THESIS

EVALUATING GENETIC MECHANISMS AND PERFORMANCE CHARACTERISTICS OF ALTERNATIVE OILSEED CROPS FOR ON-FARM BIOFUEL PRODUCTION IN COLORADO

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ABSTRACT

Dryland winter wheat (*Triticum aestivum*) cropping systems dominate most of the agricultural landscape in Colorado's semi-arid eastern plains. Since this area's climate is characterized by frequent heat and drought, it is important to maximize water use efficiency to make agricultural lands as productive as possible. Adding a spring crop in rotation with winter wheat intensifies the rotation, increasing water use efficiency by up to 37%. Recent research has explored further intensifying this rotation by adding an oilseed crop into a wheat – spring crop – fallow rotation during the fallow period. Ideally, the oilseed crop acts as a cover crop for part of the season and leaves enough time at the end of the season to regenerate water in the soil profile before planting wheat in the fall. The oil from this crop can be used to produce on-farm biofuels, offsetting petroleum diesel costs without displacing high-value food crops. Additionally, the meal from this crop acts as a value-added byproduct by providing feed for livestock.

Since traditional oilseeds such as soybean (*Glycine max*) and rapeseed (*Brassica napus*) do not perform well in Colorado, several alternative oilseeds have been tested to assess whether they can fill this niche. Camelina (*Camelina sativa*) has shown great potential, with high oil content and inherent resistance to many biotic and abiotic stressors. Other potential oilseeds include *Brassica juncea* and *Brassica carinata*, but both of these species have exhibited longer life cycles and lower yields than camelina.

A major challenge to camelina production in Colorado is a susceptibility to heat stress during reproductive periods. Both short periods of intense heat stress and longer periods of mild heat stress can cause floral and seed abortion, resulting in reduced yield. In the current study, a quantitative trait locus (QTL) approach is used to identify heat and

drought tolerance mechanisms and yield components, explore the extent of pleiotropy, epistasis, and linkage, and identify promising lines for study or production.

Genetic resources for camelina are becoming more readily available and a newly developed genetic map with improved marker density was used for QTL discovery. Replicated field trials were performed during the 2014 growing season in Fort Collins and Greeley, Colorado, under differential irrigation treatments at each site to collect phenotypic data on a variety of traits. Sixteen new QTL were discovered from this data, along with nine QTL using data from Colorado trials of the same population in 2009 and 2010 performed by Enjalbert (2011). Seven QTL were discovered for yield, however, no QTL were found in more than two environments, indicating a lack of stable QTL for this trait. This was in contrast to results from Enjalbert (2011) where stable QTL for yield across environments were detected using the original, mainly AFLP generated, genetic map by Gehringer et al. (2006). This underscores the high amount of variation that can be caused by environment. QTL for other traits, such as plant height and days to flowering, were detected that were more robust, however, no QTL were detected with either data set that spanned more than three environments. Two loci were identified that affected multiple traits, supplying evidence of either pleiotropy or close linkage of genes. Several RIL performed well in multiple environments, indicating potential for production in Colorado, however, these lines were not in common with previous studies, so further trials will be needed to confirm consistently stable yields.

In addition to the camelina QTL study, a two-year variety trial of *Brassica carinata* was performed in Fort Collins, CO during the 2013 and 2014 growing seasons under limited and full irrigation. Collaboration with the private Canadian oilseed company

Agrisoma Biosciences spurred interest in reevaluating the potential of this alternative oilseed in Colorado cropping systems. Agrisoma Biosciences developed early flowering and early maturing germplasm that performs well in the Canadian prairie and is interested in testing their germplasm in new regions with potential for production. The company provided six lines for the trial, five experimental lines and one commercial check cultivar.

Mean flowering time was over 13 days longer than previously tested African accessions that had been deemed too late flowering to be competitive in Colorado's climate. Mean yields were low as well, at 669 kg ha^{-1}. The commercial check cultivar, A100, outperformed all of the experimental lines, with a mean yield of 1081 kg ha^{-1} across environments. With a wide margin between the other lines and A100, this commercial cultivar was clearly more successful than any of the experimental lines. However, yields of this one cultivar were not sufficiently impressive to recommend on-farm testing of the crop.

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CHAPTER 1:

LITERATURE REVIEW

COLORADO DRYLAND CROPPING SYSTEMS

Coloradoans plant more acres to winter wheat (*Triticum aestivum*) than any other crop, accounting for more than 2.3 million acres of the 4.7 million acres dedicated to commodity crops (Clark, 2013). According to the most recent National Agricultural Statistics Service report, Colorado ranks as the 5th highest winter wheat producing state in the United States, generating $305,532,000 in the 2013 season (Clark, 2013). To grow winter wheat in the semi-arid environment of Colorado, a fallow period is required to maintain consistent yields. This is generally achieved by one of two rotational schemes, i.e. by a wheat-fallow rotation or a wheat-spring crop-fallow rotation.

In a wheat-fallow rotation, wheat is planted in the fall and harvested the following mid-summer. This would be followed by a year of fallow and planted the next fall. In a wheat fallow system, one wheat crop is harvested every other year. In a wheat – spring crop – fallow rotation, Colorado farmers add a spring-planted crop to the rotation, such as maize (*Zea mays*), sunflower (*Helianthus annus*), or proso millet (*Panicum miliaceum*). Intensifying the cropping rotation in this manner can increase water use efficiency (WUE) by 37% (Peterson and Westfall, 2004). This is important because 75% of the precipitation in the Great Plains falls from April to September, which months experience frequent high temperatures and low relative humidity (Peterson and Westfall, 2004). Not surprisingly, much of this water is lost to evaporation and runoff. Annual precipitation in the eastern plains of Colorado is generally low, ranging from less than 305 mm in the Arkansas valley

to almost 460 mm in the northeastern and southeastern corners of the state (Doesken, 2014). Consequently, it is very important for dryland farmers to maximize use of water for cropping whenever possible.

Recent research has begun to explore whether growing a short-season spring oilseed crop in lieu of a fallow season could further intensify Colorado cropping systems. An ideal crop would be cold tolerant so it can be planted early, drought and heat tolerant, and early maturing to leave time to regenerate water in the soil profile before wheat is planted in September. Several industrial oilseed crops fit this niche, most notably rapeseed (*Brassica napus*), camelina (*Camelina sativa*), Indian brown mustard (*Brassica juncea*), and Ethiopian mustard (*Brassica carinata*). All of these crops have known food and industrial uses, but it has been proposed that using this niche in a winter wheat cropping system to create on-farm biofuel is an attractive and economically viable option (Keske et al., 2013).

Of these species, spring-type camelina is the most promising crop to integrate into a Great Plains winter wheat cropping system (Enjalbert, 2011; Enjalbert et al., 2013). Camelina has a remarkably short growing season, reaching full maturity around 80 days (Jewett, 2015). It is a cold tolerant crop that yields best when planted early in the spring. With an optimal germination temperature of 3.3°C and a sensitivity to heat during flowering, it has been shown that a delay of planting from March to April will result in a 25% reduction in yield (Ehrensing and Guy, 2008). Therefore, it is best to plant camelina as early as weather allows, both to maximize yield and to leave as much time as possible to prepare the land and regenerate water in the soil profile before fall planting of wheat. In this way, a successful oilseed harvest can be obtained without displacing high-value food crops. Much of the opposition to using arable land for biofuel crops stems from the

argument that the crops displace food, which is commonly known as the "food versus fuel" debate, but this innovative crop rotation allows farmers to gain an additional crop without having to sacrifice acreage that would be devoted to other crops. Additionally, adding an oilseed to the rotation adds a cover crop during part of the fallow season, which reduces weed competition and soil erosion (Clark, 2012).

ON-FARM BIOFUEL PRODUCTION

Liquid fuels represent a significant portion of agricultural input expenses. Over 30.5 million gallons of fuel oil was sold specifically for on-farm use in Colorado in 2013 alone (EIA, 2013). This leaves farmers at the mercy of foreign energy markets, which can translate to unexpected fluctuations in energy input costs. Some farmers are responding to this issue with a will to produce their own fuels. This gives farmers greater control of their energy inputs, ensuring supply during times of shortage or high-prices and offsetting standard operating costs (Keske et al., 2013). How much savings a farmer will garner is dependent on usage and fluctuations in the oil market, but it has been demonstrated that growing camelina during a fallow season is an economically viable model, particularly when diesel prices are high (Keske et al., 2013).

Making several types of plant-based biofuels is possible with an oilseed crop, but the simplest fuel to produce on-farm is straight vegetable oil (SVO). Simply crushing seed, filtering, and collecting the resultant oil produces this type of fuel (Lakshminarayanan, 2014). It is possible to further process the oil, via transesterification, to make biodiesel. This will make the fuel more similar to petroleum diesel, but has the disadvantage of a loss of energy during processing and producing environmentally unfriendly byproducts such as

glycerol (Jewett, 2013). Production of high quality biodiesel requires equipment and expertise that a farmer may not have, thus requiring an investment of time and money, or the expense of a third-party processor. For making straight vegetable oil, crushing costs are relatively negligible. Farmers can opt to purchase a small-scale crushing device, i.e. a hydraulic press, or transport their seed to a local crushing facility where it can be turned into oil for a nominal charge. Colorado crushing facilities can be found in Stratton, Costilla County, and Rocky Ford (Enjalbert and Johnson, 2011).

There are known issues with running SVO in an unmodified diesel engine. Fuel properties, most notably viscosity, can be significantly different in SVO compared to petroleum diesel (Drenth et al., 2014). Engine modifications, such as adding a fuel line heater, may be made to improve the performance of SVO in a diesel engine. However, a common and practical method of bringing fuel properties closer to petroleum diesel is fuel blending, which can involve blending SVO with any number of thinning agents such as gasoline, petroleum diesel, biodiesel, ethanol, methanol, etc. (Drenth et al., 2014; Ramadhas et al., 2004). Engine modifications, blending, and other SVO concepts are extensively covered in the literature and well reviewed by Ramadhas et al. (2004). A recent variation on SVO blending, called a triglyceride blend (TGB), has shown promise at Colorado State University as a simple method of improving SVO fuel properties (Drenth et al., 2014). Loosely, blending SVO with a less viscous fuel (not petroleum diesel) creates a TGB. In concurrent research and for the purpose of this chapter, however, TGBs will be defined more narrowly as a 3:1 volumetric ratio of SVO to E10 gasoline (Drenth et al., 2014). This makes an attractive fuel for farmers because unleaded gasoline is readily available, miscible and stable with SVO without a surfactant, is inexpensive and has high energy

content (Drenth et al., 2014). Blends of this type are also an attractive alternative to biodiesel because they are quickly made, amenable to continuous processing, cold tolerant (do not gel at cold temperatures), do not require a catalyst, and do not produce associated waste products (Dunn and Bagby, 2000).

Initial results of camelina and *B. carinata* TGB engine tests are promising. Across the board, TGBs consumed less fuel than their biodiesel counterparts, with a higher thermal efficiency. TGB combustion characteristics and engine performance were also found to be similar to biodiesel (Drenth et al., 2014). This is not to say that TGBs are perfect fuels. TGBs were found to produce higher emissions than biodiesel, specifically carbon monoxide, non-methane hydrocarbons, volatile organic compounds, and formaldehyde (Drenth et al., 2014). Despite a slight increase emissions compared to biodiesel, TGB emissions were still typical compared to traditional oilseed-produced fuels, i.e. fewer emissions than petroleum diesel (Drenth et al., 2014). More studies will be required to assess the long-term effects of TGBs on diesel engines, but, due mainly to ease of production, TGBs are a very attractive solution for farmers looking to become more energy independent without requiring an investment in equipment or engine modifications.

The market for SVO and TGBs remains relatively underdeveloped. Because of this, on-farm production of biofuels is currently relegated to early adopters and farmers who are willing to pay a premium for energy security and independence (Keske et al., 2013). Camelina and *B. carinata* oil have not been approved by the Environmental Protection Agency (EPA) for on-road use, so the market will remain restricted to on-farm use unless federal rules change.

