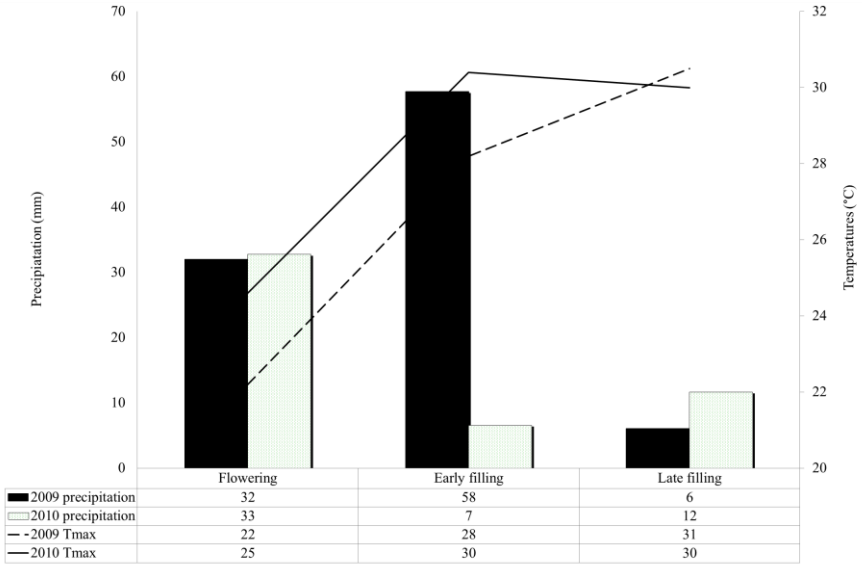
In addition to the pleiotropic effects of certain genes, the study found QTLs for plasticity. Due to the relationship between environment and phenotypic plasticity, it is crucial to look at phenotypic environmental differences, especially for physiological traits such as flowering, which express fundamental correlation with structural traits (Reich et al., 1997). In this study two methods were used to analyze differences among environments: reaction norms and QTL scan for the phenotypic difference between dry and irrigated conditions. Both techniques were successful in reflecting phenotypic

plasticity. For flowering, LWC, $\delta^{13}C$, seed yield, and linolenic acid content, the RIL population expressed strong differences in phenotypic plasticity. The reaction norms showed that some RILs had adaptive plastic response while other had maladaptive response. In addition, QTL scan of the phenotypic difference revealed six QTLs. A QTL at 43.0 cM on chromosome LG4 reduced the difference in flowering time between dry and irrigated treatments. RILs having this QTL had a more stable flowering time than the ones without. Another QTL at 69.5 cM on chromosome LG7 for seed yield and oil content increased the difference between dry and irrigated treatments when the Lindo allele was present. Thus, those QTLs reveal breeding potential for camelina varieties that are more stable or better adapted to dryland and irrigated conditions.

CONCLUSION

This study is the first known to generate QTLs for drought adaptation. Variation in plasticity was revealed among the RIL population by a wide range of trait responses to moisture. A pleiotropic effect was observed due to strong impact of flowering time on other traits like fitness, seed size, and linolenic fatty acid content. Under dry and hot environments, earlier flowering improved fitness. Linolenic acid content and seed size were linked to flowering time, implying potential improvement of seedling establishment and shoot vigor under dry conditions in short growing seasons. The positive correlation between WUE ($\delta^{13}C$) and leaf nitrogen suggested that higher LNC may delay leaf senescence and allow further uptake of soil water and nitrogen, resulting in higher yields. Twenty-nine QTLs were found. Some of them were very stable and coincided with

previous findings from Gehringer et al. (2006). When combining stable QTLs, up to 16% seed yield increase was achieved. In addition, significant flowering, seed yield, oil content and linolenic acid QTLs for the phenotypic differences between dry and irrigated conditions were found. This work demonstrated good potential for camelina improvement and the use of marker-assisted selection to create camelina cultivars adapted to dry environments, as well as adaptation to a broader range of environments.



	Flowering	Early filling	Late filling
2009 precipitation	32	58	6
2010 precipitation	33	7	12
2009 Tmax	22	28	31
2010 Tmax	25	30	30

Figure 2.1: 2009-2010 Precipitation and maximum average temperatures at flowering, early filling and late filling period.

Table 2.1: ANOVA, CV, mean, heritability and IPC values of the measured traits of a recombinant inbred line population (n = 181) evaluated in two environments in 2009 and 2010.

Effect	DF	Height	Flowering	Yield	TSW	Oil	Oleic	Linoleic	Linolenic	Eico	Erucic
Environment	3	ns	***	**	ns	ns	**	ns	*	***	ns
Rep (Env)	8	***	***	***	**	***	***	***	***	**	***
RIL	188	*	***	***	**	***	***	***	***	***	***
Env*RIL	564	*	***	***	ns	***	***	***	***	*	***
CV, %		13.1	1.9	24.0	8.9	3.3	5.8	5.5	3.8	3.8	6.9
Mean		65.2	74.8	95.9	1.6	30.2	19.1	18.4	31.0	13.6	3.0
Heritability1		0.03	0.86	0.50	0.87	0.87	0.97	0.95	0.83	0.95	0.92
Heritability2		0.00	0.39	0.10	0.36	0.42	0.78	0.72	0.40	0.64	0.52

*Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

Table 2.1: Development and growth traits, seed yield and oil summary under irrigation and dryland treatments of 181 RILs at Fort Collins, CO, in 2009 and 2010.

			Dryland					Irrigated				
Trait	year	Unit	Mean	Min	Max	Licalla	Lindo	Mean	Min	Max	Licalla	Lindo
Height	2009	cm	66.38	51.30	80.00	67.00	61.70	68.52	51.70	88.70	80.00	77.00
	2010	cm	50.06	33.67	64.36	59.60	59.30	68.62	42.33	75.67	65.30	70.30
Flowering	2009	day	79.48	76.00	84.00	79.30	81.70	79.81	76.30	83.30	81.00	80.70
	2010	day	70.11	66.00	73.67	70.70	70.70	69.50	63.67	74.00	70.30	68.70
LWC	2010	%	78.00	68.00	84.00	81.40	78.20	0.86	0.77	0.93	88.09	85.30
$\delta^{13}C$	2009	ratio	-28.10	-29.41	-26.87	-28.10	-28.09	-29.08	-30.32	-27.65	-28.93	-29.41
LNC	2009	%	2.11	0.42	3.47	2.14	2.37	2.66	1.43	4.53	3.20	2.50
Seed yield	2009	gram	96.43	45.10	163.00	86.30	106.70	123.79	73.00	189.30	148.70	157.70
	2010	gram	50.41	20.17	81.17	36.70	60.00	114.31	51.67	168.33	115.00	158.20
TSW	2009	gram	1.65	1.16	2.08	1.72	1.38	1.62	1.17	1.95	1.52	1.28
Oil content	2009	%	30.37	27.64	33.16	31.40	33.00	30.80	26.79	34.56	32.60	34.00
	2010	%	29.21	26.33	31.47	29.80	29.80	30.05	26.33	33.13	32.60	33.30
Linolenic	2009	%	30.91	26.80	36.13	32.70	29.30	31.66	27.96	35.52	32.20	30.60
	2010	%	29.14	24.98	34.33	28.00	26.30	32.19	29.02	35.07	33.90	30.50
Eicosenoic	2009	%	13.77	11.61	16.05	15.60	13.00	13.84	11.85	16.02	5.50	13.40
	2010	%	13.56	11.04	16.74	14.30	12.40	13.39	11.46	15.59	14.30	13.30

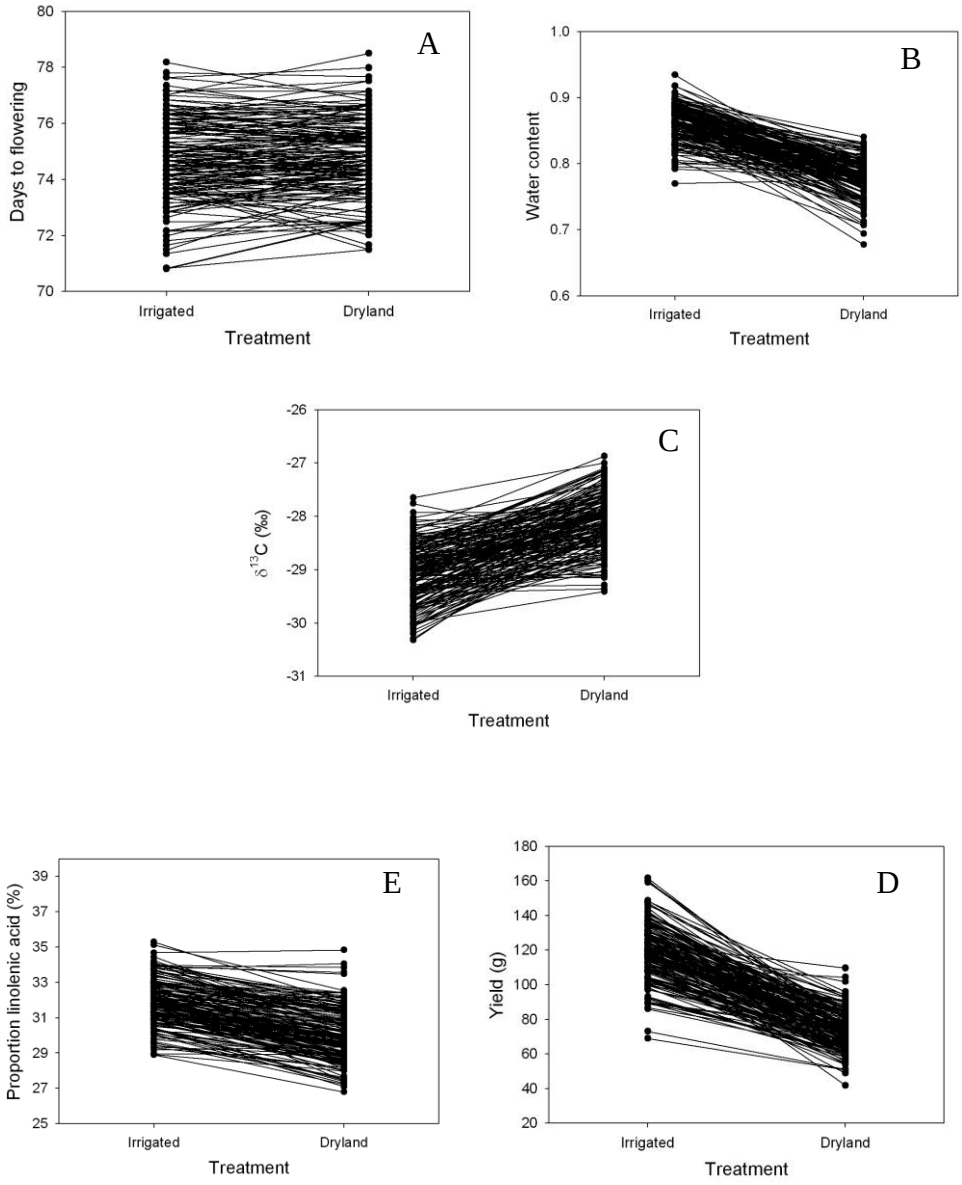


Figure 2.1: Effect of water availability on days to flowering (A) water content (B) carbon isotope (C) yield (D) and linolenic acid content (E) on 181 RILs in 2009 and 2010 at Fort Collins, CO.

Table 2.2: Summary of quantitative trait loci (QTL) effects for days to flower, leaf water content, seed yield, oil content, linolenic and eicosenoic fatty acids detected in dry (D1 and D2), irrigated (W1 and W2) treatments, trait difference between dryland and irrigation (Dif1 and Dif2) and IPC1 (QTLxE) of 181 recombinant inbred lines evaluated in Fort Collins, CO, in 2009 and 2010.

				Additive Effect							
Traits	**Marker**	**LG**	**Position**	**D1**	**D2**	**W1**	**W2**	**DIF1**	**DIF2**	**mean**	**2006**
Height (cm)	E40M49_580	4	37.84	.	-1.89	.	.	.	.	.	
	E38M62_330	19	0.00	-2.38	.	.	.	.	.	.	§
Model LOD				2.60							
Variation (%)				6.46							
Flowering (day)	E39M51_230	1	9.68	-0.95	-0.75	-0.76	-1.22	.	.	-0.92	
	E42M56_760	4	43.00	.	.	.	.	0.59	.	.	
	E33M54_370	4	24.70	.	0.79	.	-1.21	.	.	0.61	
	E43M55_243	7	32.43	-0.87	-0.62	-0.69	.	.	.	-0.71	
Model LOD				11.83	11.84	9.05	14.65	5.54		14.65	
Variation (%)				25.99	26.00	20.58	31.20	13.16		31.45	
Water content (%)	E31M60_365	7	69.50	.	2.00	.	.	.	.	.	
Model LOD					2.36						
Variation (%)					5.84						
Yield (gram)	E32M61_166	1	0.00	-10.20	.	.	.	.	.	.	
	E33M54_370	4	24.70	.	-7.90	.	-10.00	.	.	-6.10	
	E41M50_235	6	54.80	.	.	.	-10.70	.	.	-5.80	
	E31M60_365	7	69.50	.	.	.	-11.10	.	-11.60	-5.20	
	E39M52_290	17	20.50	.	5.65	.	.	.	.	.	
Model LOD				4.88	6.27		11.50	.	6.83	9.73	
Variation (%)				11.68	14.76		25.40		15.96	21.93	
TSW (mg)	E32M61_166	1	0.00	77.00	.	78.00	.	.	.	56.00	
	E31M53_345	9	14.00	-80.00	-52.00	-70.00	-80.00	.	.	-65.00	§
Model LOD				6.20	2.60	8.40	4.30	.	.	8.10	
Variation (%)				14.60	6.30	19.20	10.10	.	.	18.50	
Oil content (%)	E31M50_215	1	34.70	-0.66	-0.41	.	.	.	.	-0.46	§
	E31M60_365	7	69.50	.	.	.	-0.57	.	-0.51	.	
	E31M51_332	9	51.59	-0.63	-0.59	-0.62	-0.57	.	.	-0.64	§
	E41M50_172	12	49.13	0.39	.	.	0.58	.	0.46	0.37	
	E38M62_335	19	19.42	.	.	-0.46	.	.	.	.	
Model LOD				11.20	8.75	10.60	11.80	.	4.69	13.94	
Variation (%)				24.80	19.97	23.63	26.00	.	11.24	29.89	
Linolenic (%)	E31M51_369	1	140.40	-0.74	.	-0.94	-0.70	.	.	-0.71	§
	E33M57_150	4	35.16	-0.82	.	-0.85	-0.52	.	.	-0.67	
	E33M54_370	4	24.70	.	0.81	.	.	.	-0.89	.	
	E31M60_365	7	69.50	0.68	.	.	.	.	.	0.47	
	E43M55_243	7	32.43	.	-0.97	.	.	.	0.86	.	
	E31M53_345	9	14.00	.	-0.83	.	-0.53	.	.	-0.57	
Model LOD				8.10	7.99	9.75	8.01	.	5.40	10.66	
Variation (%)				18.60	18.39	21.90	18.45	.	12.85	23.80	
Eicosenoic (%)	E33M49_260	1	126.18	-0.33	.	-0.33	.	.	.	.	§
	E31M60_365	7	69.50	.	-0.35	-0.33	.	.	.	.	
	E34M55_225	8	61.08	-3.10	.	.	.	.	.	.	
	E38M47_505	20	10.40	-0.37	-0.27	-0.37	.	.	.	.	
Model LOD				11.91	9.26	13.10					
Variation (%)				26.10	20.90	28.50					