Another major component to making an intensified oilseed rotation profitable is the use or sale of the meal that is left over after pressing the seed. Camelina meal has been approved by the United States Food and Drug Administration (FDA) for blending in feed for chickens, cattle, and swine (Church, 2014) and is known to be a highly nutritious feed. Camelina meal is high in protein (~40%) and fiber (10 to 12%) and also raises levels of omega-3 fatty acids in chicken meat, as well as eggs laid by chickens that fed on camelina meal (Ryhanen et al., 2007). Although the feed certainly has value, there is no established market for camelina meal in Colorado. This means that a farmer will need either to have his or her own livestock to consume the meal, or to find another farmer who is looking for a meal supplement. However, the value of the camelina meal is such that it behooves the farmer to find a buyer rather than to turn the meal into compost.

CAMELINA SATIVA

Origin, Life Cycle, and Physical Characteristics

Camelina (*Camelina sativa*) is known historically as gold-of-pleasure and false flax, but is generally recognized today by its one word moniker. Archeological excavations in Europe and Scandinavia show direct evidence of cultivation of this crop dating to 1500 B.C. (Zubr, 1996). This evidence corroborates the idea that northern Europe represents the evolutionary center of origin for the species (Jewett, 2015), however, a genetic diversity study by Ghamkhar et al. (2010) suggested that Russia or the Ukraine was a genetic diversity "hotspot" and a potential center of origin as well. Camelina gained traction over many centuries as a food and oil crop in Europe, reaching its peak production in France in the 19th century (Frölich and Rice, 2005). Camelina was eventually displaced in European

agriculture by higher yielding crops, namely rapeseed and wheat (*Triticum aestivum*), becoming largely relict by the mid 20th century (Kagale et al., 2014). Interest in camelina has recently been revived due to its potential as a food crop high in omega-3 fatty acids (Zubr, 1997) and as a low-input industrial oilseed with significant applications for biofuel, jet fuel, and high-value biolubricants (Kagale, 2014).

The plant itself is a small-statured (~1 m at maturity), cruciferous-type plant that is typically grown as a spring-seeded annual (Guy et al., 2013). Fall-seeded-type camelina, which requires vernalization, is available, but these types have not been found to be suitable for Colorado agriculture due to increased insect and drought pressure in the fall and winter, when compared to spring habit camelina (Jewett, 2013). Initial growth forms a rosette of leaves, which later forms an erect stalk. This stalk will form an elongated raceme inflorescence, with pale yellow flowers that are mainly self-pollinated (Zubr, 1997; Guy et al., 2013). Camelina plants are almost entirely autogamous, with an estimated 0.01-0.28% outcrossing rate (Walsh et al., 2012). Tear-shaped pods will form at the flower sites, which hold up to 18 seeds per pod (Guy et al., 2013). The seeds are remarkably tiny, even among the *Brassicaceae*, with 1000 seeds ranging from 0.8-1.8 g (Zubr, 1997).

Camelina seeds contain a high amount of lipids with a total oil content ranging from 32 to 46% of total seed weight (Vollman et al., 2007). This oil is prized for its unusually high levels of linolenic acid (35 to 40%). Common oilseed crops such as rapeseed or soybean (*Glycine max*) only contain around 8% of this compound. Linolenic acid is an omega-3 fatty acid that is typically only found in high quantities in linseed (*Linum usitatissimum*) and fish oils (Crowley and Frölich, 1998). Because of this characteristic, camelina oil has potential to lower cholesterol in humans, as well as having a wide variety

of industrial applications, from cosmetics to low-emission biofuels (Vollman et al., 2007). Although camelina has shown promise as a health supplement, it is not currently approved for human consumption in the United States (Lakshminarayanan, 2014).

Camelina Agronomy

The small seed size of camelina requires a shallow planting depth for best emergence, with a target depth of 6.3 mm below the soil surface (Enjalbert and Johnson, 2011). As previously mentioned, it is important to plant camelina as early in spring as weather allows, preferably in late February or March. A wheat drill may be used to direct seed the camelina, if the desired depth of no more than 12 mm can be achieved, or the seed may be broadcast (Enjalbert and Johnson, 2011). A seeding rate of 5.6 to 7.8 kg ha^{-1} is recommended, with higher rates corresponding to less optimal conditions or anticipated weed pressure (Enjalbert and Johnson, 2011; Jewett, 2013). Cost of camelina seed is relatively low compared to major crops, averaging $4.40 per kilogram (Enjalbert and Johnson, 2011). Camelina fertilizer requirements are minimal as well. Generally, 2-2.7 kg of available nitrogen will produce 45 kg of seed yield, but no fertilizer may be required if the previous crop has left adequate residual nitrogen in the soil (Hulbert, 2012). As with any crop, a soil analysis is recommended before planting to ensure proper nutrient levels.

Another benefit of growing camelina, compared to other more common oilseeds, is a natural resistance to flea beetles (*Phyllotreta cruciferae*) and disease. Flea beetles are endemic to Colorado and cause significant yield losses in rapeseed and other *Brassica* crops (Johnson et al., 2008). Camelina's resistance to flea beetles and some fungal infections is

attributed to natural defensive compounds, including quercetin glycosides and phytoalexins (Onyilagha, 2012; Browne et al., 1991).

Harvest of a camelina crop should occur when the pods and at least some of the stem of the plant have ripened to a yellow-brown color (Enjalbert and Johnson, 2011). Harvesting earlier than this can cause several problems, including immature seed and green stems binding the moving parts of machinery. Later than optimal harvesting times will result in excessive shattering, either in the field or during cutting. The crop is generally direct-harvested with a combine, however, swathing is an option as well. Swathing would generally be used in the event of uneven maturity or excessive weeds and may be performed when the crop is approximately 65% mature (Lafferty, 2009).

A wide range of yield has been reported for camelina. A study of camelina yields across 18 location/year environments, in four precipitation zones in Washington, showed variation from 100 to 3,795 kg ha^{-1}, with an average yield of 1,213 kg ha^{-1} (Guy et al., 2013). Research at Colorado State University has also shown a wide yield range of 336 to 1,794 kg ha^{-1}, with a mean yield of 1,383 kg ha^{-1} (Enjalbert, 2011; Enjalbert et al., 2013) and a proposed dryland yield target of 1,344 kg ha^{-1} for farmers with tested varieties (Enjalbert and Johnson, 2011). A separate CSU trial evaluated 15 genotypes of camelina in 12 limited irrigation and dryland environments in Montana, Washington, and Colorado (Jewett, 2013). Yield varied dramatically in this study as well, from 123 to 2,037 kg ha^{-1}, with a mean yield of 813 kg ha^{-1}. Clearly, much variation has been observed for yield under drought and heat conditions.

Challenges of Camelina Production in Colorado

Although camelina has some natural advantages over better-known oilseed crops, it retains its fair share of challenges. One major issue is a susceptibility to heat stress during flowering. During yield trials in Colorado, locations that experienced very high temperatures (>32°C) during flowering resulted in large-scale floral abortion, lack of seed fill, and early ripening, which translated to drastically lower yields (Enjalbert, 2011; Jewett, 2013). During the Jewett (2013) study, heat stress was noted as a major factor in determining yield. In almost every environment that reported low yields of less than 500 kg ha^{-1}, heat stress days (maximum daily temperature over 32°C) comprised 22% or more of the growing season. The analysis of yield data for these trials showed that latitude and temperature more strongly influenced yield than precipitation, indicating that heat stress may play a more important role in camelina yield than drought stress (Jewett, 2013).

Weed control remains an issue as well. Camelina is sensitive to residual sulfonylurea herbicides, such as Ally, Amber, and triazine, which are commonly used in wheat cropping (Enjalbert and Johnson, 2011). Additionally, only one herbicide, sethoxydim or Poast, is approved for weed control in camelina. This may be applied at any point during the life cycle of camelina since it only affects grasses, but does not control common broadleaf weeds (Lafferty et al., 2009). Tillage or application of an herbicide like glyphosate directly before planting will help to control weeds, as will good stand establishment (Enjalbert and Johnson, 2011).

Lastly, awareness and marketing of the crop remain a challenge. Even though awareness of camelina as a industrial oilseed crop is growing (Kagale, 2014), significant investments in cultivar improvement and infrastructure will be required to make the crop

attractive to risk-averse farmers. Lack of an established market for camelina meal also reduces the probability that farmers can grow camelina profitably (Keske, 2013). However, early adopters can create their own fuel and feed their own livestock, circumventing the need for an established market.

Genetic Resources for Camelina

Genetics-based approaches for the improvement of camelina have only recently been tapped. Since interest in camelina waned until the 21st century, the plethora of quantitative genetics and molecular breeding techniques that have been applied to major crops, like maize, are yet to be fully exploited (Kagale et al., 2014). Genomic characteristics that are well established in other crops are only beginning to be elucidated in camelina. For instance, number of chromosomes and ploidy level has been subject to variation among accessions, ranging from $2n$=12 to $2n$=40 (Francis and Warwick, 2009), with the most common count being $2n$=40 (Warwick and Al-Shehbaz, 2006). The first high-quality reference genome sequence was published in 2014 (Kagale et al.) and represents a significant step forward in understanding the genome of this crop. This study confirmed that camelina is a hexaploid, presumably through an initial tetraploidization event, followed by a subsequent hybridization event. These hybridization events are presumed to have happened relatively recently, some 5,000-10,000 years ago, corresponding to the domestication of major crops like rapeseed, wheat, and cotton (Kagale et al., 2014). It was also confirmed that camelina is genetically very similar to the model species *Arabidopsis thaliana* and even more closely to *Arabidopsis lyrata*. Although implementing quantitative genetics approaches to improve camelina will be similarly challenging as in other hexaploid

crops, like wheat, identifying close synteny and collinearity with thoroughly researched *Arabidopsis* species will be a boon to understanding genetic control of complex traits (Kagale et al., 2014).

A small number of QTL studies have been performed on a camelina mapping population developed at the University of Geissen, identifying QTL for agronomic traits (Gehringer et al., 2006) and drought resistance (Enjalbert, 2011). The mapping population for these studies was developed from a cross of two registered German cultivars, 'Lindo' and 'Licalla' (Deutsche Saatveredelung, Lippstadt, Germany), to produce 186 recombinant inbred lines (RIL) by single-seed descent. A genetic map was also developed for this population by Gehringer et al. (2006), comprised of 157 amplified fragment-length polymorphism (AFLP) markers and three simple-sequence repeat (SSR) markers. In this initial study, 36 significant QTL were identified for seed yield, oil content, fatty-acid composition, plant height, and thousand seed mass. The following study by Enjalbert (2011) identified 29 significant QTL for seed yield, drought tolerance, and oil quality traits. Of these QTL, six were the same as those discovered by Gehringer et al.

A high-density genetic map was recently developed for this same population, using genome-wide SNP markers, by an international team led by Canadian scientist Dr. Isobel Parkin (Singh et al., 2015). Using an Illumina GoldenGate single nucleotide polymorphism (SNP) array, 533 SNP markers were mapped, along with 46 previously mapped SSR markers, to create a genetic map consisting of 579 loci. This represents a significant step forward in mapping resolution and these markers will provide useful tools for genomic improvement of this crop (Singh et al., 2015).

A limited number of studies have been performed on the genetic diversity present in the camelina gene pool. The populations studied have divided into two to four groups with varying degrees of subgrouping (Vollman et al., 2005; Ghamkhar et al., 2010; Singh et al., 2015). The overall consensus seems to be that a high amount of phenotypic diversity can be observed, but that a relatively low amount of genetic diversity is present and groupings by molecular marker do not divide by geographical center of origin, as one might expect (Singh et al., 2015). If more conclusive details of the genetic diversity present in this species are desired, more research into this subject will be necessary.