§ Gehringer et al. (2006) study

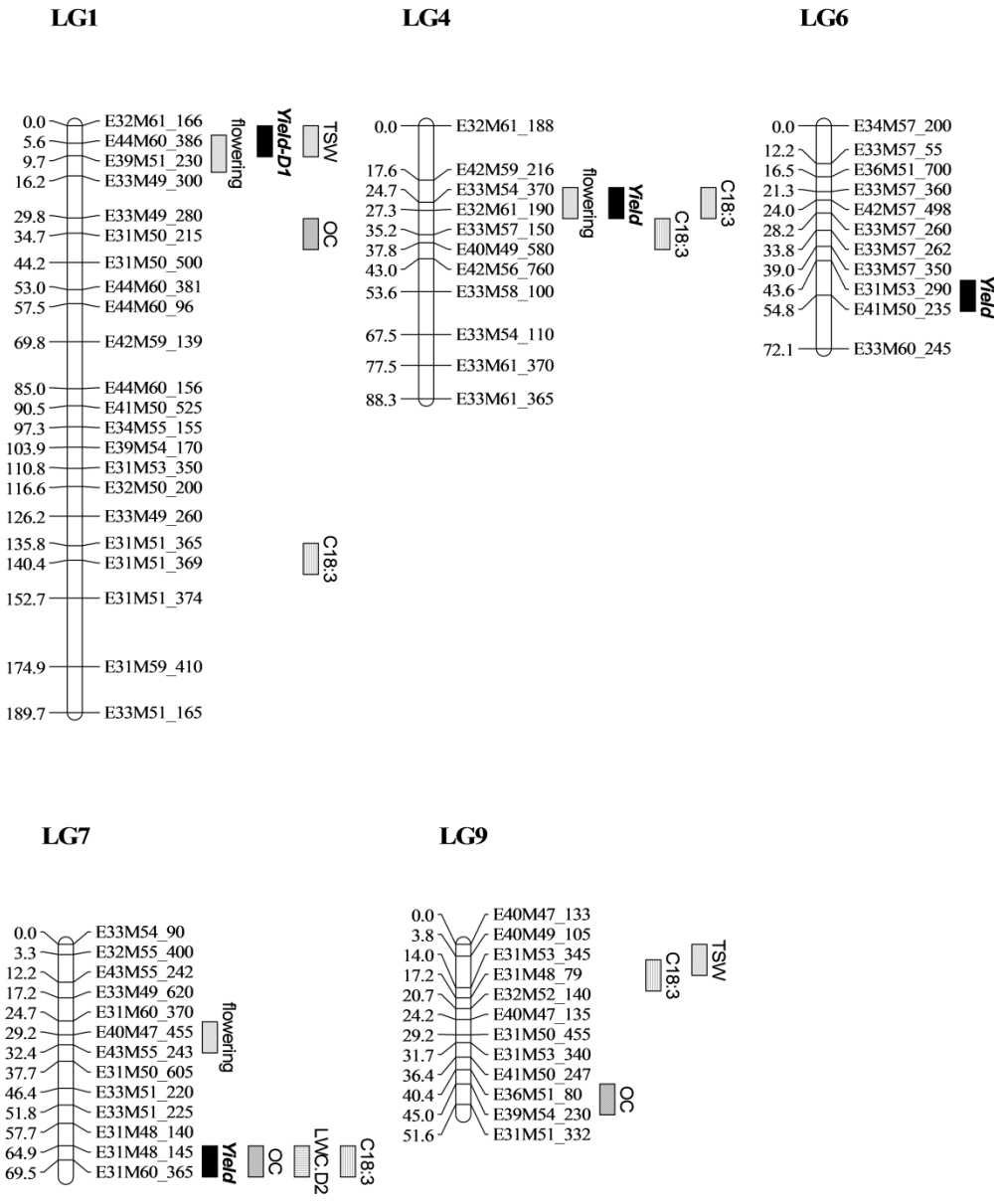


Figure 2.2: Genetic map of *Camelina sativa*, comprising 157 amplified fragment length polymorphism (AFLP) and 3 simple sequence repeat (SSR) markers, constructed using 181 single-seed-descent (SSD) lines from 'Lindo' X 'Licalla' cross. Shown on the map are the linkage groups and position of major quantitative trait loci (QTLs) for time to flowering, leaf water content (LWC), seed yield, oil content, 1000 seed weight (TSW), and linolenic fatty acid (C18:3) measured under dryland and irrigation in field trials during two years.

Table 2.3: Phenotypic correlation coefficients between traits based on trait values averaged within environment in 2009 (1) and 2010 (2) of 181 RILs at Fort Collins, CO.

		Irrigated															
	Traits	Height1	Height2	Flowering1	Flowering2	LWC2	δ^{13}C1	LNC1	SY1	SY2	TSW1	OC1	OC2	Linolenic1	Linolenic2	eicosenoic1	eicosenoic2
Dryland	Height1	**0.06**	0.01	0.25	0.10	0.07	0.02	0.03	0.17	0.01	-0.03	0.24	0.09	-0.08	0.11	0.08	0.03
		ns	ns	***	ns	ns	ns	ns	*	ns	ns	**	ns	ns	ns	ns	ns
	Height2	-0.05	**-0.01**	0.04	-0.09	0.06	0.02	0.06	0.23	0.25	0.18	0.07	0.17	0.01	0.04	-0.05	-0.01
		ns	**ns**	ns	ns	ns	ns	ns	**	**	*	ns	*	ns	ns	ns	ns
	Flowering1	0.38	-0.06	**0.71**	0.61	0.11	-0.08	0.15	0.22	0.03	-0.20	0.34	0.06	-0.27	-0.15	-0.06	-0.04
		***	ns	****	***	ns	ns	*	**	ns	**	***	ns	**	*	ns	ns
	Flowering2	0.20	0.08	0.56	**0.81**	0.15	-0.04	0.04	0.15	-0.39	-0.27	0.13	-0.14	-0.12	-0.21	-0.09	-0.15
		**	ns	***	****	*	ns	ns	*	***	**	§	§	§	**	ns	*
	LWC2	0.03	-0.04	-0.06	-0.14	**0.10**	-0.10	0.11	0.06	-0.01	-0.11	0.08	-0.01	0.06	0.07	-0.06	-0.06
		0.66	ns	ns	*	**ns**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	δ^{13}C1	0.07	0.00	-0.04	-0.03	-0.06	**0.28**	-0.27	-0.05	-0.03	0.15	0.12	0.24	-0.04	-0.06	0.22	0.26
		ns	ns	ns	ns	ns	****	**	ns	ns	*	§	**	ns	ns	**	***
	LNC1	-0.01	-0.05	0.18	0.06	0.07	0.13	**0.08**	0.05	0.03	0.02	0.11	0.12	0.02	-0.01	-0.09	-0.10
		ns	0.49	*	ns	ns	§	**ns**	ns	ns	ns	ns	§	ns	ns	ns	ns
	SY1	0.31	-0.07	0.30	0.13	0.01	0.06	0.07	**0.29**	0.19	-0.06	0.41	0.22	0.00	-0.09	0.06	-0.05
		***	ns	***	§	ns	ns	ns	****	*	ns	***	**	ns	ns	ns	ns
	SY2	-0.07	0.09	-0.05	-0.43	0.08	-0.05	-0.09	0.07	**0.30**	0.03	0.22	0.44	-0.11	0.01	0.10	0.14
		ns	ns	ns	***	ns	ns	ns	ns	****	ns	**	***	ns	ns	ns	*
	TSW1	-0.07	-0.05	-0.34	-0.25	0.03	0.16	0.00	-0.14	0.03	**0.61**	-0.10	0.01	0.27	0.16	0.03	0.00
		ns	ns	***	***	ns	*	ns	*	ns	****	ns	ns	**	*	ns	ns
	OC1	0.17	-0.13	0.20	0.09	0.01	-0.05	0.03	0.24	0.06	-0.16	**0.70**	0.68	0.00	-0.04	0.35	0.25
		*	§	**	ns	ns	ns	ns	**	ns	*	****	***	ns	ns	***	***
	OC2	0.14	-0.10	0.24	0.12	0.01	0.09	0.02	0.15	0.32	0.05	0.58	**0.55**	0.04	0.04	0.42	0.46
		*	ns	**	§	ns	ns	ns	*	***	ns	***	****	ns	ns	***	***
	Linolenic1	-0.30	-0.04	-0.35	-0.06	0.04	0.06	-0.14	-0.10	0.00	0.23	0.00	0.15	**0.81**	0.73	-0.17	-0.12
		***	ns	***	ns	ns	ns	§	ns	ns	**	ns	*	****	***	*	ns
	Linolenic2	0.17	0.08	0.40	0.50	-0.03	0.03	-0.03	0.11	-0.07	0.00	0.15	0.43	0.41	**0.42**	-0.19	-0.16
		*	ns	***	***	ns	ns	ns	ns	ns	ns	*	***	***	****	**	*
	Eicosenoic1	0.00	-0.09	-0.10	-0.13	0.03	-0.04	0.03	0.00	0.10	0.02	0.24	0.15	-0.22	-0.26	**0.92**	0.80
		ns	ns	ns	§	ns	ns	ns	ns	ns	ns	**	*	**	**	****	***
	Eicosenoic2	0.08	-0.09	0.04	0.05	0.02	0.04	0.04	0.06	0.02	0.05	0.22	0.24	-0.18	-0.09	0.83	**0.74**
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	**	*	ns	***	****

"ns" non significant§ Significant at the 0.1 level; Significant at the 0.05 probability level; *Significant at the 0.05 probability level; **Significant at the 0.01 probability level; ***Significant at the 0.001 probability level.

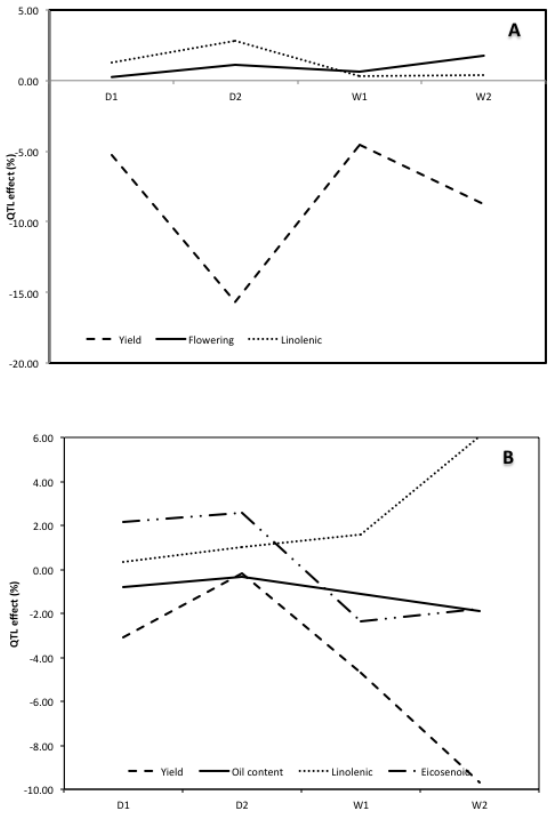


Figure 2.3: QTL effect over environment mean (%) of (A) the marker E33M54_370 on LG4 across environments for flowering, yield, and linolenic fatty acid, and (B) the marker E31M60_365 on LG7 across environments for yield, oil content, linolenic and eicosenoic fatty acids.

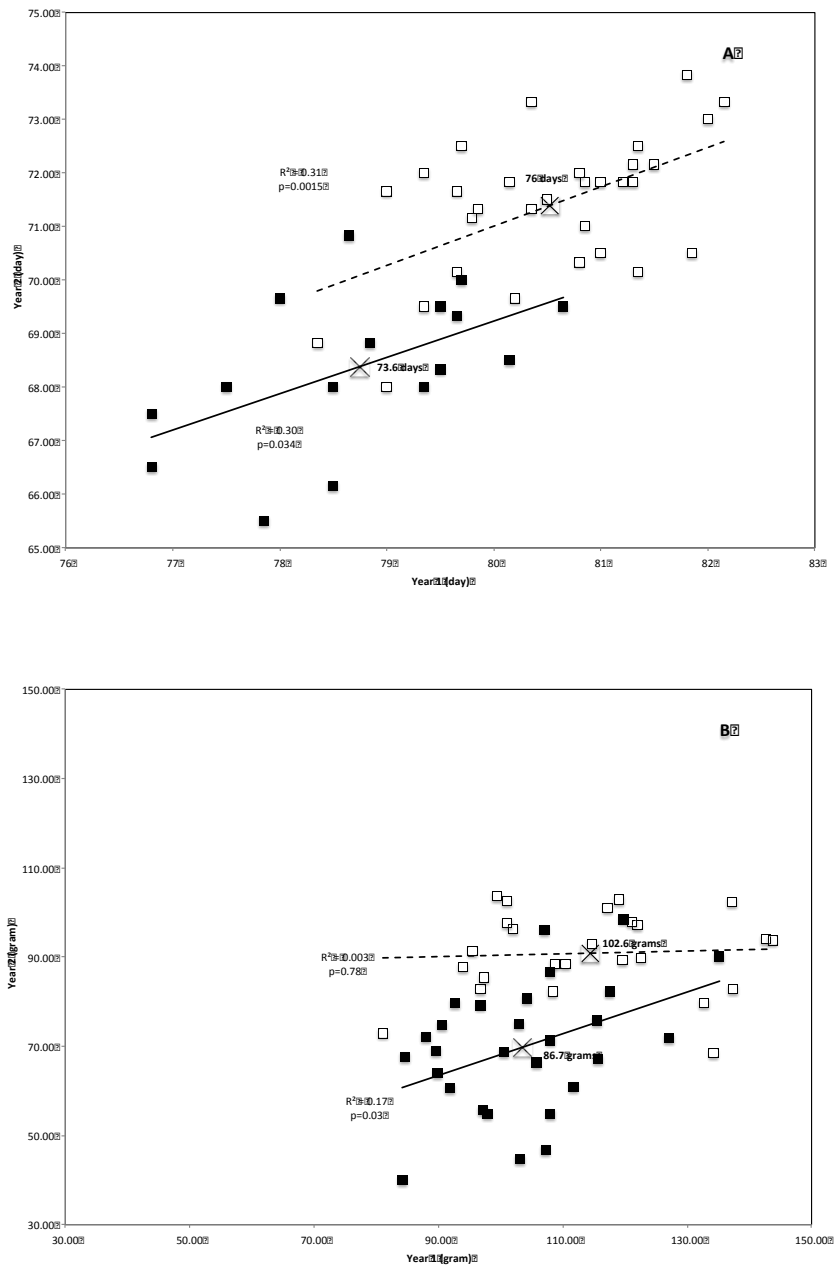


Figure 2.4: Trait prediction across treatments between 2009 and 2010 with the significant QTLs; (A) Days to flowering for RILs with alleles "aba" (empty square) versus "bab" (solid square) at the three markers E39M51_230 (LG1), E33M54_370 (LG4), E43M55_243 (LG7); (B) seed yield for RILs with alleles "aaa" (empty square) versus "bbb" (solid square) at the three markers E33M54_370 (LG4), E41M50_235 (LG6), E31M60_365 (LG7). Figure 2.5 is continued on the next page.

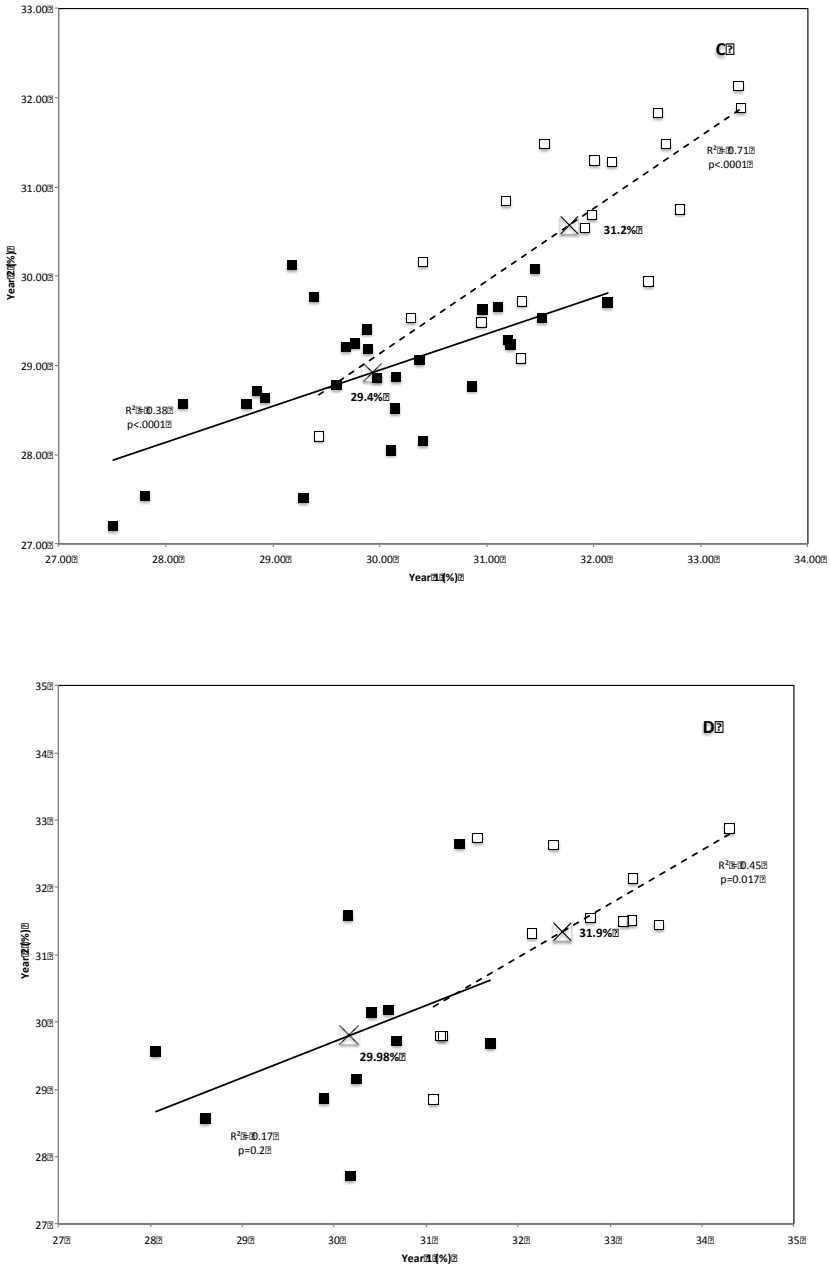


Figure 2.5 continued: Trait prediction across treatments between 2009 and 2010 with the significant QTLs; (C) oil content for RILs with alleles "aaa" (empty square) versus "bbb" (solid square) at the three markers E31M50_215 (LG1), E31M60_365 (LG7), E31M51_332 (LG9); (D) linolenic content for RILs with alleles "aaba" (empty square) versus "bbab" (solid square) at the four markers E31M51_369 (LG1), E33M57_150 (LG4), E31M60_365 (LG7), E31M53_345 (LG9).

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CHAPTER THREE:

THE EFFECTS OF GENOTYPE, ENVIRONMENT, AND GENOTYPE BY ENVIRONMENT INTERACTION ON *CAMELINA SATIVA* OIL QUALITY AND YIELD

ABSTRACT

Camelina sativa (L.) Crantz oil can be used as a sustainable biofuel or for human consumption. Its meal, high in omega-3 fatty acids, could become an important animal feed substitute for soybean meal. Camelina oil composition affects engine emissions, power and durability, and the seed composition also affects meal quality. Seed yield and oil content determine the economic feasibility of using camelina oil and meal, which could be grown in western U.S. cropping systems without competition with food crop production. The economic feasibility of using camelina oil and meal depends on the seed yield and oil content of camelina, which could be grown in western U.S. cropping systems without displacing food crops. This research studied the effects of genotype, environment, and genotype by environment interaction (GEI) on camelina seed yield, oil content, and oil quality for eight camelina varieties of different maturity grown in 12 environments in Washington, Montana, Wyoming, and Colorado in 2009 and 2010.

Environments were the major source of variation for seed yield, oil content, and linolenic fatty acid content. Water deficit and high temperatures were negatively correlated to seed yield, oil content and linolenic fatty acid content. Irrigation

increased oil linolenic acid content (p<0.0001) by 17%. Early-maturing varieties demonstrated higher seed yields in water-stressed regions whereas later-maturing varieties were high yielding in the northern locations. Genotype by environment interaction (GEI) was significant for seed yield, oil content and linolenic fatty acid content. Mega-environments, that is, the regions within which GEI was not significant for traits of interest, were determined for all traits using AMMI analysis. The northern mega-environment included Washington, Montana, Wyoming, and southwest Colorado. Northeastern Colorado constituted the second mega-environment. IPC values for yield, oil content and linolenic fatty acid content were most highly correlated to temperature and water deficit parameters and explained the different varietal responses to critical environmental variables. This study contributes understanding of how environments (and varieties) affect the yield, oil content, and oil composition of camelina.

INTRODUCTION

As the world faces increasing demand and diminishing supplies of fossil fuels, oilseed crops are emerging as a potential source of sustainable biofuel, especially for on-farm use, which could lead to energy independence of the agricultural sector. Short-season camelina (*Camelina sativa* (L.) Crantz), an opportunity crop that can be incorporated into existing low-rainfall, western dryland crop rotations with minimal inputs (Zubr, 1997), is of interest due to its earliness, drought tolerance, resistance to flea beetles, ease of direct harvest, oil quality, and high value as animal feed.

Camelina is native to Southeast Europe and Southwest Asia and known to have been cultivated for 4000 years (Putnam et al., 1993). Camelina has favorable oil

properties that include high content of polyunsaturated linolenic fatty acid (C18:3), and low erucic acid (C22:1) (Bernardo et al., 2003). Unsaturated fatty acid content can favorably influence engine performance and emissions when used as biodiesel or as straight vegetable oil (SVO) (Nettles-Anderson and Olsen, 2009; Sidibe et al., 2010). In addition, linolenic fatty acid influences both cold and drought adaptation (Kodama et al., 1994; McConn et al., 1994; Repellin et al., 1997; Triboi-Blondel and Renard, 1999; Murakami et al., 2000; Dyer et al., 2001; Izquierdo et al., 2002; Matsuda et al., 2005; Merrien et al., 2007) and is the source of omega-3 fatty acid desired for human food and animal feed.

The camelina production region ranges from eastern Washington to the southern high plains of Arizona, generally dry environments with a short growing season, with high daily temperatures and significant year-to-year variation. These environments vary greatly with regard to temperatures, altitudes, and soils, which would be expected to result in significant genotype by environment interactions (GEI) for seed yield, oil content, and fatty acid composition.

The goal of this study was to test diverse camelina varieties in a wide range of environments to determine the magnitude of environment, genotype and GEI effects on camelina yield, oil content, and linolenic fatty acid.

MATERIALS AND METHODS

Field trials

The study was conducted in four western U.S. states, Colorado, Wyoming, Montana and Washington, under irrigated and dryland conditions. Three trials were irrigated and the remaining trials were conducted under dryland or rainfed conditions.

Three sites had both irrigated and dryland treatments. In 2009 and 2010, a total of 12 trials were conducted. Elevation, latitude, irrigated or dryland trial conditions, planting and harvest dates, amount of irrigation, seasonal precipitation from April to July, seasonal evapotranspiration (ET), crop water deficit, seasonal days above 32°C, seasonal days below 5°C, and growing degree days (GDD) are reported for each environment (Table 3.1). Daily rainfall, temperature and evapotranspiration were taken from the closest Coagmet, AgriMet or USDA meteorological station to the trial. The growing season ASCE Standardized Penman reference evapotranspiration (ET) was measured from planting to harvest. The difference between P-ASCE ET and available water was defined as water deficit with runoff and winter ET considered as zero. Available water was calculated as total precipitation from the previous crop harvest to camelina harvest plus the amount of irrigation.

Heat and cold were calculated following the Baux et al. (2008) method. Heat was established by summing each day with maximum temperature above 32°C, and called days above 32°C. Cold days were measured by summing the number of days with minimum temperature below 5°C. Plot size varied by location from 8 to 10 square meters. Dates of planting conformed to acceptable dates for planting camelina for each trial region. The seeding rate was 6.5 kg ha^{-1}. Application of fertilizer varied from site to site based on yield potential and crop requirements.

Plant materials

Eight varieties from four sources were selected for testing on the basis of expected maturity. Suneson and Blaine Creek, both medium maturities, came from Montana State University. BSX-G74, BSX-G22 and Cheyenne, respectively late, early and medium maturity, came from Blue Sun Biodiesel Company in Colorado.

Celine and Ligena, respectively medium and late maturity, were of European origin. Yellowstone, a very early-maturing variety, came from Great Plains Oil and Exploration in Ohio.

Collected data

Seed yields were calculated in all locations except Bozeman, MT, with the yield adjusted to 8.5% seed moisture content. The Bozeman, MT, trial, the 2009 Yellow Jacket, CO, irrigated trial and the 2010 Pullman, WA, trial suffered from pre-harvest pod shattering and weed infestations, which reduced yield. Seed samples were taken for oil content and oil profile analyses. Samples were oven-dried to approximately 8% seed moisture at 50°C. Fatty acid profile analyses were carried out by gas chromatography mass spectrometry (GC-MS). Following the procedure outlined by Hammond (1991), oil content was determined on single 12-gram samples using a Newport MKIIIA Nuclear Magnetic Resonance (NMR) Analyzer (Oxford Instruments Inc., Concord, MA). The NMR was calibrated with a single reference sample of known oil content and the sample analysis carried out as described by Howard and Daun (1991). This procedure, and that of the Canadian Grain Commission, is based on the International Organization for Standardization procedure ISO 5511-1984 (Oilseeds - Determination of oil content - Low resolution nuclear magnetic resonance spectrometric method).

Experimental design and statistical analysis

The experimental design was a randomized complete block design (RCBD) of eight varieties with three replicates in 12 environments. All statistical analyses were performed with the R statistical software (The R Development Core Team, 2008) using the Agricolae program packages (De Mendiburu, 2009) for Additive Main

Effects and Multiplicative Interaction (AMMI) analysis. Error variances were not heterogeneous across environments as measured by Bartlett's (1937) test. Then, R software was used to compute the ANOVA using the Additive Main Effects and Multiplicative Interaction (AMMI) statistical model. Replicates, environments, and GEI were considered as random variables while varieties were considered as a fixed effect. The genotype by environment data analysis was done using AMMI analysis according to the following model equation (Gauch, 1992):

$$Y_{ger} = \mu + \alpha_g + \beta_e + \Sigma_n \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

where, Y_{ger} is the phenotype of the genotype (g) in environment (e) for replicate (r); μ is the additive parameter, grand mean; α_g is the additive parameter, or genotype deviation from the grand mean; β_e is the additive parameter, or environment deviation from the grand mean; λ_n is the multiplicative parameter, or singular value for PCA axis n; n is the number of PCA axes retained in the model; γ_{gn} is the multiplicative parameter, or genotype eigenvector for PCA axis n; δ_{en} is the multiplicative parameter, or environment eigenvector for PCA axis n; ρ_{ge} is non-additive residual remaining if not all PCA axes are used; and ε_{ger} is the error term.

Since, environment was of great interest in this study, AMMI1 analysis was a better choice than a genotype and genotype-by-environment (GGE) biplot, which does not include Environment as a separate effect (Gauch et al., 2008). Because Interaction Principal Component 1 captured most of the variability, AMMI1 biplot was used.

Pearson's correlation coefficients among different traits and environmental variables were calculated from the LSMEAN values of each environment generated by PROC CORR of SAS, version 9.2 (SAS Institute, 2008).

RESULTS

The combined ANOVA for yield, oil content (OC), and six FAs (means given only for linolenic FA) of eight varieties over 12 environments revealed highly significant interactions between environments and varieties for all traits except total saturated fatty acid (Table 3.2). Environments were a major source of variation and Principal Component Analyses (PCA) of three environmental variables (water deficit, number of days above 32°C, and number of days below 5°C) suggested two clusters of trial environments with potential biological significance (Fig. 3.1). PC1 captured mainly water deficit (Table 3.4). AMMI analysis was used to create two mega-environments (ME1 and ME2) without significant GEI for yield, oil content, and linolenic FA content (Fig. 3.2 A, B, C). The vertical dotted line in each figure indicates the grand mean for the trait. Varieties and environments to the right of the dotted line have trait levels above average. Any variety with an IPC1 (absolute) value close to zero shows a general adaptation to the tested environments. A variety with a large IPC1 score reflects more specific adaptation to environments with IPC1 scores of the same sign.

The data for yield of eight varieties over 11 environments and for OC and FA composition of eight varieties over 12 environments are shown in Table 3.2. Environment mean yield over eight varieties in 11 environments ranged from 572 kg ha^{-1} in a dryland treatment at Fort Collins, CO, to 3407 kg ha^{-1} in a dryland treatment at Pullman, WA. Environment mean OC of eight varieties over 12 environments ranged from 30.8% in a dryland treatment in Fort Collins, CO, to 39.1% in a dryland treatment in Pullman, WA. Mean linolenic fatty acid content (percent of oil content) varied from 26.6% in two dryland treatments at Pullman, WA, to 37.2% in a dryland

treatment at Bozeman, MT. Variety mean yield over 11 environments ranged from 1272 kg ha^{-1} for Yellowstone to 1620 kg ha^{-1} for Celine. Variety mean OC over 12 environments ranged from 32.5% for BSX G-74 to 33.9% for BSX G-22. Variety mean linolenic fatty acid content over 12 environments ranged from 29.5% for BSX G-22 to 33.7% for Yellowstone. In Yellow Jacket and Fort Collins, CO, trials were conducted under both irrigated and dryland conditions. Linolenic content was 17% higher under irrigation (Fig. 3.3). ME1 and ME2 include different environments for yield, OC, and linolenic oil content (see notes below Table 3.2).