BRASSICA CARINATA

Origin and History

The cultivation of *Brassica carinata*, a.k.a. Ethiopian mustard, Abyssinian mustard, or gomenzer, is an ancient practice dating back to 4,000-5,000 B.C. (Taylor et al., 2010; Alemayehu and Becker, 2002). The plant is traditionally used as a food crop for its edible leaves and oil from the seeds. *B. carinata* is well adapted to the Ethiopian highlands and, until recently, was rarely cultivated elsewhere (Taylor et al., 2010). Like the other two amphidiploid *Brassica* species, *B. napus* and *B. juncea*, *B. carinata* was the product of a spontaneous hybridization event between two diploid progenitor species (Zuo et al., 2014). These events have been well characterized in the now famous Triangle of U (U, 1935). U showed that *B. carinata* ($2n = 4x = 34$, genome BBCC) was formed from the hybridization of *Brassica nigra* ($2n = 2x = 16$, genome BB) and *Brassica oleracea* ($2n = 2x = 18$, genome CC). The economically important species *B. napus* ($2n = 4x = 38$, genome AACC) shares half of its genome with *B. carinata*. *B. napus* is well adapted to moist, cool areas in northern (and very

southern) latitudes, but performs poorly in hotter and drier regions (Taylor et al., 2010). Because of this, *B. carinata* is increasingly being researched as a possible drought tolerant oilseed crop, intended for production in more arid regions.

Agronomic Characteristics and Practices

The physical features of *B. carinata* are similar to *B. napus* or *B. juncea* and an untrained eye would not be able to distinguish the species. Plants emerge from the soil as cotyledons, which grow into rosettes. Single stalks bolt from the rosette and initiate flowering. Predominantly self-pollinated flowers form elongated siliques, which contain seeds along both sides of a thin membrane. Plants are ready for harvest when the stems change from green to brown (Agrisoma, 2014).

Agronomic practices, for all intents and purposes, are the same as spring rapeseed and are well documented. Equipment for planting and harvesting rapeseed is sufficient to manage a *B. carinata* crop without any modification. *B. carinata* is amenable to no-till systems, but stubble should be managed, so as to not interfere with planting depth. A consistent depth of 1.3-2.5 cm should be maintained for best stand establishment (Agrisoma, 2014). An ideal seeding rate to aim for is 86 to 183 plants per square meter, depending on seedbed conditions (Agrisoma, 2014). Several herbicides and insecticides have been approved for use on *B. carinata*. A complete list of approved pesticides is contained in the Agrisoma Resonance Carinata Production Manual, which is available at www.agrisoma.com (Agrisoma, 2014). Fertility requirements are similar to other *Brassica* species, with a target nitrogen rate of 112 kg ha^{-1}, adjusted for soil analysis results. *B. carinata* is sensitive to seed-placed fertilizer, so any banded fertilizer should be placed at

least 2.54 cm away from seeds. Direct combining of plants with a wheat or rapeseed combine is the best way to harvest *B. carinata*, although swathing is an option if uneven maturity is observed.

Development and Testing of Modern *Brassica carinata* Varieties

Initial trials of Ethiopian varieties of *B. carinata* performed poorly in the rapeseed producing regions of Canada, mainly due to an average extra 19 days to maturity (Getinet et al., 1996). It was concluded, however, that *B. carinata* had many positive attributes, including insect, disease, and drought tolerance and could become a new oilseed or protein crop if earlier maturing varieties could be identified or developed. An initiative by Agriculture and Agri-Food Canada (AAFC) resulted in the development of several "early to maturity" or ETM strains of *B. carinata* (Taylor et al., 2010). These strains still took 5-7 days longer than rapeseed to mature, but represented a significant improvement over the 10-14 day margin exhibited by the base populations, with no observed loss in yield (Taylor et al., 2010). This was the start of a "real" *B. carinata* oilseed industry in Canada and, later, the United States.

Rapeseed is cultivated in small quantities in Colorado, but a lack of sufficient cold tolerance, a sensitivity to heat, and a susceptibility to flea beetles make the crop unattractive to local farmers (Johnson et al., 2008). Taking a cue from the Canadian researchers, trials of *B. carinata* were run at Colorado State University to evaluate its potential compared to other alternative dryland oilseed crops. Although the *B. carinata* plants produced large amounts of biomass, seed production was poor and it was determined that camelina was a much better adapted crop for Colorado's eastern plains.

The poor performance of *B. carinata* was attributed to a lack of adaptation, late flowering, and flea beetle damage (Enjalbert et al., 2013). After these trials, *B. carinata* research at Colorado State University was suspended.

In early 2013, interest in *B. carinata* was renewed by collaboration with a private Canadian company. Mechanical engineering PhD candidate, Aaron Drenth, was looking for vegetable oil feedstocks to perform engine tests and made contact with the private company Agrisoma Biosciences. Agrisoma is a Saskatoon, Saskatchewan-based biotechnology company that specializes in renewable, *B. carinata* diesel and jet fuels. The company is a leader in the industrial oilseeds field and collaborates with many institutions, private and public. Agrisoma provided *B. carinata* oil for engine testing and during those negotiations expressed interest in evaluating some of their new, early-maturing lines of *B. carinata* in Colorado. This began a new round of evaluation of *B. carinata* as a potential alternative oilseed for dryland agriculture.

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CHAPTER 2:

QUANTITATIVE TRAIT LOCUS (QTL) MAPPING OF HEAT AND DROUGHT TOLERANCE TRAITS AND YIELD COMPONENTS IN *CAMELINA SATIVA*

SUMMARY

New genetic resources are being developed for camelina (*Camelina sativa*) due to its potential as a specialty food, feed, and fuel crop. Previous research at Colorado State University identified camelina as an alternative oilseed with potential for production during the fallow period of dryland winter wheat (*Triticum aestivum*) cropping systems, which will increase water use efficiency and allow the production of on-farm biofuel without displacing valuable food crops.

One of the major challenges experienced when growing camelina in Colorado is susceptibility to heat stress. High temperatures (>32°C) during reproductive periods can cause widespread floral abortion and yield loss. This study explored genetic mechanisms of heat and drought tolerance and yield components using a quantitative trait locus (QTL) approach and explored the extent of pleiotropy, epistasis, and linkage among QTL, and identify promising lines for study or production. Only two previous QTL studies have been published about camelina, both using the same 'Lindo x Licalla' recombinant inbred line (RIL) population (n=186) as the current study. These two studies explored agronomic traits and drought tolerance and used the original genetic map developed by Gehringer et al. (2006), comprised of 154 amplified fragment length polymorphism (AFLP) markers and 3 simple sequence repeat (SSR) markers. The current study makes use of an improved,

high-density genetic map, consisting of 579 loci, 533 single nucleotide polymorphism (SNP) markers and 46 SSR markers.

The population was evaluated in four Colorado environments in 2014, two well-watered and two drought stressed. QTL analysis was conducted with Rqtl, using a genome-wide significance level of $p<0.05$. In addition, phenotypic data from Enjalbert (2011) was analyzed for QTL using the current marker data.

Twenty-five QTL were detected, 16 using data from the current study and nine using data from Enjalbert (2011). Seven QTL were detected for yield, however, no QTL were found in more than two environments, indicating a lack of stable QTL for this trait. The QTL discovered for days to flowering and plant height that were more robust, but no QTL were detected in every environment. Two loci were detected that were associated with multiple traits, indicating pleiotropic effects or close linkage. No evidence of epistasis was detected using Hayley-Knott regression. Several lines from the population performed well in multiple environments, showing promise for production. However, none of these lines were in common with high performers from previous studies, indicating a large effect of environment on performance of individual lines.

INTRODUCTION

Interest in camelina (*Camelina sativa*) has been rekindled in recent years due to its potential as a nutritional food crop, high in omega-3 fatty acids (Zubr, 1997), and as a low-input industrial oilseed that is amenable to production of biofuels, jet fuel, and high-value biolubricants (Kagale et al., 2014). Camelina has performed well as a dryland crop in semi-arid environments (Angelini et al., 1997) and has been shown to be more adapted to

cropping in colder and drier climates than the more common commercial oilseed crop, canola (*Brassica napus*). This is largely attributed to an innate tolerance to biotic and abiotic stress factors (Singh et al., 2015).

Weather conditions in Colorado's eastern plains are challenging for any dryland crop production. The weather in this region is characterized by frequent drought, low humidity, and seasonal high temperatures, which can result in soil evaporation commonly exceeding precipitation (Peterson and Westfall, 2004). The most economically important commodity crop in Eastern Colorado is winter wheat (*Triticum aestivum*) (Clark, 2013), which requires a fallow period to maintain consistent yields. Recent research on alternative cropping systems at Colorado State University has shown promising results, indicating that growing camelina as an on-farm biofuel crop during the early part of a fallow period, particularly in a wheat-spring crop-fallow rotation, can be a sustainable and economically attractive option (Keske et al., 2013). By intensifying wheat-based crop rotations, water use efficiency is improved (Peterson and Westfall, 2004) and food crop acreage is not displaced by an industrial oilseed. This will theoretically enable farmers to produce their own fuel for use on-farm and offset petroleum diesel costs, while gaining a higher degree of energy independence.

Although it may seem that fuel production is beyond the scope of a typical family farm, turning camelina into a diesel substitute can be a simple and straightforward process. Crushing camelina seed with a hydraulic press and filtering the resultant oil produces a straight vegetable oil (SVO), which may be used in a diesel engine with minor modifications (Drenth et al., 2014, Lakshminarayanan, 2014). To avoid the need for engine modifications, the oil may be blended at a rate of 3:1 volumetric ratio of SVO to E10 unleaded gasoline to

create a triglyceride blend (TGB) with improved fuel characteristics (Drenth et al., 2014). These types of fuels are easy to produce and do not require specialized equipment or caustic chemicals. In addition, after pressing the camelina seed for oil, the leftover camelina meal is a valuable byproduct, as it is a livestock feed high in protein and omega-3 fatty acids (Ryhanen et al., 2007).

While camelina is frequently praised for its stress tolerance and multitude of uses, formidable challenges to production in Colorado remain. The most notable of these challenges include stand establishment under dry spring conditions, weed control, and susceptibility to heat stress during flowering. Yield trials in Colorado have shown that when very high temperatures (>32°C) occur during flowering, yields can be drastically affected due to large-scale floral abortion, lack of seed fill, and early ripening (Enjalbert, 2011; Jewett, 2013). Hence, breeding for heat stress tolerance is an important goal for improving yield stability of camelina in Colorado. This can be challenging because heat stress response is an interplay of physiological and phenological responses throughout the ontology of a species that are deeply tied to drought stress responses (Wahid et al., 2007). Since heat and drought stress tolerance are both complex, quantitative traits, understanding the genetic mechanisms that confer tolerance provides information for targeted crop improvement.

Since camelina had fallen out of production before its potential as an industrial oilseed was recognized, genetic resources were not exploited in this crop as in major commodity crops and its agronomic and breeding potential remains largely untapped (Kagale et al., 2014). However, new genetic resources are being developed for camelina at a rapid pace. Nguyen et al. (2013) produced the first attempt at sequencing the camelina

seed transcriptome, while Kagale et al. (2014) published the first high-quality reference genome sequence the following year. Two previous QTL studies have been performed on a camelina recombinant inbred line (RIL) population (Lindo x Licalla, n=186) developed in Germany at the University of Giessen. The original study identified QTL for agronomic characters and also produced the first genetic map of camelina, comprised of 157 amplified fragment-length polymorphism (AFLP) markers and three simple-sequence repeat (SSR) markers (Gehringer et al., 2006). The following study identified QTL for drought tolerance, yield, and oil quality traits using the same genetic map (Enjalbert, 2011). Singh et al. (2015) recently developed a new genetic map for this same population, which offers improved mapping resolution. This map utilizes more reliable single-nucleotide polymorphism (SNP) markers and a smaller number of SSR markers and covers 579 loci. The current study utilized the original RIL population, combined with the improved power of the higher-density genetic map, to detect new QTL.