Several camelina variables were correlated with environmental variables (Table 3.4). Yield, OC, and linolenic acid content were negatively correlated to water deficit. Yield, OC, and linolenic oil content were all significantly and positively correlated to one another and to latitude, increasing in value from south to north in the study area.

In ME1, the low-yielding mega-environment including dryland Fort Collins, CO, Iliff, CO, 2010, and dryland Yellow Jacket, CO, the varieties Celine, Ligena, and BSX G-22 were significantly higher yielding. Celine was highest yielding in ME2, which included Pullman, WA, Torrington, WY, and Iliff, CO, 2009 (Table 3.3). BSX G-22, Ligena, Yellowstone, and Blaine Creek had significantly higher oil content in ME1 (with Iliff, Fort Collins dryland, and 2009 dryland Yellow Jacket) and there was no significant difference among varieties in ME2 (Table 3.3). For linolenic FA content, the two varieties Ligena and BSX G-22 were significantly lower yielding in both ME1 and ME2 (Table 3.3). Ligena, the latest-maturing variety, had the lowest linolenic acid content over environments, 29.7%, whereas Yellowstone, the earliest variety, had the highest, 33.7% (Table 3.3).

DISCUSSION

The effects of genotype, environment, and GEI were significant in this study. Environments were the largest source of variation for all measured traits due to the wide range in environmental variables in trial locations from Washington to Colorado. There were only relatively minor differences among genotypes as the camelina improvement programs providing the varieties draw, for the most part, from similar parental material.

Two mega-environments were determined for yield, OC, and linolenic oil content from PCA and AMMI analysis. For yield, a northern mega-environment included sites from southern Wyoming to northeast Washington and a second, central/south, mega-environment extended from northeast Colorado to southwest Colorado. The main environmental variables separating these two mega-environments were water deficit and temperature. Due to year-to-year variation, trials at the same location in two different years might be in different mega-environments. GEI was not significant within mega-environments, which were delineated by the PCA and AMMI analyses.

Celine and Ligena were high-yielding varieties in both mega-environments, showing wide adaptability. Within the lower-yielding mega-environment characterized by higher temperature and higher water deficit, the two early varieties, BSX G-22 and Yellowstone, ranked first and seventh, showing that early maturity, per se, does not confer adaptability to stressful environments although early-maturing varieties can avoid some heat and drought stress. Earlier flowering has been shown to favor higher yield in dry regions because plants are the most susceptible to water and heat stress at the reproduction stage (Siddique et al., 1990).

Considerably higher oil content was observed in the cooler and wetter northern mega-environment including a high-elevation trial location at Torrington, WY. Similar results were found in *Brassica napus*: the observed oil content and seed yield of *B. napus* were positively correlated to higher rainfall and lower temperature during seed development (Si et al., 2003; Pritchard et al., 2000).

GEI for oil content was successfully minimized within oil content mega-environments. OC was significantly higher in the early maturing varieties in ME1, the hotter and drier ME, and there was no significant difference in OC among varieties within ME2.

Linolenic FA was studied in more detail than other fatty acids because it seems to have an adaptive advantage over other unsaturated and saturated FA. When linolenic FA content increases, the oil melting point decreases, enabling active plant metabolism in colder environments. Higher levels of linolenic FA can hasten germination, which will improve overall fitness and stand establishment under harsh environments (Enjalbert et al., unpublished; Weiner, 1985).

Like OC, significantly higher linolenic FA accumulation was observed in ME2, with cool and wet conditions. The linolenic FA enzyme, fatty acid desaturase three (FAD3), is down-regulated when temperatures increase (Baux et al., 2008). A strong positive correlation was observed between linolenic FA and latitude. Linder (2000) demonstrated that the higher the latitude and/or elevation, the more linolenic FA in the seed and biomass.

In addition to the effects of heat stress, results have shown that water stress can reduce linolenic FA content by up to 17% compared to a well-watered trial. Similar results have been demonstrated in other *Brassica* species (Canvin, 1965;

Williams et al., 1992). The 2010 Pullman trial suffered from a weed infestation and lack of nitrogen that resulted in lower linolenic content than the 2009 trial. Linolenic content appears to be influenced by multiple plant stresses. Linolenic FA has been shown to play a role in cell survival and is a precursor of jasmonic FA, a potent lipid signal molecule essential for defense (Mene-Saffrane et al., 2009). Rucker and Robbelen (1996) indicated that, under certain conditions, high linolenic FA content could be favorable for seed yield.

The average linolenic FA content among varieties ranged from 29.8 to 33.7% over all locations which appears to be less than the genetic variation of linolenic FA content in other Brassica species (Schierholt and Becker, 2001; Aslam et al., 2009; Bachlava et al., 2008). Yellowstone, the earliest-maturing variety, was observed to have the highest level of linolenic fatty acid whereas Ligena, the latest-maturing variety, had the lowest level of linolenic FA content in both ME1 and ME2. By flowering earlier than other varieties, Yellowstone potentially avoided some high temperatures and enjoyed cooler temperatures during its seed-filling period, which occurred before the seed-filling stage in the other varieties. However, as noted earlier, the late-maturing BSX G-74 also ranked high for linolenic acid content and the early-maturing BSX G-22 ranked low for linolenic acid content, so maturity date was not the only factor involved.

Correlations among environmental and response variables paint a clear picture of inter-relationships. Water deficit (crop water – ET) integrates both water availability and temperature. Yield, OC, and linolenic FA content are all significantly and negatively related to water deficit. Based on the results of this study, one would

expect camelina grown at northern (low ET), higher elevation (low ET), and irrigated (high crop water) locations to result in higher yield, OC, and linolenic FA content.

CONCLUSION

The study demonstrated that oil quality and quantity are highly dependent on the environment. Northern and/or cooler environments produced higher seed yields, as well as elevated oil and linolenic FA contents. AMMI analysis outlined mega-environments as well as the best-adapted and stable varieties for these mega-environments. Higher linolenic FA content was observed in cooler environments that did not experience water, weed, pest and nutrient-related stresses. Food and feed marketers, looking for high omega-3, could target low-stress environments due to the high levels of linolenic FA expected in these areas. For use of camelina SVO as fuel, published studies are not in agreement in terms of the impact of different oil profiles on engine emissions and engine durability. Older studies suggested increased levels of NOx emissions and engine build-up when using fuels with higher linolenic FA content. Newer studies, in more advanced direct injection engines, show no difference, or fewer emissions, with high linolenic FA content fuel. It is known that higher linolenic FA content results in lower fuel viscosity at lower temperatures, which would presumably be advantageous in colder regions. Linolenic FA content could play an important role in plant adaptation. We hypothesize that under stressed environments, higher FA linolenic content could improve camelina fitness and improve cold and drought tolerance while development of low linolenic germplasm, currently in demand for better oxidative stability, could lead to lower yields.

Table 3.1: Years, locations, and environmental variables of the multi-environment trial.

Year	Site	Environment code	Elevation	Latitude	Irrigated/Dryland	Planting	Harvest	Amount of irrigation	Seasonal precipitation	Evapotranspiration	Crop water deficit	Heat = Days >32C	Cold = Days< 5C
			meters	degree		date	date	mm	mm	mm	mm	day	day
2009	Bozeman MT	BnMT09	1509	45.68	Rainfed	5/3/09	8/15/09	.	431	625	194	2	58
2009	Pullman WA	PnWA09	717	46.73	Rainfed	5/8/09	8/5/09	.	599	577	-22	2	36
2009	Illif CO	IfCO09	1165	40.77	Dryland	4/5/09	7/21/09	.	529	643	114	14	5
2009	Torrington WY	TnWY09	1251	42.06	Rainfed	3/19/09	8/5/09	112	381	610	117	17	60
2009	Yellow Jacket CO	YtCO09D	2103	37.53	Dryland	5/17/09	8/12/09	.	230	584	354	6	6
2009	Yellow Jacket CO	YtCO09I	2103	37.53	Irrigated	5/11/09	8/27/09	407	230	711	74	6	6
2010	Pullman WA	PnWA10	717	46.73	Rainfed	3/16/10	7/26/10	.	449	497	48	4	59
2010	Ft Collins CO	FsCO10D	1557	40.65	Dryland	4/22/10	7/28/10	.	245	673	428	9	17
2010	Ft Collins CO	FsCO10I	1557	40.65	Irrigated	4/22/10	7/30/10	225	245	685	216	9	17
2010	Illif CO	IfCO10I	1165	40.77	Irrigated	4/9/10	7/14/10	42	295	662	325	28	33
2010	Illif CO	IfCO10D	1165	40.77	Dryland	4/9/10	7/14/10	.	295	662	367	28	33
2010	Yellow Jacket CO	YtCO10D	2103	37.53	Dryland	4/16/10	8/18/10	.	298	812	514	5	35

Table 3.2: ANOVA of yield, oil content, and FA content across environments and mega-environments.

			Fatty Acids				
Traits	Seed Yield	Oil content	C18:1	C18:2	C18:3	C20:1	C22:1
Overall	kg h^{-1}	%	%	%	%	%	%
Environment	***	***	***	***	***	***	***
Variety	***	***	***	***	***	***	***
GxE	***	*	*	**	**	*	***
CV, %	15.7	3.359	4.8	5.8	3.7	4.01	6.94
Mean	1402	33.4	17.55	19.9	31.4	14.01	2.99
IPC1, %	73.9	44.9	44.6	46.1	49.6	54.1	43.7
IPC2, %	13	21.9	21.3	30.2	26.9	21.6	24.8
Mega-Environment one							
Environment	**	*	*	**	ns	ns	ns
Variety	***	***	***	***	***	***	***
GxE	ns	ns	*	*	ns	ns	ns
CV, %	17.47	2.6	1.85	3.64	2.54	2.32	6.73
Mean	842	31.8	19.2	21.1	28.9	13.5	2.8
Mega-Environment two							
Environment	***	***	***	***	***	***	***
Variety	***	ns	***	***	***	***	***
GxE	ns	ns	ns	**	ns	ns	**
CV, %	9.1	3.05	6.29	4.48	2.79	3.64	6.73
Mean	2369	34.9	16.0	17.8	35.0	14.4	3.2

· Significant at the 0.1 probability level.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

Table 3.3: Variety mean and rank for yield, oil content and linolenic fatty acid content across environments and two mega-environments (ME1 and ME2).

Variety	FsCO10D	FsCO10I	BnMT09D	IfCO09D	IfCO10D	IfCO10I	PnWA09D	PnWA10D	TnWY09I	YtCO10D	YtCO09D	YtCO09I	Overall	ME1	ME2
								Yield							
								kg ha^{-1}							
Celine	630	1660	.	1910	1100	1383	3875	1704	2479	827	635	.	1620	915	2755
Ligena	599	1890	.	1754	1212	1393	3574	1527	2187	873	473	.	1548	910	2505
BSX G-22	867	1526	.	1478	1242	1356	3122	1663	1949	580	693	.	1448	948	2183
BlaineCreek	628	1444	.	1603	1085	1391	3495	1711	1902	762	551	.	1457	883	2333
Suneson	534	1411	.	1826	1118	1178	3680	1420	2081	459	685	.	1439	795	2529
Yellowstone	453	1729	.	1444	1018	1129	2668	1300	1728	696	557	.	1272	770	1947
Cheyenne	417	1286	.	1685	1070	1284	3350	1419	1819	638	495	.	1346	781	2285
BSX G-74	446	1236	.	1689	1015	1141	3493	1505	2070	594	491	.	1368	737	2417
Mean	572	1523	.	1674	1107	1282	3407	1531	2027	679	573		1437	842	2369
$LSD_{0.05}$	ns	444		ns	ns	ns	387	318	363	243	ns		282	114	204
CV, %	34	17		12.9	11.6	11.4	6.5	11.8	10.2	20.5	20.2		38.6	18.7	9.1
								Oil Content							
								%							
BSX G-22	31.3	31.3	34.3	32.7	32.2	33.3	33.7	39.0	34.9	35.9	32.4	35.7	33.9	32.3	35.5
Ligena	31.6	33.3	35.2	33.6	31.7	33.0	34.0	39.2	35.2	32.1	32.1	34.6	33.8	32.1	35.7
Suneson	30.7	31.8	36.1	33.3	31.1	32.0	34.5	39.4	35.5	33.8	31.5	34.1	33.6	31.3	35.9
Yellowstone	30.5	31.1	35.4	32.8	32.1	32.6	33.5	38.7	34.3	32.5	32.7	34.2	33.4	31.7	35.2
Cheyenne	30.3	30.9	35.6	33.5	31.0	32.2	33.7	39.1	34.5	32.7	32.1	34.8	33.4	31.2	35.5
BlaineCreek	30.5	32.9	35.7	31.9	31.8	32.8	33.8	39.9	34.4	32.3	31.7	32.3	33.3	31.7	35.2
Celine	31.3	31.0	32.7	32.2	30.0	32.6	34.3	39.2	35.5	33.1	31.1	34.8	33.2	31.3	35.3
BSX G-74	30.4	31.2	35.3	31.6	29.6	31.0	33.8	38.0	34.8	31.6	30.3	32.3	32.5	30.3	34.8
Mean	30.8	31.7	35.0	32.7	31.2	32.4	33.9	39.1	34.9	33.0	31.7	34.1	33.4	31.5	35.4
$LSD_{0.05}$	ns	ns	3.9	ns	1.1	0.6	0.5	0.5	ns	2.0	0.6	ns	0.8	0.7	ns
CV, %	3.5	2.6	6.3	3.8	2.1	1.1	0.8	0.7	1.2	3.4	1.1	6.4	5.1	2.9	6.4
								Linolenic (C18:3)							
								%							
Yellowstone	29.8	34.5	39.0	32.4	30.1	28.8	35.4	33.5	37.8	34.7	31.5	37.1	33.7	31.8	36.6
BSX G-74	32.2	33.7	38.5	31.4	27.2	27.8	35.0	30.4	36.4	30.5	29.7	35.1	32.3	29.5	35.1
Celine	30.6	33.7	37.0	30.8	26.7	27.4	34.1	31.4	36.4	31.3	29.0	35.0	32.0	29.4	34.8
Cheyenne	29.3	35.0	37.3	30.0	26.7	26.5	34.9	31.6	35.4	30.5	28.8	34.6	31.7	29.0	34.8
Suneson	29.3	34.5	37.2	30.3	25.6	25.8	34.6	31.0	35.7	31.1	28.5	34.2	31.3	28.7	34.5
BlaineCreek	28.1	33.3	37.4	29.7	26.6	26.8	33.8	32.1	35.2	29.9	27.9	34.3	31.3	28.8	34.5
Ligena	29.2	31.6	36.2	28.0	24.3	25.2	33.0	29.7	33.9	28.6	24.9	32.6	29.8	26.8	33.1
BSX G-22	26.2	32.4	35.2	27.6	25.0	24.6	32.3	29.4	33.4	29.1	25.7	32.0	29.5	26.9	32.5
Mean	29.3	33.6	37.2	30.0	26.6	26.6	34.1	31.1	35.5	30.7	28.3	34.4	31.5	28.9	34.5

$LSD_{0.05}$	3.5	2.6	1.3	1.5	1.0	1.1	0.6	0.8	1.1	2.1	0.9	0.7	0.6	0.7	0.7
CV, %	6.8	4.4	1.9	2.7	2.1	2.3	1.1	1.4	1.8	3.8	4.8	2.9	4.0	2.8	2.6

† Seed Yield ME1: YtCO09D, YtCO10D, FsCO10D, IfCO10D, IfCO10I; Seed yield ME2: PnWA10D, IfCO09D, TnWY09I, PnWA09D; FsCO10I and PnWA10D not in either ME.

Oil Content ME1: YtCO09D, YtCO10D, FsCO10D, IfCO10D, IfCO10I, IfCO09D; Oil Content ME2: PnWA10D, BnMT09D, TnWY09I, PnWA09D; FsCO10I and YtCO09I not in either ME.

Linolenic ME1: YtCO09D, YtCO10D, IfCO10D, IfCO10I, IfCO09D, PnWA10D; Linolenic ME2: BnMT09D, TnWY09I, PnWA09D, YtCO09I, FsCO10I; FsCO10D not in either ME.

Table 3.4: Pearson correlation of the measured traits and environmental variables;

	PC1	Latitude	Water deficit	Days>32C	Days<5C	Yield	OC	Oleic	Linoleic	Linolenic	Eicosenoic
Latitude	-0.60 * 12										
Water deficit	1.00 *** 12	-0.60 * 12									
Days>32C	0.29 ns 12	-0.23 ns 12	0.29 ns 12								
Days<5C	-0.21 ns 12	0.69 * 12	-0.20 ns 12	-0.02 ns 12							
Yield	-0.77 ** 11	0.75 ** 11	-0.77 ** 11	-0.12 ns 11	0.41 ns 11						
OC	-0.63 * 12	0.61 * 12	-0.63 * 12	-0.38 ns 12	0.66 * 12	0.37 ** 88					
Oleic	0.49 . 12	-0.14 ns 12	0.49 . 12	0.37 ns 12	-0.09 ns 12	-0.40 *** 88	-0.12 ns 96				
Linoleic	0.58 * 12	-0.39 ns 12	0.58 * 12	0.64 * 12	-0.23 ns 12	-0.39 ** 88	-0.27 ** 96	0.49 *** 96			
Linolenic	-0.58 * 12	0.40 ns 12	-0.58 * 12	-0.61 * 12	0.37 ns 12	0.46 *** 88	0.37 ** 96	-0.71 *** 96	-0.82 *** 96		
Eicosenoic	-0.35 ns 12	0.18 ns 12	-0.35 ns 12	-0.05 ns 12	-0.03 ns 12	0.37 ** 88	0.09 ns 96	-0.34 ** 96	-0.45 *** 96	0.08 ns 96	
Erucic	0.03 ns 12	-0.26 ns 12	0.03 ns 12	0.21 ns 12	-0.45 ns 12	0.16 ns 88	-0.45 *** 96	-0.43 *** 96	-0.26 ** 96	-0.01 ns 96	0.75 *** 96

· Significant at the 0.1 probability level.

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

***Significant at the 0.001 probability level.

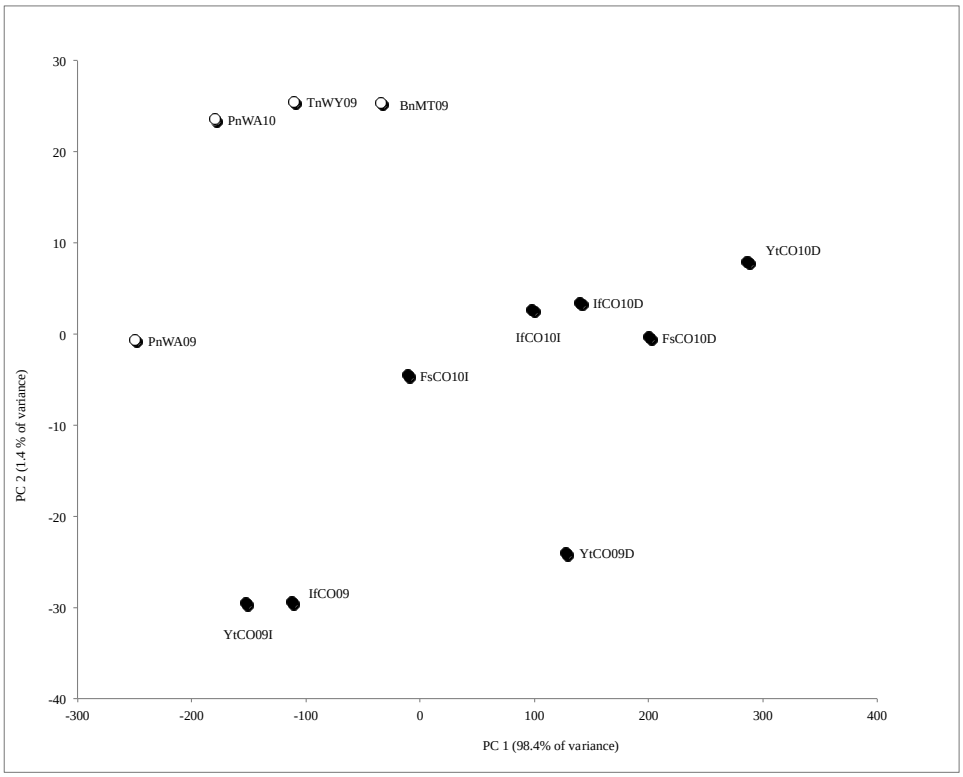

Figure 3.1: Biplot of the environmental variables: water and temperature, based on 12 environments; darkened circles are the central mega-environment, empty circle are the northern region. PC = principal component.

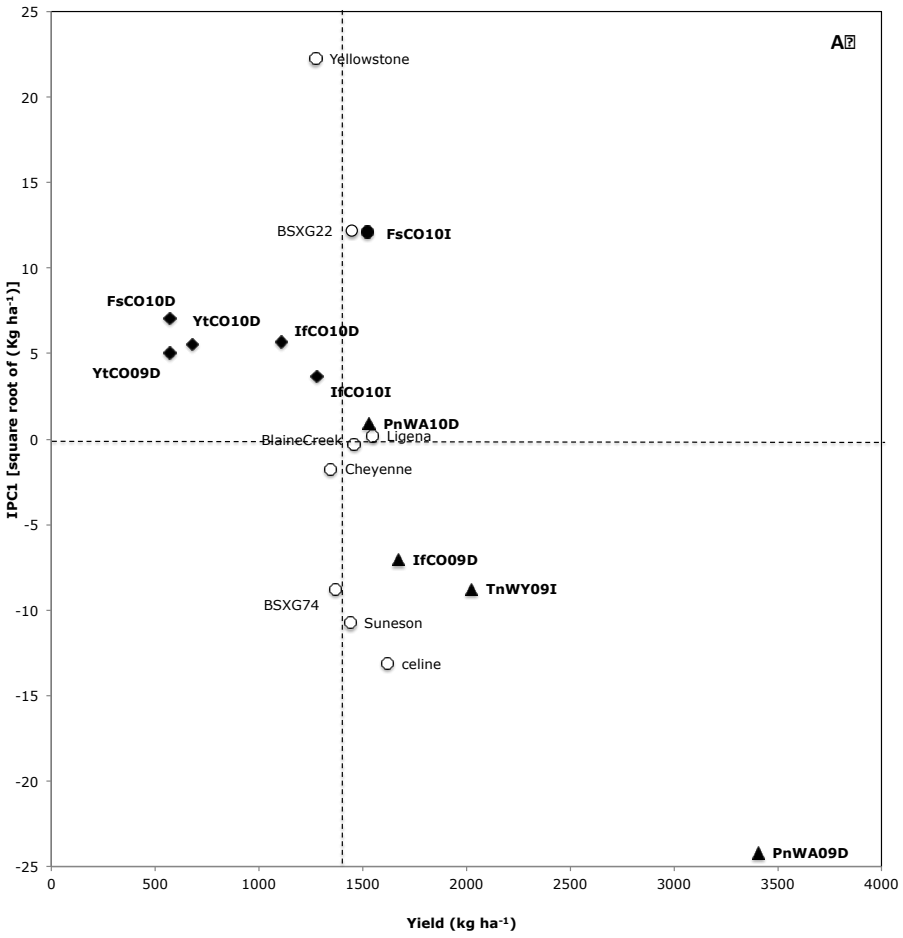

Figure 3.2A: AMMI1 biplot based on data of eight camelina varieties (empty symbols) across 12 environments (solid symbols) for yield with vertical line showing grand mean of 1402 kg ha^{-1}. The main additive effects (average trait score for varieties and environments) are on the x-axis and IPC1 scores on the y-axis. Varieties and environments, on the right side of the biplot, show trait levels above average. Any variety with an IPC1 (absolute) value close to zero shows general adaptation to the tested environments. A variety with large IPC1 score reflects more specific adaptation to environments with varieties having IPC1 scores of the same sign.

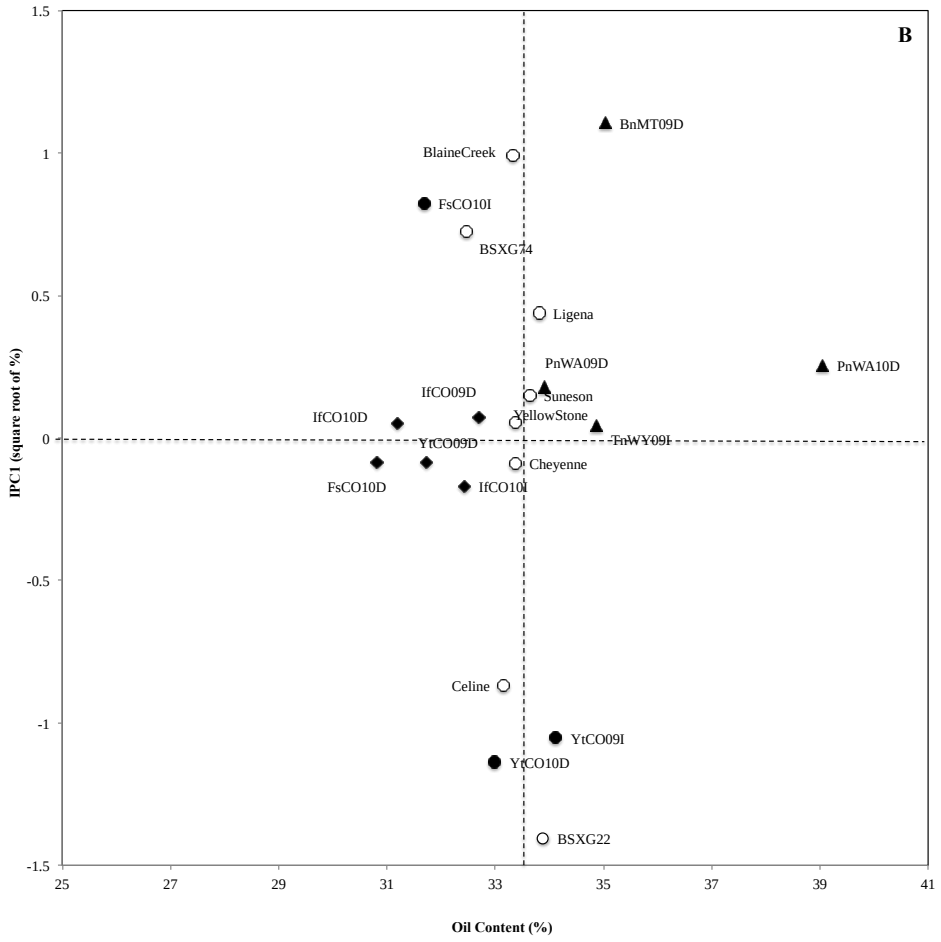

Figure 3.2B: AMMI1 biplot based on data of eight camelina varieties (empty symbols) across 12 environments (solid symbols) for oil content with grand mean of 33.4%. The main additive effects (average trait score for varieties and environments) are on the x-axis and IPC1 scores on the y-axis. Varieties and environments, on the right side of the biplot, show trait levels above average. Any variety with an IPC1 (absolute) value close to zero shows general adaptation to the tested environments. A variety with large IPC1 score reflects more specific adaptation to environments with varieties having IPC1 scores of the same sign.

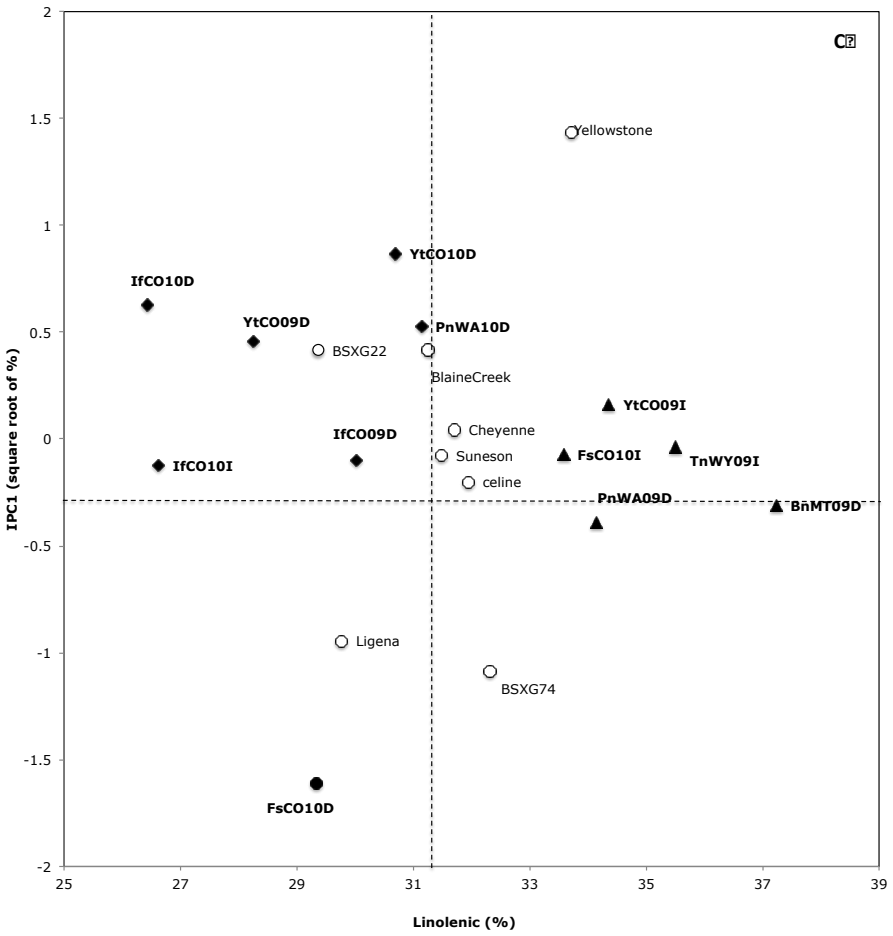

Figure 3.2C: AMMI1 biplot based on data of eight camelina varieties (empty symbols) across 12 environments (solid symbols) for linolenic fatty acid content with grand mean of 31.4%. The main additive effects (average trait score for varieties and environments) are on the x-axis and IPC1 scores on the y-axis. Varieties and environments, on the right side of the biplot, show trait levels above average. Any variety with an IPC1 (absolute) value close to zero shows general adaptation to the tested environments. A variety with large IPC1 score reflects more specific adaptation to environments with varieties having IPC1 scores of the same sign.