The main objectives of this research are to (1) identify QTL for traits associated with heat and drought tolerance and yield components using a newly developed, high-density genetic map; (2) assess epistatic, pleiotropic, and linkage effects within the RIL population; and (3) identify lines of camelina with potential for local production, breeding, or genetic research.

MATERIALS AND METHODS

Plant Materials

Two registered German camelina varieties, 'Lindo' and 'Licalla' (Deutsche Saatveredelung, Lippstadt, Germany) were crossed to produce F_1 plants, from which a total

of 186 RIL were derived by single seed-descent to the F_6 generation. The parents used in the cross exhibited variation in seed yield, thousand seed mass, plant height, fatty-acid composition, and seed-oil content (Gehringer et al., 2006), as well as drought tolerance and oil quality characteristics (Enjalbert, 2011). Aliquots of seed from the RIL population were acquired from Dr. Isobel Parkin at Agriculture and Agri-Food Canada (AAFC) in Saskatoon, Saskatchewan. Seed was increased to field quantities during winter 2013/2014 in CSU greenhouse facilities, using pollination bags to ensure self-pollination.

Environments

Two locations were chosen to evaluate performance of the RIL population. One location was Colorado State University's primary research farm, the Agricultural Research Development and Education Center (ARDEC). This facility is on the northeast edge of Fort Collins, Colorado at latitude 40.65 and longitude -105.00, with an elevation of 1,557 m and average annual precipitation of 408 mm. The soil texture at this location is a sandy clay loam. ARDEC is equipped with an overhead linear sprinkler irrigation system, which was used throughout the study.

The second location was at the Limited Irrigation Research Farm (LIRF) in Greeley, Colorado. LIRF is a United States Department of Agriculture-Agricultural Research Service (USDA-ARS) research site. This facility is in northern Greeley at latitude 40.45 and longitude -104.64, with an elevation of 1,427 m and average annual precipitation of 373 mm. The soil texture at this location is a sandy loam. Sprinklers were used to germinate seeds at LIRF and then drip line tubing was installed by hand, with drip tubes running in between the two rows of each plot. Weather data for both locations were obtained from the

Colorado Agricultural Meteorological network (COAGMET) and the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center (NCDC).

Seedbed preparation was as similar as possible at both sites. The soil was disked and approximately 39 kg ha^{-1} of nitrogen in the form of urea and 2.34 L ha^{-1} of the herbicide Sonolan (ethafluralin) were incorporated into the soil with a roller harrow before planting. The soil was then smoothed with rakes and furrows were made by hand to achieve a planting depth of approximately 6.3 mm. A uniform seeding rate was used of 0.8 g (approximately 643 seeds) per square meter.

Experimental Design

Two side-by side experiments were grown at each site, one fully irrigated to avoid serious moisture stress throughout the season and one with limited irrigation to provide a moderate level of drought stress. For each experiment, a Latinized row-column design was implemented. Two-row plots, 1.5 m in length, were hand-planted for each of the 186 RILs as well as three occurrences of each parent, acting as checks, for a total of 192 plots in each block. Each parent was entered in triplicate to obtain accurate estimations of parental trait values. Individual plots, with bordering space included, were 2 m long and 0.5 m wide. The planted rows were 1.5 m in length, leaving 0.5 m in between ranges to allow access for data collection. The two rows of a plot were spaced 0.2 m apart with 0.3 m between adjacent plots.

Two replications of the experiment were planted, for a total of four blocks (two fully irrigated and two with limited irrigation) at each site. The fully irrigated treatments received 25.4 mm of irrigation weekly, unless there was 25.4 mm or greater rainfall in a

given week. The limited irrigation treatments were mostly rain-fed. After germination, these treatments were irrigated once to establish seedlings and then not again, unless the plants were visibly drought stressed, i.e., wilting. If wilting was observed, a half-rate (12.7 mm) irrigation was applied as "rescue irrigation".

Data Collected

Days to flowering (DTF) were calculated as the number of days between the date of planting and the date when 50% of the plants in a given plot showed at least one open flower. Days to maturity (DTM) were similarly calculated as the number of days between the date of planting and the date when 50% of the plants in a given plot reached physiological maturity. A plant was considered to be physiologically mature when the main stem of the plant changed from green to brown.

Plant height (PLHT) was measured from the soil surface to the highest point of the plant at maturity to the closest centimeter. Five plants were randomly chosen per plot and measured to estimate plant height. Five pods per plot were also randomly sampled to estimate seeds per pod (SPP).

At the end of the season, plots were hand-harvested with rice knives, cutting plants at the soil surface. Plants were placed into paper bags and air-dried before weighing. Plant biomass (BIOMASS) was considered to be the dry mass of all aboveground plant matter from each plot at harvest. Plots were meticulously weeded by hand throughout the season and directly before harvest to avoid weeds confounding weed and biomass measurements. The plants were mechanically threshed, using an Almaco (Nevada, IA) SBT thresher, to separate the seed from the rest of the plant biomass. After cleaning, the seed was weighed

to determine total seed yield (YIELD). A harvest index (HI) ratio was calculated from these terms as (YIELD/BIOMASS). Thousand seed mass (TSM) was also measured, by weighing 1000 randomly sampled seeds from each plot.

Weather data collected for the growing season from COAGMET included precipitation, maximum daily temperature and minimum daily temperature. From these data several other parameters were calculated, including number of heat stress days (HSD, days during the growing season where maximum temperatures exceeded 32°C), %HSD (number of HSD/total number of days in season) and total growing degree-days (GDD), calculated as $((T_{max}+T_{min})/2)-T_{base}$ with T_{base}=4.4°C (McMaster and Wilhelm, 1997) for each day and then a sum was taken over the season.

Dr. Jean-Nicolas Enjalbert (2011) adapted a method from rapeseed literature to determine threshold temperatures that affect camelina performance, specifically oil profile and vegetative growth (Baux et al., 2008). The parameters for HSD, as previously defined, were adopted from Enjalbert (2011).

Genetic Maps

Two genetic maps have been developed for the Lindo x Licalla camelina RIL population. Gehringer et al. (2006), who published the first QTL study on this population, developed the first genetic map. This map is comprised of 157 AFLP markers and three *Brassica* SSR markers. The markers were organized into 20 linkage groups, which supported the reported chromosome count of *n*=20. The markers were distributed over a total length of 1,385.6 cM, with 8.6 cM average distance between markers.

Singh et al. (2015) published a higher-density genetic map for this same population. This new map represents a significant improvement in mapping resolution, covering 579 loci. Of these, 533 are SNP markers and 46 are SSR markers. The 579 markers covered a total of 1,808.7 cM on 20 linkage groups and were distributed at an average of one locus per one Mb of genome sequence. Extensive details on how this map was developed can be found in Singh et al. (2015). Both maps were used in the analysis of this study, with the Singh et al. (2015) map being the primary source for QTL discovery and the Gehringer et al. (2006) map used for comparison to previous studies.

Statistical Analyses

The genetic map developed by Singh et al. (2015) utilized 180 RIL. Consequently, the analysis of phenotypic data includes measurements of all 186 lines and both of the parents, while the QTL analysis only includes 180 lines. Phenotypic data were analyzed using the statistical web-based program SAS Studio (SAS Institute, Inc., Cary, NC). The PROC MIXED function of SAS was used to create a mixed model, with treatment, location, row, and column as fixed effects and genotypes as random effects. To account for spatial variation, an anisotropic spatial power model was applied to calculate adjusted LSMEANS. The LSMEANS were then used to generate Pearson correlation coefficients among the traits using the PROC CORR function of SAS, as well as in the subsequent QTL analysis. Due to significant variation among locations and treatments, each treatment at each location was analyzed as a separate environment, for a total of four environments. Broad-sense heritability for means over environments was calculated by hand for all traits, using variance components from the SAS function PROC VARCOMP with the 'method=REML'

option, according to a formula provided by Clay Sneller from the Ohio State University (personal communication) adapted from Hallauer et al. (1988) for plot-mean data. The broad-sense heritability formula is as follows:

$$\text{Heritability} = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma^2}{re} + \frac{\sigma_{GE}^2}{e}}$$

In this formula, σ^2_G represents genotypic variance, σ^2 represents error variance, σ^2_{GE} represents genotype x environment variance, *r* represents replications, and *e* represents number of environments.

QTL discovery was performed using the add-on library Rqtl for the statistical analysis software R, version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). A penalized likelihood approach was used on QTL and pairwise interactions to control false positive rates (Broman et al., 2003; Manichaikul et al., 2009). Logarithm of Odds (LOD) thresholds were determined for each trait by permutation analysis, using a 5% type I error rate and 1000 permutations. Model selection for QTL was performed using Haley-Knott regression (Haley and Knott, 1992). Multiple QTL terms detected during Haley-Knott regression provided evidence of multiple QTL affecting the trait, while interaction terms were indicative of epistasis. Each locus was tested for interaction with pairwise comparisons across the marker set. QTL with significant LOD scores are reported according to environment unless otherwise indicated.

RESULTS

Environmental Conditions

Growing seasons in Colorado's eastern plains are characterized by frequent heat and drought and the 2014 season was no exception. Weather data for the overall growing season at each location is provided in Table 2.1, along with a breakdown of precipitation by month in Table 2.2.

As is typical in a Colorado summer, high temperatures were experienced sporadically and average daily maximum temperatures at both sites were similar to 30-year averages. The long-term average for Fort Collins from April to August is 25.54°C and the 2014 season average daily maximum temperature was 25.35°C. Similarly, the long-term average for daily maximum temperature in Greeley is 25.97°C and the 2014 season average was 26.27°C. Heat stress during reproductive periods was of interest, so Figure 2.1 and Figure 2.2 show the average maximum temperatures during reproductive stages and the proportion of heat stress days during those times. Extreme heat stress was minimal during flowering, with more severe stress during early and late seed fill.

Precipitation was less typical than temperature, with certain months experiencing severe drought and other months experiencing far higher than average rainfall. April was a particularly dry month, with the Fort Collins site reporting 6.70% of average rainfall and Greeley reporting 34.81% of the monthly average. However, July was an uncharacteristically wet month, with Fort Collins receiving 144.92% of average rainfall and Greely receiving an astonishing 307.75% of the long-term average. Overall, the growing season at ARDEC experienced a higher than average level of drought, while LIRF had a wetter than average season.

At both sites, irrigation was performed once in the beginning of May to establish the seedlings and rescue irrigation was performed during a particularly hot and dry stretch in June when plants were visibly heat and drought stressed.

Phenotypic Analyses

ANOVA and Simple Statistics

Since treatments and locations were statistically significantly different, at the p=0.001 probability level, for the majority of traits, each location/treatment combination was analyzed as a unique environment, for a total of four environments. These four environments were ARDEC limited irrigation (ARDEC Dry or AD), ARDEC full irrigation (ARDEC Wet or AW), LIRF limited irrigation (LIRF Dry or LD), and LIRF full irrigation (LIRF Wet or LW). An analysis of variance (ANOVA) was performed over these four environments that displayed significant differences, at the p=0.001 probability level, between the genotypes and environments for all traits studied. Results of the ANOVA, as well as coefficient of variation (CV), trait means, and heritability can be found in Table 2.3. Environment and genotype were determined to be highly significant variables, with no evidence of genotype by environment interaction.