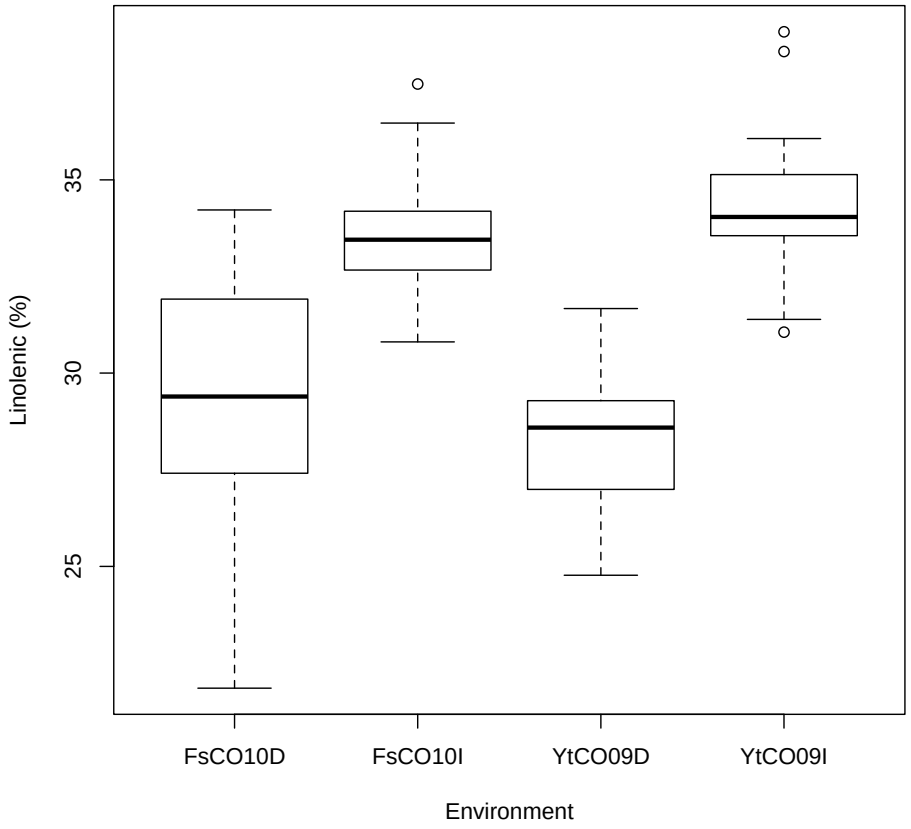

Figure 3.3: Boxplot of the linolenic content between irrigated (I) and dry (D) treatment at Fort Collins, Colorado (FsCO10), and Yellow Jacket, Colorado (YtCO09).

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CHAPTER FOUR:

GUIDE FOR PRODUCING DRYLAND CAMELINA IN EASTERN COLORADO

AUTHORSHIP

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Camelina is an annual crop with small seed and it has been cultivated in Europe for more than a thousand years. Eastern Colorado's wheat-based, cropping system covers more than 4 million acres and spring-planted camelina would fit well into a dryland crop rotation. Camelina is a short, relatively shallow-rooted, short-season, crop requiring 85 to 100 days from emergence to maturity. The seed contains approximately 30 – 35% oil and can produce 40 or more gallons per acre of clean vegetable oil and potentially 900 lb/ac of high-protein animal meal.

POTENTIAL BENEFITS OF GROWING DRYLAND SPRING CAMELINA IN EASTERN COLORADO

- Camelina production requires the same equipment as wheat.
- Typical seeding rates are 5 to 7 lb/ac and seed cost is approximately $2/lb.
- Camelina requires little fertilization. See *Fertilization* below.
- Unlike spring canola and Indian brown mustard, camelina is tolerant of flea beetles and other insects.
- Camelina has been relatively disease-free in eastern Colorado.
- Camelina branches easily, fills in blank spaces, and is more drought and heat-tolerant than other brassica crops.
- Until its pods ripen, camelina is less susceptible to hail damage than most other crops grown in eastern Colorado.
- Camelina can be direct-harvested given its pods mature quite uniformly on the plant.
- Camelina meal contains approximately 40% protein, is high in Omega-3 fatty acid content, and low in erucic acid content and glucosinolates.

POTENTIAL PROBLEMS FOR GROWING DRYLAND SPRING CAMELINA IN EASTERN COLORADO

- The primary production challenges are stand establishment and weed control.
- An effective weed control method is to plant camelina in late winter/early spring into a clean field. Camelina can be planted from the end of February to the first week of April in eastern Colorado
- Camelina is sensitive to damage from sulfonylurea (SU) herbicide residuals such as Ally, Amber, and triazine herbicides.
- Shallow (1/4 in.) planting is critical to good stand establishment and good seed-to-soil contact.
- Weed control in open areas is more of a threat than lower yields due to poor stands.
- Camelina seed yields have been variable in eastern Colorado, ranging from 300 to 1600 lb/ac in experimental plots. Crop yields vary but camelina yields have been more variable than what we have observed in many other crops in eastern Colorado and the reasons for this yield variation of camelina has not been identified.

- Reliable markets for camelina need to be developed.

ROTATION

Following summer crops (corn, sunflower, sorghum, or proso millet), camelina can be planted early. Harvested from mid-to-late July, the soil can store moisture from late July to mid-September before planting winter wheat. When moisture allows, it's possible to harvest three crops in three years instead of two crops in three years. Camelina would

replace fallow, provide an energy crop, and not compete with a critical food crop. Insufficient precipitation after camelina harvest and before wheat planting could reduce subsequent wheat yields. Camelina crop residue decomposes readily and is not expected to be a problem for tilled or no-till wheat planting.

FIELD SELECTION

Some residual (carry over in the soil) herbicides from preceding crops are important to avoid. Camelina is damaged by the residual sulfonylurea (SU) herbicides below.

Herbicide (active ingredient)	Weeds controlled	Labeled Crop	Residuals for camelina
Ally/ Escort (Metsulfuron)	Broadleaf weeds	Wheat/Barley and CRP land	>24 months
Amber (Triasulfuron)	Broadleaf weeds	Winter and spring wheat	>24 months
Express (Tribenuron)	Annual broadleaf weeds	Wheat/Barley	>24 months
Atrazine (triazine)	Broadleaf weeds	Corn/Fallow	>18 months

In areas with a soil pH higher than 7.0 and with prolonged periods of low soil temperature and low annual rainfall, the SU herbicides can remain active in the soil for 2-4 years or more and they can injure non-tolerant rotational crops such as camelina.

Camelina can be grown under tilled or no-till dryland conditions as long as there is no residual SU or triazine herbicides remaining in the soil, that good seed-to-moist soil contact must be obtained at planting, and an effective weed control in camelina can be difficult due to lack of registered products. Excessive crop residue can reduce emergence so seeding rates might need to be increased.

VARIETY SELECTION

Camelina variety selection is highly dependent on seed availability. This spring (2011), the only regionally available camelina variety is *Pronghorn*. It is an early-maturing variety bred and released by Charlie Rife (High Plains Crop Development, LLC) in Torrington, WY. Colorado State University has conducted camelina variety trials for multiple years at Akron, Iliff, and Fort Collins under dryland and irrigated conditions. Camelina varieties Ligena, Celine, Cheyenne, and Blaine Creek have performed well but seed of these varieties would have to be found from outside the region.

Pronghorn – Early maturity, will be in Colorado yield trials for first time in 2011.

Cheyenne – Medium to late maturity, stable yield and 32.4% oil content in CSU trials (Johnson and Enjalbert, 2011)

Ligena – European variety, late maturity, tall, high yielding, and 31.8% oil content in CSU trials.

Celine – European variety, medium maturity, highest yielding variety, 32.4% oil content in CSU trials.

Blaine Creek – Medium maturity, average yield, and 32.3% oil content in CSU trials.

CAMELINA BREEDING PROGRAMS, VARIETIES, AND CONTACT INFORMATION

High Plains Crop Development, LLC. Varieties: *Cheyenne* and *Pronghorn*. Breeder: Charlie Rife. Location: Torrington WY. Phone: 307-575-4580. Price: 2$/lb

Montana State University. Varieties: *Blaine Creek*, *Sunesson* and *Celine*. Breeder Biobased Products Institute. Site: www.montana.edu/biobased/

Sustainable Oils (Target Growth, Inc.). Varieties: SO-10, SO-20 and SO-30. Contact: Mike Waring

Phone: 406 788 2433, email: Mike.Waring@susoils.com: www.susoil.com

Great Plains Oil. Variety: Yellow Stone. Contact: Great Plains Oil & Exploration, LLC. 1 Enfield Street, Cincinnati, OH 45218. Site: www.camelinacompany.com

PLANTING CONSIDERATIONS

Camelina is cold-tolerant and can be planted from late February to mid-April in eastern Colorado without too much risk of experiencing freeze damage. Early planting can help to control weeds. Planting into moisture is critical. Camelina seed will germinate and emerge at soil temperatures at or above 36°F. Early planting leads to early emergence, flowering, and maturity which improves the chances of escaping high temperatures during pollination. Planting after April 15 in eastern Colorado may subject the crop to high temperatures during flowering, which will likely reduce yields. Planting early, in

cool temperatures, reduces the risk of drying out the soil surface and reducing emergence. Plant camelina ¼-inch deep and use the press-wheels on the planter to ensure good seed-to-soil contact. Camelina can be planted with a wheat drill, or broadcast and then pressed into moist soil. Seed can also be broadcast with fertilizer. Some producers may prefer to plant camelina seed with an alfalfa seed attachment on the wheat drill or plant with an alfalfa seeder. Camelina should be planted at 5–7 lb/ac. There are about 500,000 camelina seed/ lb. Seeding rate should be increased to 7 lb/ac if soil moisture in not optimal, if the field has a history of crusting, or if there is excessive crop residue.

WEED CONTROL

The best weed control approach is to plant early into a field that was relatively weed-free. Early planting and good stand establishment, allows camelina to compete well with weeds so herbicide applications may not be necessary. Emerged weeds can be controlled with glyphosate applied immediately before planting camelina. Currently, Poast® (a BASF post-emergence grass control product) is the only herbicide labeled for camelina and is a grass herbicicde. Therefore, no broadleaf weed control is available in crop.

FERTILIZATION

Soil sampling is always recommended. Soil nitrogen and organic matter content and expected yield determine how much nitrogen should be applied. Assuming a common residual nitrogen level in a dryland wheat based cropping system and a camelina seed yield target of 1200 lb/ac, 20-40 lb N/ac may need to be incorporated prior to planting.

Camelina has not been shown to respond to phosphorus, potassium, or sulfur applications at current yields in our cropping systems.

HARVEST CONSIDERATIONS

- **Timing:** Camelina should be harvested when the pods turn yellow brown. The lower stems can still be light green but the upper stems should be yellow. Optimum seed moisture for storing camelina is 8%. Seed moisture contents higher than 8.5% may increase the rise of spoilage.
- **Direct harvest or Swathing:** Pod shattering is less with camelina than in canola. Camelina is typically direct harvested. However, it can be swathed to limit possible pod shattering. Oilseeds can be harvested at night to limit shattering.
- **Combine settings:** Set combine settings initially for canola and adjust accordingly. When direct combining camelina the header height should be set as high as possible and open the concave to avoid plugging the combine with moist stems. Producers have reported that a 9/64 in. screen installed over the lower sieves produces good separation of the seed from pod and stem pieces. Due to the small seed size, **reduce the airflow** to minimize seed loss. Transportation and storage equipment as well as the combine should be checked for leaks and sealed with tape or caulking as necessary.
- **Residue management:** Camelina residue contains high levels of glucosinolates, which can be a nematicide and be beneficial to a subsequent wheat crop.

MARKETING CAMELINA

A reliable market for camelina should be identified prior to planting. This fact sheet targets on-farm production of camelina as a petroleum fuel substitute and on-farm use of camelina meal. Mechanically extracted camelina meal contains approximately 13% fat and 40% protein, making it similar to other oilseed meals. The quantities of glucosinolates and other anti-nutritional factors are relatively low in camelina meal. (Böhme, and Flachowsky, 2005). The Food and Drug Administration (FDA) has approved camelina meal use in cattle feed at a level of 10% of the total ration. High omega 3 content in camelina meal indicates that it might be more beneficial than other oilseed meals for human and livestock health.

COLORADO CRUSHING FACILITIES:

Rocky Ford. Big Squeeze. Managers/Owners: Hal Holder (Phone: 719 241-1128) and Joel Lundquist. Contact: Dr. Perry E. Cabot. Extension Water Resources Specialist. Phone: (719) 549-2045.

Burlington (Stratton). Chuck Clapper. Phone: (719) 349-2183.

San Luis Valley (Costilla County). Ben Doon. Phone: (719) 672-0320. Email: doon@amigo.net

ADDITIONAL RESOURCES

Lafferty R., C. Rife, G. Foster. 2009. Spring Camelina Production Guide for the Central High Plains. Blue Sun Energy.

http://www.extsoilcrop.colostate.edu/CropVar/oilseeds.html

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CHAPTER FIVE:

MAKING YOUR OWN STRAIGHT VEGETABLE OIL (SVO) AS FUEL

AUTHORSHIP

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Farmers are suffering economically due to rising prices of diesel, fertilizers and transportation. Farmers are directly dependent on fossil fuels and a consolidated energy system. Producers need some way to dampen the effects of increased fuel costs. Growing fuel on the farm with alternative crops could provide a buffer to rising energy costs for entire rural communities.

Oilseeds can be a source of fuel for farms. Straight Vegetable Oil (SVO) is oil obtained from oilseed crops by simple cold pressure crushing of the seeds, and filtering without solvents or chemical modification. The development of SVO for use on the farm can provide efficient and environmentally attractive fuel alternatives for individual producers. It could contribute to community-based economic development, improve energy security for US rural communities and allow producers to be self-sufficient for on farm fuel consumption to reduce overall production costs.

This fact sheet provides advice on how to crush and use SVO for fuel. It describes oil quality, crushing, filtering, oil storage and engine modifications.

To produce and use SVO as a fuel, five points are involved:

1. Oilseed choice that fits rotation and fuel requirements
2. Cold pressing
3. Filtering
4. Oil and meal storage
5. SVO uses and engine modifications

SELECTING A VEGETABLE OIL TYPE

Oilseed crops selection in eastern Colorado is restricted to winter and spring canola, camelina, mustard and sunflower. They have different characteristics that are important to be aware of.

Research has shown that high oleic content improves oil stability and engine performance. However, polyunsaturated oils with high antioxidants level, such as camelina, have shown high stability (Crowley and Fröhlich, 1998) and low emissions. This question is being investigated at Colorado State University (CSU) at the Engines and Energy Conversion Lab (EECL). So far canola, camelina, sunflower, and mustard oils emissions fulfilled emissions EPA requirements for on farm uses with common-rail injection, tier 2 engine (Olsen et al, unpublished). In addition to oil profile, gums and waxes levels impact the SVO production and engine efficiency and durability. For best results, these factors should impact oilseed selection.