To provide a more nuanced view of trait values, a table of simple statistics was generated from LSMEANS and broken down by environment. These statistics include trait means, minimum and maximum values, and parental values, all of which are reported in Table 2.4 and Table 2.5. These statistics highlight the transgressive segregation that is evidenced in this population. For every trait measured, maximum and minimum values were well outside of the ranges exhibited by the parental genotypes. Performance of

parental genotypes is important in the context of a RIL population because genetic diversity in a RIL population is strictly dependent on the divergence of the two parents for traits of interest. Indeed, some of the measured traits exhibited large differences. For instance, Licalla produced an average of 2,180 kg ha^{-1} more biomass than Lindo under limited irrigation and 1,878 kg ha^{-1} more biomass under full irrigation. Licalla also produced an average of 264 kg ha^{-1} more seed yield than Lindo under limited irrigation and 224 kg ha^{-1} more seed yield than Lindo with full irrigation.

Physiological Traits

The RIL exhibited a wide range of values for dry plant biomass, from as low as 5,096 kg ha^{-1} under limited irrigation conditions at ARDEC and up to 15,661 kg ha^{-1} under full irrigation at LIRF. As would be expected, larger amounts of biomass were produced under full irrigation on average, with the highest mean biomass of 11,531 kg ha^{-1} observed in the fully irrigated treatment at LIRF. This corresponded to the highest total of precipitation and irrigation of any environment studied. Environment and genotype were both highly significant for biomass and heritability was calculated to be 0.50.

Plant height was the most highly heritable trait measured, with a heritability of 0.78. Mean plant heights were higher at LIRF than at ARDEC, with taller plants in the respective irrigated treatments.

Phenology Traits

The number of days to flower was another trait with a high heritability at 0.75. A low CV% of 2.61% was calculated for this trait as well. The mean number of days it took to

reach flowering across environments was 59.04 days. The environment that induced flowering the fastest was LIRF Dry, with a mean DTF of 55.50 days. The most delayed flowering was observed at ARDEC Dry, with a mean DTF of 63.59 days.

The amount of time that it took to reach physiological maturity was also hastened at LIRF. Under limited and full irrigation, it took 93.62 days and 94.04 days to reach maturity, respectively. A striking difference was observed at ARDEC where the limited irrigation treatment took 114.27 days to reach maturity and the full irrigation treatment took 111.07 days on average. The lowest CV% of all traits, 2.40%, was calculated for this trait. The heritability of DTM, however, was lower than DTF with a calculated heritability of 0.54. The timing of late season rains induced an indeterminate flowering response in many of the lines at ARDEC in both treatments. This caused later maturity dates and some missing data for this trait.

Seed Traits

Seed yield was relatively high in this study, with a mean yield of 1,270 kg ha^{-1} across all environments. A high amount of variation was observed for this trait, especially at ARDEC where both the lowest yield of 351 kg ha^{-1} and the highest yield of 2,908 kg ha^{-1} were measured in the limited and fully irrigated treatments, respectively. A high CV% of 31.27% reflected this high variability. The heritability of the trait was still quite high, however, at 0.67.

Seeds per pod ranged from 3.86 to 14.78, with the maximum and minimum counts both coming from the limited irrigation treatment at ARDEC. The lowest amount of heritability was observed for this trait with a value of 0.44. This was the only trait that did

not show a significant difference of replication within environments, indicating a higher amount of uniformity within environments, but the high CV% of 32.02% indicates a high amount of variation among environments.

Thousand seed mass averaged 1,243.00 mg across all environments. Means for TSM were lower under limited irrigation and higher with full irrigation. There was a range of slightly more than 1,000 mg with the lowest TSM in the environment with the least precipitation and irrigation, 836.00 mg at ARDEC Dry, and the highest TSM in the environment with the most precipitation and irrigation, 1,858.00 mg at LIRF Wet. Heritability for this trait was moderate at 0.58.

Harvest index was a calculated trait, rather than a directly measured trait, represented as a ratio of seed yield to total biomass. The heritability for this trait was 0.75, which is among the highest calculated, as well as being higher than the heritability of both of the traits from which it was derived. In general, harvest index was low, ranging from 0.06 to 0.21 and a mean over environments of 0.12.

Correlation Among Traits

A correlation analysis was performed to describe relationships among the variables in all environments. Four tables were created to show trait correlation coefficients and significance level within each of the four environments. Tables 2.6 and 2.7 show correlations for both full and limited irrigation treatments at ARDEC and LIRF, respectively. Two additional tables were created to visualize correlations across irrigation treatments. Table 2.8 shows correlations of all measured traits by irrigation treatment at ARDEC and Table 2.9 shows correlations of all measured traits by irrigation treatment at LIRF.

Yield showed a strong, positive correlation with biomass in all four environments, from 0.73 to 0.89 (p<0.0001). A strong, negative correlation with days to flowering was also apparent, ranging from -0.34 to -0.84 (p<0.0001). A surprising result was that seed yield was also significantly positively correlated to days to maturity, from 0.20 to 0.42, (p<0.0001) in all four environments. This indicates that an earlier flowering date and a later maturity date are both associated with higher yields. Yield was positively correlated with both thousand seed mass (r=0.25 to 0.68, p<0.0001) and seeds per pod (r=0.17 to 0.21, p<0.05). The stronger correlations with thousand seed mass show that higher seed mass was more of a contributing factor to yield than more seeds per pod.

In general, correlations were much stronger within environments than across environments. Although statistically significant correlations were observed for each trait with its counterpart in other environments, the results support analyzing each of the four environments separately.

Promising Genotypes

Four lines from this RIL population, SSD 10, SSD 177, SSD 87, and SSD 138, were previously identified as having potential for production in Colorado under both irrigated and dryland cropping systems (Jewett, 2013). However, ranking lines based on yield in all four environments did not identify any of these lines among the top performers. A full list of the 10 highest and lowest yielding lines in each environment can be found in Tables 2.10 and 2.11. Of the highest yielding lines, only one line was present in more than two environments. SSD 65 was ranked among the top five yielding lines in three environments, AD, LD, and LW. Other lines with potential are SSD 57, which ranked as number 2 at AD and

number 1 at AW and SSD 212, which ranked as the third highest yielding line at both LD and LW. Performance of the previously identified lines was extremely variable, both among lines and across environments.

QTL Analysis

A logarithm of odds (LOD) threshold from 2.74-3.12 was determined significant to detect QTL in this study at the genome-wise probability level of 0.05. The LOD scores were developed using a permutation analysis of 1000 permutations of the phenotypic data for each trait in each environment. Using these LOD thresholds, 16 QTL were identified from the current study. A summary of the QTL discovered and their effects can be found in Table 2.12. Additionally, data from Enjalbert (2011) was analyzed against the newly available genetic map (Singh et al., 2015) to detect nine more QTL. A summary of those QTL can be found in Table 2.13. The data from Enjalbert (2011) was also collected from four environments, ARDEC Dry 2009 (AD09), ARDEC Wet 2009 (AW09), ARDEC Dry 2010 (AD10), and ARDEC Wet 2010 (AW10). For the purpose of this thesis, QTL analysis of data from Enjalbert (2011) was restricted to traits in common with the current study. For more details on experimental design and data analysis from that study please refer to Enjalbert (2011).

QTL for Biomass and Plant Height

One significant QTL was detected in the AW environment for biomass on chromosome 9 at position 80.0, associated with marker Cs114391p534. This QTL accounts for 9.08% of the variation observed in the trait.

QTL were detected in three environments for plant height in this study. A QTL on chromosome 2 at position 48.0, corresponding to marker Cs111492p1088, was detected at AD, accounting for 9.73% variation in height. Another QTL was detected on chromosome 2 that was present in both LD and LW. That QTL was located at position 63.0 in the LD environment and position 65.0 in LW, both associated with marker Cs18343p1410. These loci accounted for 8.90% and 10.15% of the variation observed for plant height at LD and LW, respectively. QTL for other traits were also detected at this locus, including a QTL for yield in AW, harvest index in AW, and thousand seed mass in AW09. Another QTL for plant height was also detected in AW10. This QTL is on chromosome 13 at position 50.0, associated with marker Cs101470p1312, and accounts for 7.82% of the trait variation in that environment.

Days to Flowering QTL

A two-QTL model best explained variation in days to flowering at AD. A QTL on chromosome 7, position 38.4, associated with marker Cs12365p1897 and another QTL on chromosome 20, position 49.0, associated with marker Cs104210p1429, together explained 20.45% of the variation in days to flowering at that location. The QTL on chromosome 7 was also detected in the environment LD, explaining 12.15% of the trait's variation. A different locus on chromosome 7, position 17.0, associated with marker Cs94681p1024, explained 11.33% of the variation in days to flowering at LW.

Three QTL were also detected for this trait in the Enjalbert (2011) data. Two QTL in AD09 were detected on chromosomes 10 and 11, at positions 29.0 and 145.8, corresponding to markers Cs15186p639 and Cs117396p287. Together, these two QTL

explained 20.24% of the variation observed in this trait in this environment. A single QTL, explaining 8.54% of trait variation was detected in AW09 on chromosome 7, position 43.0, associated with marker Cs108120p486. Due to its close proximity to the QTL detected in AD and LD for the same trait, it is likely that this is the same QTL. This locus is also detected as a QTL for yield in LW.

QTL for Yield

Three QTL were detected for yield in the current study. No significant QTL were detected under limited irrigation, but QTL were detected in both fully irrigated environments. A two-QTL model explained 15.32% of the variation in yield at AW. One QTL was on chromosome 2, position 63.0, associated with marker Cs18343p1410. The other QTL was on chromosome 20, position 61.0, associated with marker Cs101720p130. A third QTL for yield was detected in LW on chromosome 7, position 36.3, associated with marker Cs196712p213. This QTL explained 7.82% of the observed variation in the trait.

Four QTL for yield were also detected in the Enjalbert (2011) data, one in each of the four environments. A QTL on chromosome 11, position 140.0, at marker Cs109651p703 explained 9.73% variation in yield in AD09. This QTL had a positive additive effect, where "aa" alleles contributed 91.33 kg ha^{-1} to yield. Interestingly, the other three QTL all exhibited a negative additive effect, where "aa" alleles decreased yield by 41.67 kg ha^{-1}, 5.50 kg ha^{-1}, and 61.67 kg ha^{-1} in AW09, AD10, and AW10 respectively. The QTL detected in AW09 is on chromosome 10, position 83.7, associated with marker *CS5G225. The other two QTL are both on chromosome 19, at positions 5.0 and 14.0, associated with markers CE_16501.184 and Cs119319p333 for environments AD10 and

AW10. Given the close proximity of the two QTL for yield from AD10 and AW10, it is possible that this is the same QTL.

QTL for Thousand Seed Mass and Harvest Index

Two QTL were detected for thousand seed mass in the 2014 study and one was detected in the Enjalbert (2011) data. The two QTL from 2014 were both detected in fully irrigated conditions. One was detected on chromosome 7, position 72.0, at marker Cs132446p317 in the AW environment and the other was detected in the LW environment on chromosome 16, position 48.0, at marker *CS1G57. The QTL explained 8.19% and 8.01% trait variation at AW and LW, respectively. The third QTL was also detected under full irrigation at AW09 on chromosome 2, position 63.0, at marker Cs18343p1410, explaining 11.60% of trait variation.

Three new QTL were also discovered for harvest index in the current 2014 study. One QTL was detected at AD and two at AW. The single QTL in AD was on chromosome 6, position 51.0, associated with marker Cs49966p165 and explained 7.96% of the variation in harvest index. A two-QTL model best explained trait variation at AW, with a LOD score of 6.68 and 15.54% of the phenotypic variation attributed to the model. A smaller effect QTL was on chromosome 2, position 56.0, at marker Cs18343p1410, while a larger effect QTL was detected on chromosome 6, position 42.5, at marker *CS2G88. It is possible that, due to a reasonably close proximity, the QTL on chromosome 6 in AW is the same QTL detected in AD for the same trait.

Pleiotropy, Epistasis, and Linkage

There were several QTL that were co-located in the same or very close positions for multiple traits. The QTL on chromosome 2, near position 63.0, were detected for plant height, yield, and harvest index in the 2014 data and for thousand seed mass in the Enjalbert (2011) data. Another QTL, on chromosome 7, near position 38.4, was detected for days to flowering in AD, LD, and AW09, as well as for yield in LW. It is impossible to tell, without further fine mapping efforts, whether or not these are single genes with pleiotropic effects, or multiple closely linked genes.

Scans for epistatic effects were performed during the Haley-Knott regression for QTL model selection, where all pairwise marker interactions were tested. Under this framework, interactions of QTL would be indicative of epistasis. However, there was no significant QTL interactions detected, so it was determined that there was no evidence of epistasis occurring for any of the traits measured.

DISCUSSION

In previous studies, heat and drought stress during reproductive periods have had a dramatic negative effect on camelina yields (Enjalbert, 2011; Jewett, 2013). Although heat and drought stress were not as severe in the 2014 growing season as in previous seasons, the effect of drought stress is evident in the differential yield, biomass, and thousand seed mass observed between the limited irrigation and full irrigation treatments at each location. Heat stress response can be more difficult to quantify. Heat and drought are also tied to one another, so separating the effects of the two in the field is a challenging task. Angadi et al. (2000) noted that heat stress is an exceptionally important and understudied

abiotic stress and that both short periods of extreme temperatures during reproductive stages and longer periods of milder temperature stress throughout the season can have significantly negative impacts on yield. Additionally, the threshold used for heat stress days (>32°C) was adapted for camelina by Enjalbert (2011) from a Baux et al. (2008) study, where effects of temperature on fatty acid composition and vegetative growth were of prime interest. Other studies in *Brassica* species have reported lower temperature thresholds for yield loss, such as Morrison and Stewart (2002), who determined that temperatures above 27°C negatively impacted yield in *Brassica* crops. Perhaps future studies will determine a more precise heat stress threshold for camelina, but it is widely acknowledged that temperate field crops are under heat stress during much of their growing season and that yield losses are due to a combination of heat stress and other abiotic and biotic stress factors (Angadi et al., 2000). However, heat stress is known to reduce flower numbers, flower fertility, and a plant's ability to support seed and pod growth (Morrison and Stewart 2002), so identifying genetic sources of heat tolerance in camelina is an important goal for future breeding programs.

The primary objective of this study was to identify QTL for traits associated with heat and drought tolerance and yield components and 25 previously unknown QTL for six different traits were identified. Two of the QTL identified in the 2014 data were also identified in the Enjalbert (2011) data. No single QTL was detected across all environments, suggesting that environmental factors significantly influence gene expression. Because of this, any QTL utilized from this study will require further verification of efficacy before implementation in a breeding program. Furthermore, this research is at the beginning of exploring heat and drought stress response in camelina.

Additional studies will need to be performed, assessing heat and drought response at various growth stages across controlled and field environments. Development of RIL populations with parents that are divergent for heat and drought stress response specifically would also help to elucidate these traits.

In the current study, seven QTL were identified for yield, with the most successful two-QTL combination accounting for 15.32% of variation in yield, i.e., conferring a yield boost of 86 kg ha^{-1}. However, this combination was detected under full irrigation, so other QTL may be more appropriate to improve yield for dryland production via marker-assisted selection.

Another trait that may have more breeding potential than yield *per se* is days to flowering. In all environments, days to flowering had a strong negative correlation with yield, indicating that earlier flowering lines produced higher yields. This correlation was stronger in both fully irrigated environments than in the limited irrigation environments, which may indicate this is more of a heat escape response than a drought escape response. With five different QTL identified for this trait and a high heritability (0.75), a combination of molecular breeding and traditional breeding methods may be used to further shorten days to flowering.

Two QTL were identified that have potential pleiotropic effects and/or close linkage. One of these QTL can be found on chromosome 2, in the region around 63 cM. This QTL significantly affected plant height, yield, thousand seed mass, and harvest index. Although yield and harvest index are directly related and all of the traits share some degree of correlation, it is highly unlikely that this same QTL would be detected for several different traits in different environments by chance. This suggests that a single locus is influencing

multiple traits and exhibiting pleiotropy, or that multiple loci controlling plant and seed development are in close linkage on chromosome 2. The other locus that was detected for multiple traits was on chromosome 7, near 38.4 cM. This QTL was significant for days to flowering in AD, LD, and AD09, and for yield in LW. This locus confers a robust effect on days to flowering, with a less stable pleiotropic effect on yield. Again, it is not possible to say with any degree of certainty whether this is one locus or multiple closely linked loci. Although the genetic map used in this study represents a significant advance in resolution compared to the Gehringer et al. (2006) map, it is only comprised of 579 loci. This number is very small compared to the maps available for major crops that are comprised of many thousands of SNP markers. Extensive fine mapping would need to be performed to identify individual genes controlling these complex traits.

Lastly, this study identified the 10 highest and lowest yielding lines in a variety of environments. Unfortunately, the four lines previously identified as being consistently high yielding lines (Jewett, 2013) did not appear in the top 10 lines from the 2014 environments. Additionally, one of those lines, SSD 138, turned up in the lowest yielding list, with the eighth lowest yield observed at AW. This underscores the dramatic effect of environment on trait expression. However, the lines that performed well, or poorly, across multiple environments may warrant more study. SSD 65 was in the top five yielding lines in three out of four environments and had a mean yield of 1,890 kg ha^{-1} across all four environments. Conversely, SSD 113 was in the 10 lowest yielding lines in three out of four environments, with a mean yield of 688 kg ha^{-1} across all four environments. More analysis of these outliers could act as an additional genetic resource to enhance understanding of specific allelic combinations (haplotypes) that contribute to, or detract from, yield stability.

In the short term, SSD 65 is worth testing more extensively to determine whether it is appropriate for production in Colorado without further breeding efforts.

Although genetic resources for camelina are coming online more rapidly in recent years, there is still much work to be done before camelina regularly outperforms other industrial oilseeds and is recognized as anything other than a niche crop. Few universities perform research on this crop and even fewer private companies devote resources to the species. There are, however, some positive aspects to camelina's underexploited nature. Close synteny and collinearity has been identified between camelina and well-researched *Arabidopsis* species (Kagale, 2014). The extensive genetic resources available for *A. thaliana* and *A. lyrata* can be mined to identify and manipulate agronomically important genes in camelina. Combined with new genomic sequence data, powerful resources are available to would-be researchers and breeders. And unlike major crops, camelina has not been extensively bred and exploited.

This underutilization of camelina may be viewed as a great opportunity. Whether the objective is to improve yield, oil quality traits, herbicide tolerance, or resistance to abiotic and biotic stress, the potential for making genetic gains remains mostly untapped. The scope of production in Colorado is small, with only a handful of early adopters willing to try the crop in on-farm trials. Camelina has great potential as a biofuel crop and specialty foods crop, but reliably high-yielding varieties for Colorado's eastern plains are not available. Effort and resources are required to realize the potential of this little known crop. Hopefully, breeding efforts will increase alongside the exciting genetic research that is being performed on this crop to elevate its status beyond an academic curiosity and secure camelina a place among the more widely cultivated oilseeds.

Table 2.1: Cultural and environmental conditions for the 2014 growing season at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, CO and the Limited Irrigation Research Farm (LIRF) in Greeley, CO.

Environmental Conditions						
Environment	Date of Planting	Date of Harvest	Precipitation and Irrigation[1] (mm)	Avg. Max. Temperature[2] (°C)	%HSD[3]	GDD[4](°C)
ARDEC Limited Irrigation	4/17/14	8/18/14	233.55	25.13	10.57	1574.51
ARDEC Full Irrigation	4/22/14	8/19/14	312.42	25.35	10.92	1556.78
LIRF Limited Irrigation	4/25/14	8/11/14	340.87	26.27	12.96	1489.23
LIRF Full Irrigation	4/24/14	8/12/14	391.67	26.25	13.64	1513.13

[1]Precipitation and irrigation includes rainfall and irrigation from date of planting to date of harvest

[2]Average Maximum Temperature is the mean of daily maximum temperatures from planting to harvest

[3]Percentage of Heat Stress Days during the growing season (where maximum temperatures exceeded 32°C)

[4]GDD=Growing degree days $(T_{max}+T_{min})/2-T_{base}$. T_{base}=4.4°C (McMaster and Wilhelm, 1997)

Table 2.2: 2014 growing season precipitation and long-term average precipitation at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, CO and the Limited Irrigation Research Farm (LIRF) in Greeley, CO.

Precipitation by Month						
ARDEC 2014 Growing Season						
	April	May	June	July	August	Season Total
Rainfall (mm)	3.56	78.74	39.12	68.83	26.42	216.66
Monthly avg. (mm)	53.09	66.04	50.55	47.50	35.56	252.73
% of avg.	6.70	119.23	77.39	144.92	74.29	85.73
LIRF 2014 Growing Season						
	April	May	June	July	August	Season Total
Rainfall (mm)	16.00	123.95	60.45	111.00	18.54	329.95
Monthly avg. (mm)	45.97	64.77	45.72	36.07	29.97	222.50
% of avg.	34.81	191.37	132.22	307.75	61.86	148.29

Table 2.3: Analysis of variance (ANOVA) results, coefficient of variation, mean, and broad-sense heritability estimates for the Lindo x Licalla camelina recombinant inbred line (RIL) population grown at Fort Collins and Greeley, Colorado in 2014.

		PHENOTYPIC TRAITS[1]							
Effect	DF	BIOMASS	PLHT	DTF	DTM	YIELD	SPP	TSM	HI
Environment (E)	3	***	***	***	***	***	***	***	***
Rep(E)	4	***	***	***	***	***	ns	***	***
Genotype (G)	187	***	***	***	***	***	***	***	***
G*E	561	ns	ns	ns	ns	ns	ns	ns	ns
CV%		18.07	5.48	2.61	2.40	31.27	32.02	12.95	20.83
Mean		10105.42	63.29	59.04	103.25	1270.00	8.21	1243.00	0.12
Heritability		0.50	0.78	0.75	0.54	0.67	0.44	0.58	0.75

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Phenotypic traits: BIOMASS= Dry plant biomass in kg ha^{-1}, PLHT= Plant height in cm, DTF= Days to flowering, DTM= Days to maturity, YIELD= Seed yield in kg ha^{-1}, SPP= Seeds per pod, TSM= 1000 seed mass in mg, HI= Harvest Index (YIELD/BIOMASS)

Table 2.4: Lindo x Licalla camelina recombinant inbred line (RIL) population descriptive statistics compared to parental values in a trial conducted at Fort Collins, Colorado in 2014.

Trait[1]	Treatment[2]	Unit	Mean	Sig[3]	Min	Max	Lindo	Licalla
Biomass	DRY	kg ha^{-1}	8436.26		5096.00	11909.00	6520.33	8839.33
	WET		10662.96	***	6854.67	13840.00	9488.33	11380.67
PLHT	DRY	cm	53.10		45.99	62.34	49.80	53.90
	WET		60.95	***	52.25	72.31	59.50	61.18
DTF	DRY	days	63.59	***	58.37	66.97	62.92	62.33
	WET		60.45		56.73	63.88	59.63	59.89
DTM	DRY	days	114.27	***	106.11	119.63	114.87	112.22
	WET		111.07		103.47	117.33	112.89	107.96
YIELD	DRY	kg ha^{-1}	1128.00		350.70	1964.00	948.07	1200.73
	WET		1453.00	***	596.00	2908.00	1593.00	1657.67
SPP	DRY	count	8.65	*	3.86	14.78	6.83	8.60
	WET		8.21		3.59	13.51	6.75	7.93
TSM	DRY	mg	1225.00		836.00	1659.00	1025.94	1245.71
	WET		1282.00	***	1022.00	1544.00	1201.49	1261.34
HI	DRY	ratio	0.13	ns	0.06	0.20	0.14	0.13
	WET		0.13		0.08	0.21	0.15	0.13

[1]Traits: BIOMASS= Dry plant biomass in kg ha^{-1}, PLHT= Plant height in cm, DTF= Days to flowering, DTM= Days to maturity, YIELD= Seed yield in kg ha^{-1}, SPP= Seeds per pod, TSM= 1000 seed mass in mg, HI= Harvest Index (YIELD/BIOMASS)

[2]Treatment: Dry=Limited irrigation treatment, WET= Fully irrigated treatment

[3]Sig indicates significance level of difference between environmental means, "ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

Table 2.5: Lindo x Licalla camelina recombinant inbred line (RIL) population descriptive statistics compared to parental values in a trial conducted at Greeley, Colorado in 2014.