Highly saturated crops

Highly-saturated crops are not adapted to the High Plain climate. Palm oil, cotton oil, and coconut oil are the most common saturated oil in the global market. Cuphea is under investigation in Colorado. Its oil composition contains more than 80% short saturated fatty acids. At room temperature, saturated oils are solid. For example, palm oil becomes solid below 50°F. Due to heating requirements, and despite their low cost on the market, they are not a crop of choice for cold climates such as Colorado.

High oleic crops

Today, many oilseed species have been bred to have high oleic fatty acid content. Sunflower, canola, safflower, and some Indian Brown Mustard have oleic content above 50%. Those oils solidify below 25°F (Sanford et al., 2009).

High polyunsaturated crops

As a result of breeding, canola, safflower and sunflower have high-oleic cultivars. These species used to have high polyunsaturated fatty acid (PUFA) prior to breeding efforts. High-oleic varieties have been bred to satisfy high market demand for stable oil to replace hydrogenated oil. Camelina still has a high PUFA level (around 50%) due to a short history of breeding effort. High PUFA oils remain liquid above 20°F.

Common potential oilseed crops oil profile

Fatty Acid	Palmitic (C16:0)	Stearic (C18:0)	Total saturated	Oleic (C18:1)	Linoleic (18:2)	Linolenic (C18:3)	Total polyunsaturated
Unit	%	%	%	%	%	%	%
Soybean	5	2	10	65	18	2	22
Sunflower	5	2	8	75	8	2	12
Safflower	6	2.5	10	37	40	0.1	45
Canola	5	2	8.5	62	18	6	25
Camelina	6	2.5	11.5	19	20	30	51

Wax and gum crops

Gums, also called mucilage or phospholipids, come from the seed coats of oilseeds such as canola and camelina. The amount of gum in the oil depends on the crushing temperature and the type of seed. In the oil analysis, they are characterized by the phosphorus content. Wax is mainly encountered in sunflower. Waxes are mainly located

on the hull surface of sunflower seeds. Dehulling prior to crushing reduces the wax content (Dorrel and Vick, 1997). The wax content in the oil can change according to the seed variety, the percentage of hull removed, and the crushing temperature (Brevedan et al., 2000; Carelli et al., 2002).

Wax and gum origin and solution

Parameters	Wax	Gum
Definition	Impurity	Phospholipids
Origin	Mainly sunflower seed	Seed coat of canola and camelina
Solution	Filter at <56°F because wax crystallizes at low temperatures. However, viscosity increases at low temperatures and filtering becomes harder.	Crushing temperatures under 135°F so phospholipids remain in seed coat

High-oleic canola and sunflower varieties are preferred for engine performance and oil stability. When choosing camelina, no standard oil profile choice is available yet. Therefore, seed yield performance is the main selection criterion. In addition, camelina has shown the best performance in the dryland wheat based no-till cropping system, bringing a third crop in the rotation (Johnson and Enjalbert, 2011).

Potential dryland oil production per acre

Species		Seed yield	Oil Extracted*	Oil
		lbs/ac	%	gal/ac
Sunflower	Average	1200	35%	56
	Max	2200	40%	103
	Min	700	30%	33
Winter Canola	Average	1250	30%	58
	Max	2200	35%	103
	Min	700	25%	33
Camelina	Average	1200	25%	40
	Max	1800	30%	60
	Min	700	20%	23

*When mechanically crushed, 5 to 10% of the oil remains in the meal.

EXTRACTING AND PROCESSING VEGETABLE OIL

Multiple oil extraction systems, such as hexane, solid, and mechanical extraction, are used. For the purpose of local and small-scale production, only the mechanically cold pressed extraction will be addressed due to economic feasibility and environmental considerations. The cold press is best adapted for small-scale production and produces a vegetable oil quality that can be used as fuel. The SVO production process is composed of the seed storage and cleaning, the actual crushing, two filtering stages, and SVO and meal storage (Fig. 1).

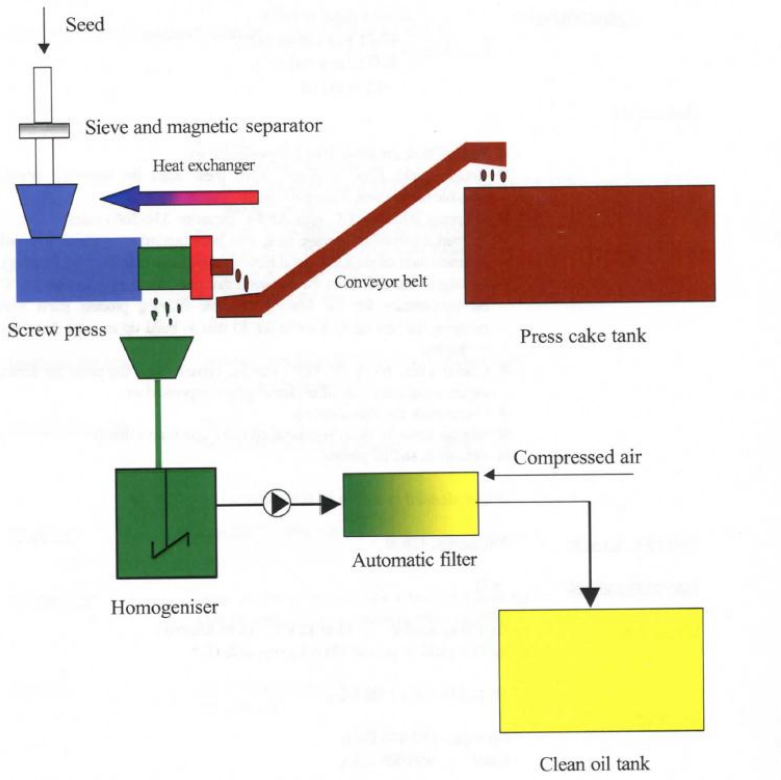

Figure 1. Complete oil crushing facility (Source: Fercheau, 2000)

Seed conditions and steps prior to crushing

- Very clean seed: <2% dockage. A seed cleaner is necessary in the crushing facility to prevent damage to the oil press.
- Seed moisture: from 6% to 8%.
- Above 25% seed oil content will facilitate the crushing and avoid plugging issues in the crusher.
- Heating seed system is recommended prior to extraction for crushing ease: 70°F to 120°F. A warm airflow can be blown into the conveyor. Also, a double-walled

drag conveyor can be installed, through which hot water is conducted in the opposite direction of the seed flow.

- A dry and warm (>55°F) crushing facility can improve overall oil extraction yield.
- Sieve and magnetic separator: any metal object going into the crusher will cause damage.

Selecting your crusher

Crushing volumes vary widely among crusher type and size going anywhere from 10 lbs/hour to 2 tons/hour with price ranging from $1500 to $150,000. Any common oilseed under required crushing conditions can be fed into a crusher. It is very important to assess the fuel requirements to choose an appropriate crusher size and type. The following questions will assist in the selection process:

1. What is your annual diesel consumption? How much diesel would you like to replace?
2. How many oilseed acres would you be able to plant in your cropping system?
3. What size crusher would you need to achieve your ultimate SVO production goal?

Two types of crushers exist:

- Hole cylinder (screw press)
- Strainer (plate crusher) and hybrid strainer

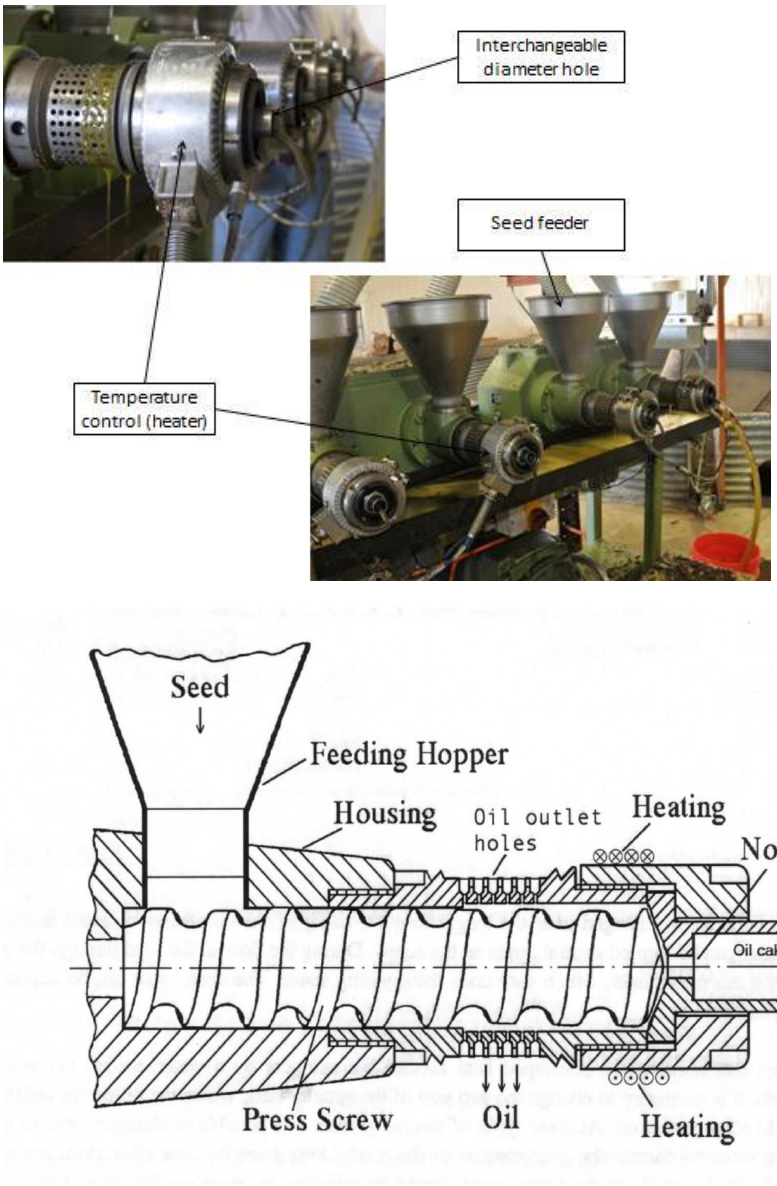

Figure 2: Hole cylinder crusher (screw press) (Source: Fercheau, 2000; Enjalbert, 2010)

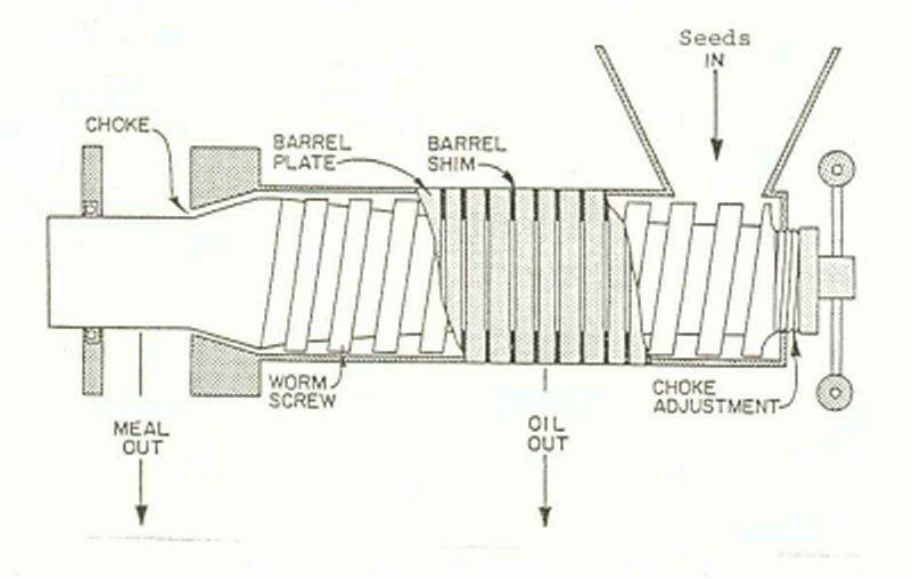

Figure 3: Strainer crusher (plate crusher) (Source: Fercheau, 2000)

Hole cylinder vs. strainer crusher

Criteria	Hole cylinder	Strainer
Gum level	Low: crushing temperatures allow low gum level.	High: when too high temperatures. It is harder to keep temperatures low.
Oil yield	This will depend on crusher settings (speed, temperatures). There is no difference between the two types.	
Meal oil content remaining	Between 7% and 15% for both types. Strainer tends to have lower residual oil.	
Oil sediment	Low but bigger size	High: filtering system needs to be well designed to deal with higher sediment level
Crushing capacity	Low (from 10 to 200 lbs/hour)	High (from 50 lbs to few tons/hour)
Crushing temperature	Around 120°F	From 110 to 200°F
Meal shape	Pellets	Flake and pellets

The farm size strainer crushers available in the United States are all made in China. They are in fact a hybrid of strainer and hole cylinder crusher. From experience, these types of presses require a lot of time to set up and produce low oil quality. Temperature control is difficult, resulting in higher gum production when crushing Brassicaceae crops, high particles level, and polymerization risk in the crusher especially with sunflower. The Chinese crusher goes up to 210°F. These types of crushers are high maintenance and require a lot of labor to clean after each use. However, not all strainer type crushers are made in China. There are successful European strainer crushers. The presses Komet, Kern, Mecanique Moderne and Reinartz produce high quality oil, and are durable.

How to optimize oil yield when crushing

1. Cleaned seed: <2% dockage

2. Crusher speed: The lower the speed, the more oil will be extracted of a better quality for fuel use. However, the hourly oil yield will be lower (Perrin, 2010).
3. End diameter holes: the smaller the holes, the more oil will come out of the seed. However, the seed becomes harder to crush and the risk of plugging the crusher increases. Typically, the higher the seed oil content, the smaller the diameter of the holes that can be used. In addition, it is recommended to start with a bigger diameter and reduce it as soon as the crusher gets warm and runs well.
4. Temperatures: When temperatures are high, oil yield increases. However, to have premium oil quality, it is requires to crush below 135°F (temperature of the exiting oil). At temperatures in excess of 150°F, phospholipids are removed from the seed coat and create gums. These will plug engine injectors.

FILTERING

After a cold press oil extraction, the oil remains cloudy. A filtering process is required to clean the oil for fuel use. This stage is very important, since poorly filtered oil could damage the engine. Filtering is done in two stages. The first stage removes most of the particles. This can be done with settling tanks or with a direct filtering system. The second stage removes the finest particulates using cartridge filters.

Not all filters are created equal. The pore size rating of a filter system is identified by the diameter of the particles that it can be expected to retain with a defined efficiency. Pore sizes are reported in microns (μm). Two size ratings exist:

- A **nominal** size rating indicates a filter retains 50% or more of a certain particle size. For example, if a filter has a nominal rating of 25 microns, that filter will retain at least 50% of all particles 25 microns and larger.

- An **absolute** size rating indicates a filter retains 98.7% or more of a certain particle size. For example, if a filter has an absolute rating of 6 microns, it indicates that the filter will retain at least 98.7% of all particles 6 microns and larger.

Because many products in the market are rated according to their nominal rating, it is crucial to understand the difference between the two ratings, and to insist on knowing the absolute rating of the filter you install.