Trait[1]	Treatment[2]	Unit	Mean	Sig[3]	Min	Max	Lindo	Licalla
Biomass	DRY	kg ha^{-1}	9791.90		7328.67	12869.67	7530.67	9570.67
	WET		11530.57	***	8827.00	15661.33	9887.00	11750.33
PLHT	DRY	cm	67.89		60.44	75.71	64.18	67.86
	WET		71.20	***	62.67	80.10	68.31	72.12
DTF	DRY	days	55.50		52.94	57.68	55.36	55.29
	WET		56.63	***	54.00	59.69	56.59	56.69
DTM	DRY	days	93.62		89.07	100.88	92.81	92.94
	WET		94.04	***	89.49	100.24	94.10	93.06
YIELD	DRY	kg ha^{-1}	1159.00		397.70	2160.00	807.00	1082.80
	WET		1340.00	***	562.27	2351.00	1183.00	1566.07
SPP	DRY	count	8.44	***	4.22	13.22	9.60	10.67
	WET		7.53		3.21	12.14	8.80	7.34
TSM	DRY	mg	1188.00		914.01	1539.00	947.77	1221.39
	WET		1277.00	***	997.87	1858.00	1262.49	1409.55
HI	DRY	ratio	0.12	ns	0.06	0.19	0.10	0.11
	WET		0.11		0.06	0.17	0.12	0.13

[1]Traits: BIOMASS= Dry plant biomass in kg ha^{-1}, PLHT= Plant height in cm, DTF= Days to flowering, DTM= Days to maturity, YIELD= Seed yield in kg ha^{-1}, SPP= Seeds per pod, TSM= 1000 seed mass in mg, HI= Harvest Index (YIELD/BIOMASS)

[2]Treatment: Dry=Limited irrigation treatment, WET= Fully irrigated treatment

[3]Sig indicates significance level of difference between environmental means, "ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

Table 2.6: Trait correlations of a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at Fort Collins, Colorado in 2014.

Full Irrigation

Limited Irrigation

Trait	YIELD	BM	HI	TSM	PLHT	SPP	DTF	DTM
YIELD	-	0.90 ***	0.81 ***	0.68 ***	0.60 ***	0.21 ***	-0.84 ***	0.21 ***
BM	0.73 ***	-	0.52 ***	0.63 ***	0.79 ***	0.13 **	-0.84 ***	0.26 ***
HI	0.86 ***	0.30 ***	-	0.59 ***	0.20 ***	0.23 ***	-0.66 ***	0.03 ns
TSM	0.55 ***	0.44 ***	0.46 ***	-	0.45 ***	0.12 *	-0.69 ***	0.16 **
PLHT	0.03 ns	0.17 ***	-0.09 ns	0.03 ns	-	0.08 ns	-0.64 ***	0.26 ***
SPP	0.17 **	0.08 ns	0.16 **	0.05 ns	0.03 ns	-	-0.13 *	0.01 ns
DTF	-0.65 ***	-0.46 ***	-0.60 ***	-0.38 ***	0.07 ns	-0.08 ns	-	-0.09 ns
DTM	0.36 ***	0.40 ***	0.21 ***	0.23 ***	0.12 *	0.10 ns	-0.15 **	-

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: BM=Biomass, SPP=Seeds per pod, DTF=Days to flowering, DTM=Days to Maturity, PLHT=Plant height, YIELD=Seed yield, TSM=1000 seed mass, HI=Harvest index

Table 2.7: Trait correlations of a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at Greeley, Colorado in 2014.

Full Irrigation

Limited Irrigation	YIELD	BM	HI	TSM	PLHT	SPP	DTF	DTM
YIELD	-	0.75 ***	0.89 ***	0.25 ***	0.25 ***	0.23 ***	-0.41 ***	0.42 ***
BM	0.73 ***	-	0.39 ***	0.16 **	0.42 ***	0.23 ***	-0.43 ***	0.30 ***
HI	0.91 ***	0.41 ***	-	0.25 ***	0.05 ns	0.17 ***	-0.32 ***	0.36 ***
TSM	0.51 ***	0.33 ***	0.50 ***	-	-0.03 ns	-0.09 ns	-0.13 **	0.19 ***
PLHT	0.18 ***	0.47 ***	-0.04 ns	0.09 ns	-	0.06 ns	0.03 ns	0.17 ***
SPP	0.13 *	0.06 ns	0.14 **	-0.07 ns	0.09 ns	-	-0.09 ns	0.11 *
DTF	-0.34 ***	-0.17 ***	-0.35 ***	-0.12 *	0.19 ***	-0.16 **	-	0.03 ns
DTM	0.20 ***	0.25 ***	0.12 *	0.19 ***	0.23 ***	-0.07 ns	0.24 ***	-

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: BM=Biomass, SPP=Seeds per pod, DTF=Days to flowering, DTM=Days to Maturity, PLHT=Plant height, YIELD=Seed yield, TSM=1000 seed mass, HI=Harvest index

Table 2.8: Trait correlations between limited irrigation and full irrigation treatments of a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at Fort Collins, Colorado in 2014.

		Full Irrigation							
	Trait[1]	BM	SPP	DTF	DTM	PLHT	YIELD	TSM	HI
Limited Irrigation	BM	**0.40**	0.14	-0.07	0.10	0.24	0.28	0.04	0.12
		***	ns	ns	ns	**	***	ns	ns
	SPP	0.06	**0.32**	-0.12	-0.13	-0.06	0.17	-0.03	0.22
		ns	***	ns	ns	ns	*	ns	**
	DTF	-0.01	-0.11	**0.55**	0.13	0.22	-0.35	-0.29	-0.53
		ns	ns	***	ns	**	***	***	***
	DTM	0.21	0.07	0.09	**0.21**	0.33	0.03	-0.16	-0.12
		**	ns	ns	**	***	ns	*	ns
	PLHT	0.13	0.03	0.15	0.13	**0.46**	-0.05	0.05	-0.18
		ns	ns	*	ns	***	ns	ns	*
	YIELD	0.26	0.20	-0.44	-0.03	-0.05	**0.50**	0.20	0.56
		***	**	***	ns	ns	***	**	***
	TSM	0.07	0.01	-0.16	-0.12	0.01	0.11	**0.46**	0.12
		ns	ns	*	ns	ns	ns	***	ns
	HI	0.06	0.15	-0.52	-0.10	-0.24	0.46	0.22	**0.66**
		ns	*	***	ns	***	***	**	***

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: BM=Biomass, SPP=Seeds per pod, DTF=Days to flowering, DTM=Days to Maturity, PLHT=Plant height, YIELD=Seed yield, TSM=1000 seed mass, HI=Harvest index

Table 2.9: Trait correlations between limited irrigation and full irrigation treatments of a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at Greeley, Colorado in 2014.

Limited Irrigation	Full Irrigation							
Trait[1]	**BM**	**SPP**	**DTF**	**DTM**	**PLHT**	**YIELD**	**TSM**	**HI**
BM	**0.39**	0.10	0.06	0.20	0.35	0.31	0.11	0.17
	***	ns	ns	**	***	***	ns	*
SPP	0.02	**0.08**	-0.10	-0.04	0.08	0.09	0.05	0.12
	ns	**ns**	ns	ns	ns	ns	ns	ns
DTF	-0.12	0.03	**0.67**	0.13	0.20	-0.28	-0.18	-0.34
	ns	ns	***	ns	**	***	*	***
DTM	0.24	0.05	0.25	**0.42**	0.35	0.05	-0.04	-0.08
	**	ns	***	***	***	ns	ns	ns
PLHT	0.20	0.03	0.27	0.26	**0.70**	0.06	0.10	-0.06
	**	ns	***	***	***	ns	ns	ns
YIELD	0.33	0.25	-0.25	0.06	0.10	**0.42**	0.10	0.36
	***	***	***	ns	ns	***	ns	***
TSM	0.12	-0.10	-0.13	0.02	0.11	0.06	**0.22**	0.02
	ns	ns	ns	ns	ns	ns	**	ns
HI	0.22	0.25	-0.36	-0.03	-0.05	0.36	0.06	**0.36**
	**	***	***	ns	ns	***	ns	***

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: BM=Biomass, SPP=Seeds per pod, DTF=Days to flowering, DTM=Days to Maturity, PLHT=Plant height, YIELD=Seed yield, TSM=1000 seed mass, HI=Harvest index

Table 2.10: Top 10 yielding lines from a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at Fort Collins and Greeley, Colorado under limited and full irrigation in 2014.

Top 10 Yielding Lines in Each Environment[1]				
Rank	**AD**	**AW**	**LD**	**LW**
1	SSD 20	SSD 57	SSD 156	SSD 65
2	SSD 57	SSD 126	SSD 241	SSD 55
3	SSD 13	SSD 82	SSD 212	SSD 212
4	SSD 65	SSD 24	SSD 64	SSD 233
5	SSD 119	SSD 175	SSD 65	SSD 30
6	SSD 55	SSD 152	SSD 170	SSD 167
7	SSD 62	SSD 226	SSD 201	SSD 157
8	SSD 221	SSD 242	SSD 173	SSD 119
9	SSD 64	SSD 76	SSD 226	SSD 171
10	SSD 156	SSD 125	SSD 126	SSD 180

Lines coded with colors represent lines that are present in the top 10 yielding lines in more than one environment. The colors do not indicate performance.

[1]Environments: AD=Agricultural Research Development and Education Center (ARDEC) under limited irrigation, AW=ARDEC under full irrigation, LD=Limited Irrigation Research Farm (LIRF) under limited irrigation, LW=LIRF under full irrigation.

Table 2.11: Ten lowest yielding lines from a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at Fort Collins and Greeley, Colorado under limited and full irrigation in 2014.

10 Lowest Yielding Lines in Each Environment[1]				
Rank	**AD**	**AW**	**LD**	**LW**
1	SSD 69	SSD 37	SSD 228	SSD 113
2	SSD 237	SSD 2	SSD 191	SSD 73
3	SSD 199	SSD 225	SSD 130	SSD 142
4	SSD 73	SSD 136	SSD 145	SSD 3
5	SSD 176	SSD 59	SSD 176	SSD 92
6	SSD 88	SSD 238	SSD 36	SSD 90
7	SSD 219	SSD 14	SSD 81	SSD 46
8	SSD 58	SSD 138	SSD 117	SSD 75
9	SSD 238	SSD 113	SSD 3	SSD 99
10	SSD 113	SSD 93	SSD 72	SSD 72

Lines coded with colors represent lines that are present in the top 10 yielding lines in more than one environment. The colors do not indicate performance.

[1]Environments: AD=Agricultural Research Development and Education Center (ARDEC) under limited irrigation, AW=ARDEC under full irrigation, LD=Limited Irrigation Research Farm (LIRF) under limited irrigation, LW=LIRF under full irrigation.

Table 2.12: QTL results and effects for a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, CO and at the Limited Irrigation Research Farm (LIRF) in Greeley, CO in 2014.