Filtering conditions

Filtering is done at room temperature (65°F), preferably in an insulated and dry shed. Most oils require two stages of filtering. However, it is recommended, for sunflower oil, to do a pre-filtering around 55°F to remove waxes, followed by the standard two- filter process at room temperature to remove solid sediments.

First filtering stage

Two types of filtering system can be used for this first stage:

1. Settling tanks combined with filter press.
2. Direct filtering system after crushing with vertical leaf filter.

Filter system comparison

	Settling tank system	**Direct filtering system**
Principle	A month in settling tanks to remove biggest particulates at a temperature above 68°F. Then the oil is filtered in a press filter.	Vertical leaf filter used right after crushing.
Advantages	Low investment	Time saving, automatic functions, easy to set up
Weaknesses	Space required, time consuming	Investment cost

Settling tank process

Two steps are required:

- A set of settling/sedimentation tanks: the oil remains in the tank system for a month at a temperature above 68°F. Insulation can be used around the settling tanks.
- Press filter (Fig. 4). First, the oil has to recirculate in the press as a closed loop to build the filtering membranes. Then, oil can flow through to the final filtering steps. The filter press can have manual or automatic cleaning systems.

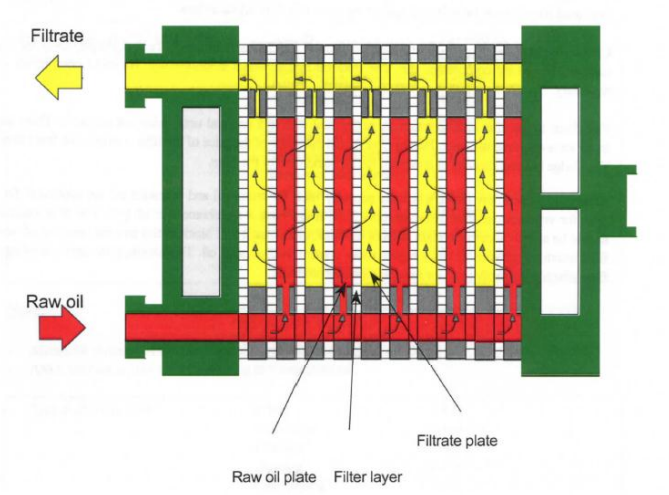

Figure 4: Press filter (Source: Fercheau, 2000)

Direct filter

A vertical leaf filter system is used for direct filtering (Fig. 5). This process is for larger units. This filtering system has automatic cleaning by a vibrator with compressed air. It is more costly than the previous system but less time-consuming.

The leaf system mechanism is as follows: The sediments, in the first tank need to be maintained in suspension using a homogenizer. First, the raw oil is pumped through a

close loop in order to establish filtering membranes from oil particles. Then, the SVO can be filtered and sent to the last filtering stage. Control of the raw oil temperature is required. When temperatures are too low (<50°F), the filter can be plugged by gum formation and/or by high oil viscosity, decreasing the filter flow.

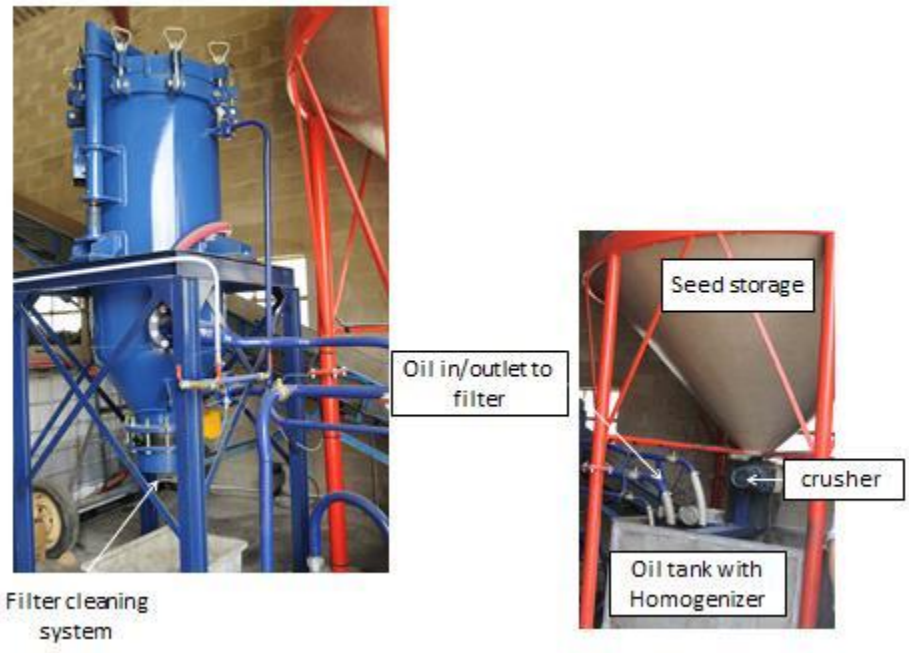

Figure 5: Vertical leaf filter (Source: Institut Français des Huiles Végétales Pures)

Final filtering stage

A set of cartridges is used for the final filtering stage. Cartridge size rating can descend from 100 to 50 to 20 to 10 µm ending with the fine filter of 2 to 6 µm (absolute size) (Fig. 6). Cartridges can retain a limited amount of particles indicated by the manufacturer. When this limit is reached, the cartridge must be replaced. For example, the HYDAC 2 µm abs fine filter has 125 grams of retention equivalent to 1,585 gallons of filtered oil.

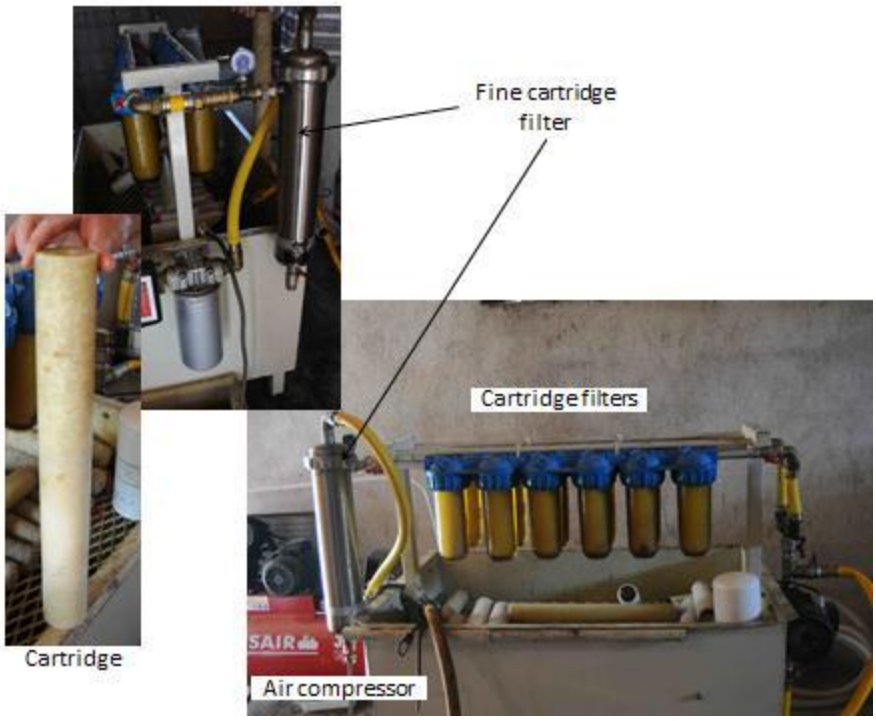

Figure 6: Set of cartridge filters ending with a fine filter (Source: Institut Français des Huiles Végétales Pures)

STORAGE

1. Oil storage: The oil has to be protected from air contact, dust, humidity and light. Polyethylene high-density (PEHD) tank storage is usually used. In these conditions, SVO can be stored for at least one year. With time, light, and air contact, oxidation level goes up increasing oil detergency.
2. Meal storage: The cooling time right after crushing is important. Pellet-format meal tends to cool faster than flakes, thus can be stored for a longer time period. It has to be stored in a cool, dry and dark place. Meal can be stored from 4 to 6 months depending on meal shape.

SVO QUALITY AND CERTIFICATION

SVO quality, low gums and low sediment, is essential for engine efficiency and durability. Every step of SVO production is crucial to maintain quality. The following table shows SVO characteristics such as acidity, viscosity and potential gum, phosphorous, wax, and particles.

SVO characteristics

Parameters	Comments
11% to 17% oxygen (Diesel has 5%)	Increases combustion and can potentially lower consumption.
SVO is acidic	It can wear bearings and pipes due to its detergency.
SVO miscible to diesel	Diesel/SVO blend works. Issues can be faced at low temperatures.
10-fold more viscous	Need to add heating system around fuel line, filters, and needs separate fuel pumps. Optimum engine efficiency is met between 60 and 85% load (Perrin, 2010).
High cetane and flash point number	Combustion requires higher temperatures. Power is slightly lower (10-15%), but better torque, up to 10%.
Wax and gum	If SVO is not well crushed and filtered, it will accelerate plugged injectors and induce coking.
Impurity	Creates unburned particulates. Requires good filtering system.

Because SVO quality can vary greatly from site to site, SVO standards have been established in Europe. Following these standards will reduce engine complications and improve emissions. Any laboratory doing biodiesel analyses can do those analyses. In the U.S., the standard procedures are listed with ASTM code.

Diesel and SVO fuel parameters limits required by European (EN 590 for diesel and DIN 51605 for rapeseed oil only), French standards (developed by IFHVP: production agreement called "Vegetole®", including production process) and ASTM equivalent code.

Parameter	Diesel EN 590	DIN 51605 (06/05)	Vegetole SVO	ASTM code[1]
Viscosity (mm2/s)	3	36	32	D-445
Water (ppm)	25	750	548	D-1533B
TAN[2] (mg KOH/g)	0.03	2	0.78	D-2274
Ash (%)	<0.001	0.01	<0.001	D-482
Masse vol (kg/m3)	834	930	925	D-1298
P (ppm)	0	12	0 to 9	D-6595
S (ppm)	31	10	1 to 9	D-6595
Particles (ppm)	9	24	26	D-524

[1]American Society for Testing and Materials standard code

[2]Total Acid Number

RUNNING SVO IN YOUR ENGINE

So far, no warranty has been extended from engine manufactures to use SVO in engines. Until a U.S. SVO standard exists, companies will not offer a warranty. However, when SVO is used properly, risks are very low. Viscosity is a major difference between SVO and diesel. At 140°F (60°C), diesel and SVO have similar viscosity. Therefore, when running SVO, fuel lines have to be heated, and/or insulated. Some diesel injection systems are more adept than others at using SVO. SVO fuel pumps must be added to handle the increased viscosity and acidity of the SVO.

Diesel engine

A diesel engine, also called a compression ignition engine, uses high compression to self-ignite the fuel. Two major types of diesel engine can be encountered: indirect injection and direct injection diesel engine.

1. Indirect injection engine: the fuel is injected into a secondary combustion chamber. This engine is common in older vehicles. It is suitable for SVO.
2. Direct injection engine: the fuel is injected into the main combustion chamber. There are two types. The common rail system has a single fuel pump supplying all the injectors. This system has high pressure and is electronically controlled. The pump-line-injector system, a mechanical system, has a fuel pump for each cylinder with pump and injector as a single unit.

These different types of engines will require adequate modifications and appropriate SVO/diesel blend. With a common rail engine it is not recommended to have more than 30% SVO blend.

30% SVO blend and under

Under 30% SVO incorporation, modifications are limited. It is recommended to control injector pressure, which can be increased, and also to have a heated diesel filter.

100% SVO

When running with 100% SVO, a retrofit kit is required. Two tanks are used with heated fuel lines. Companies such as ATG and Ellsbett make proper modifications. The retrofit kit makes the switch between diesel and SVO according to fuel temperatures and engine load. It can be manual or automatic.

The lubricating oil needs to be analyzed to detect premature engine wear. Fuel pump type and engine conditions will determine the SVO use feasibility. According to many experts, engines with the following fuel pumps are less compatible with SVO: CAV, Lucas, Stanadyne and Delphi.

CONCLUSION AND RECOMMENDATIONS

An SVO production facility investment is not limited to a crusher and filter. It is important to be aware of the additional cost involved for storage tanks, augers, air compressor, panel control, electric set-up, and automation. Comparisons should include life-time costs, replacement price, ease of use and cleaning, and maintenance when evaluating the cost-effectiveness of an SVO production facility.

Producing your own fuel is not an easy task, but it provides many advantages. It will make your farm less subject to fuel price fluctuations and more resilient to any major fuel crisis. It will protect the environment, create a better local economy, and produce meal for your livestock, poultry or dairy. It also enhances personal empowerment.

RESOURCES

Kurki A., Bachmann J., Hill H. (2008) Oilseed Processing for Small-Scale Producers. NCAT. Piedmont Biofuels Cooperative © 2008 NCAT. http://attra.ncat.org/attra-pub/oilseed.html

Lenntech, 2009. Absolute rating vs. nominal rating for filters. http://www.lenntech.com/library/fine/absolute/absolute-nominal-filters.htm

Filter companies:

- Lenntech: http://www.lenntech.com/replacement/atlas-filtri.htm
- Hydac: http://www.hydac.com.au/filtersystemtechnology.aspx
- MAHLE:

  http://www.mahle.com/C12571AE0031126E/CurrentBaseLink/W26FCDMN691GANDFR

Retrofit kit companies

- Bioltec, inc: http://www.bioltec.de/index.php?hp=157&
- Elsbett: http://www.elsbett.com
- ATG conversion kit

Oil press companies

Brand	Origine	Type	Link	Address
Kern Kraft	Germany	Hole cylinder	http://www.oelpresse.de/ http://www.aquasolaer.eu/natural_oil_production/applications.html	M. Jon Becker-Schickel, 1531 A Owl Creek Road, Thermopolis, WY 82443 Phone: (307) 867-2233 Cell: (563) 581-0048
Komet	Germany	Hole cylinder	www.oekotec.ibg-monforts.de	An der Waldesruh 23 D-41238 Mönchengladbach, Germany Tel. (+49 (2166) 8682- 62 Fax. (+49 (2166) 8682- 44
Reinartz	Germany	Strainer	http://www.reinartz.de/index.php	
La Mecanique Moderne Crusher	France	Strainer	http://www.la-mecanique-moderne.com/english_meca.html	A. Bosquet - abosquet@lamecaniquemoderne.com B.Z.A.C Artoipole B.P. 42015 62060 Arras Cedex 9 FRANCE +33(0)3.21.55.36.00
Mammoth	China	Strainer	http://www.trimlinedesigncentre.com/page.php?103	Trimline Design Centre, Inc., 6772-99 Street, Edmonton, Alberta, Canada, T6E 5B8 Phone: (780) 466-9034
Cropland Biodiesel Oilseed	China	Hybrid Strainer	www.croplandbiodiesel.com	2003 Pangborn Rd., Lynden, WA 98264
Biopress Oilseed	China	Hybrid Strainer	www.centralbiodieselhtp.com	Central Biodiesel, 8208 NW 6th St., Coral Springs, FL 33071

SVO analysis laboratories:

- Analysts, Inc: www.analystsinconline.com
- CSU EECL: http://www.eecl.colostate.edu/

Dr. Anthony Marchese. Mechanical Engineering. Anthony.Marchese@colostate.edu (970) 491-4796

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