				Additive Effect						
Trait	Marker	Chromosome	Position	AD	AW	LD	LW	Std. Error	Individual LOD	Individual % Variation
BIOMASS (kg ha^{-1})	Cs114391p534	9	80.0	.	496.67	.	.	115.00		
Model LOD					3.76					
% Variation					9.08					
PLHT (cm)	Cs111492p1088	2	48.0	0.93	.	.	.	0.21		
	Cs18343p1410	2	63.0	.	.	0.89	.	0.21		
	Cs18343p1410	2	65.0	.	.	.	1.10	0.23		
Model LOD				4.04		3.68	4.23			
% Variation				9.73		8.90	10.15			
DTF (days)	Cs12365p1897	7	38.4	-0.11	.	.	.	0.10	5.24	11.28
	Cs12365p1897	7	38.4	.	.	-0.09	.	0.08		
	Cs104210p1429	20	49.0	0.44	.	.	.	0.10	4.445	9.47
	Cs94681p1024	7	17.0	.	.	.	-0.03	0.09		
Model LOD				9.04		5.12	4.75			
% Variation				20.45		12.15	11.33			
YIELD (kgha^{-1})	Cs18343p1410	2	63.0	.	32.20	.	.	28.90	3.261	7.282
	Cs101720p130	20	61.0	.	53.60	.	.	31.60	3.681	8.266
	Cs196712p213	7	36.3	.	.	.	94.8	24.30		
Model LOD					6.57		3.22			
% Variation					15.32		7.82			
TSM (mg)	Cs132446p317	7	72.0	.	29.50	.	.	7.32		
	*CS1G57	16	48.0	.	.	.	48.90	12.20		
				.	.	.	.			
Model LOD					3.38		3.30			
% Variation					8.19		8.01			
HI (ratio)	Cs49966p165	6	51.0	0.008	.	.	.	0.002		
	Cs18343p1410	2	56.0	.	0.0002	.	.	0.002	3.509	7.841
	*CS2G88	6	42.5	.	0.007	.	.	0.002	3.83	8.595
Model LOD				3.28	6.68					
% Variation				7.96	15.54					

AD=ARDEC Dry, AW=ARDEC Wet, LD=LIRF Dry, LW=LIRF Wet

Traits: BIOMASS=Dry plant biomass, PLHT=Plant height, DTF=Days to flowering, YIELD=Seed yield, TSM=1000 seed mass, HI=Harvest index (YIELD/BIOMASS)

Table 2.13: QTL results and effects for a Lindo x Licalla camelina recombinant inbred line (RIL) population grown at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, CO in 2009 and 2010 (Enjalbert, 2011) using a newly available genetic map (Singh et al., 2015).

				Additive Effect						
Trait	**Marker**	**Chromosome**	**Position**	**AD09**	**AW09**	**AD10**	**AW10**	**Std. Error**	**Individual LOD**	**Individual % Variation**
PLHT (cm)	Cs101470p1312	13	50.0	.	.	.	-0.04	0.33		
Model LOD							3.09			
% Variation							7.82			
DTF (days)	Cs15186p639	10	29.0	0.07	.	.	.	0.05	4.72	10.54
	Cs117396p287	11	145.8	0.22	.	.	.	0.11	4.40	9.80
	Cs108120p486	7	43.0	.	0.07	.	.	0.09		
Model LOD				8.60	3.39					
% Variation				20.24	8.54					
YIELD (kg ha^{-1})	Cs109651p703	11	140.0	91.33	.	.	.	1.37		
	*CS5G225	10	83.7	.	-41.67	.	.	1.54		
	CE_16501.184	19	5.0	.	.	-5.50	.	1.00		
	Cs119319p333	19	14.0	.	.	.	-61.67	1.66		
Model LOD				3.89	3.01	3.31	3.17			
% Variation				9.73	7.63	8.34	8.01			
TSM (mg)	Cs18343p1410	2	63.0	.	53.00	.	.	10.90		
Model LOD					4.69					
% Variation					11.60					

AD09=ARDEC Dry 2009, AW09=ARDEC Wet 2009, AD10=ARDEC Dry 2010, AW10=ARDEC Wet 2010
Traits:PLHT=Plant height, DTF=Days to flowering, YIELD=Seed yield, TSM=1000 seed mass

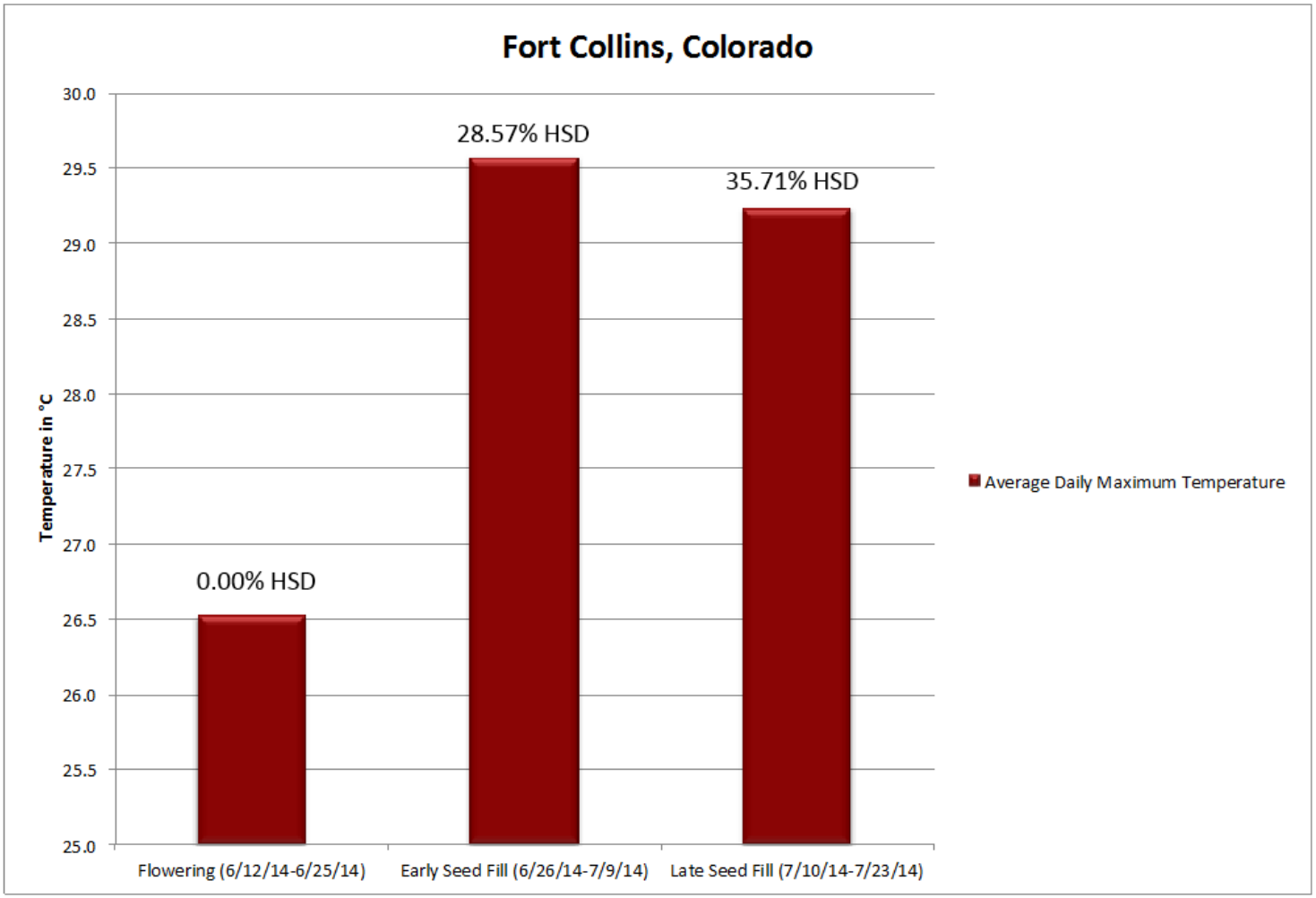

Figure 2.1: Average daily maximum temperatures, with percentage of heat stress days (HSD, days with maximum temperature >32°C), during reproductive growth stages of a Lindo x Licalla camelina recombinant inbred line (RIL) population at Fort Collins, CO in 2014.

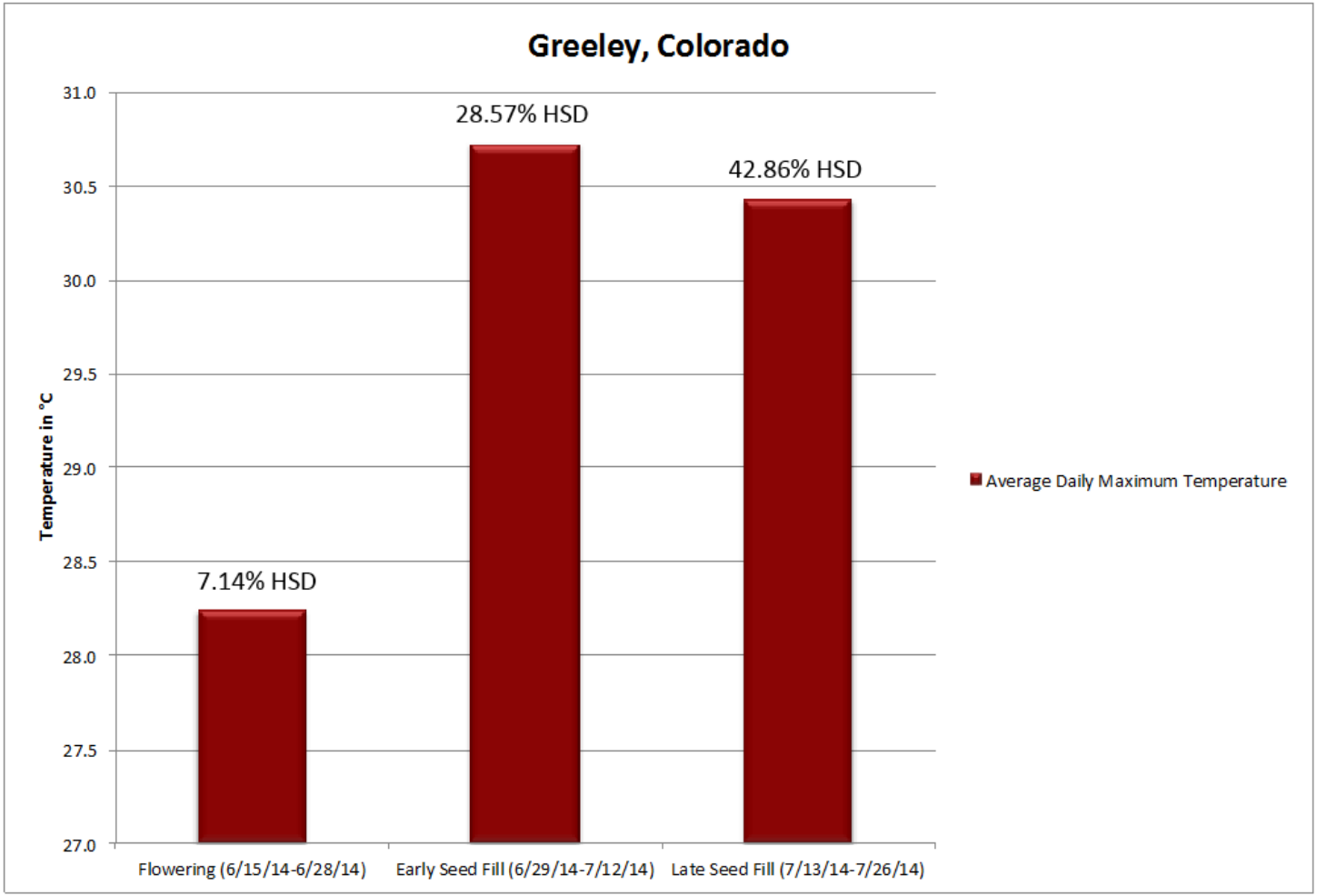

Figure 2.2: Average daily maximum temperatures, with percentage of heat stress days (HSD, days with maximum temperature >32°C), during reproductive growth stages of a Lindo x Licalla camelina recombinant inbred line (RIL) population at Greeley, CO in 2014.

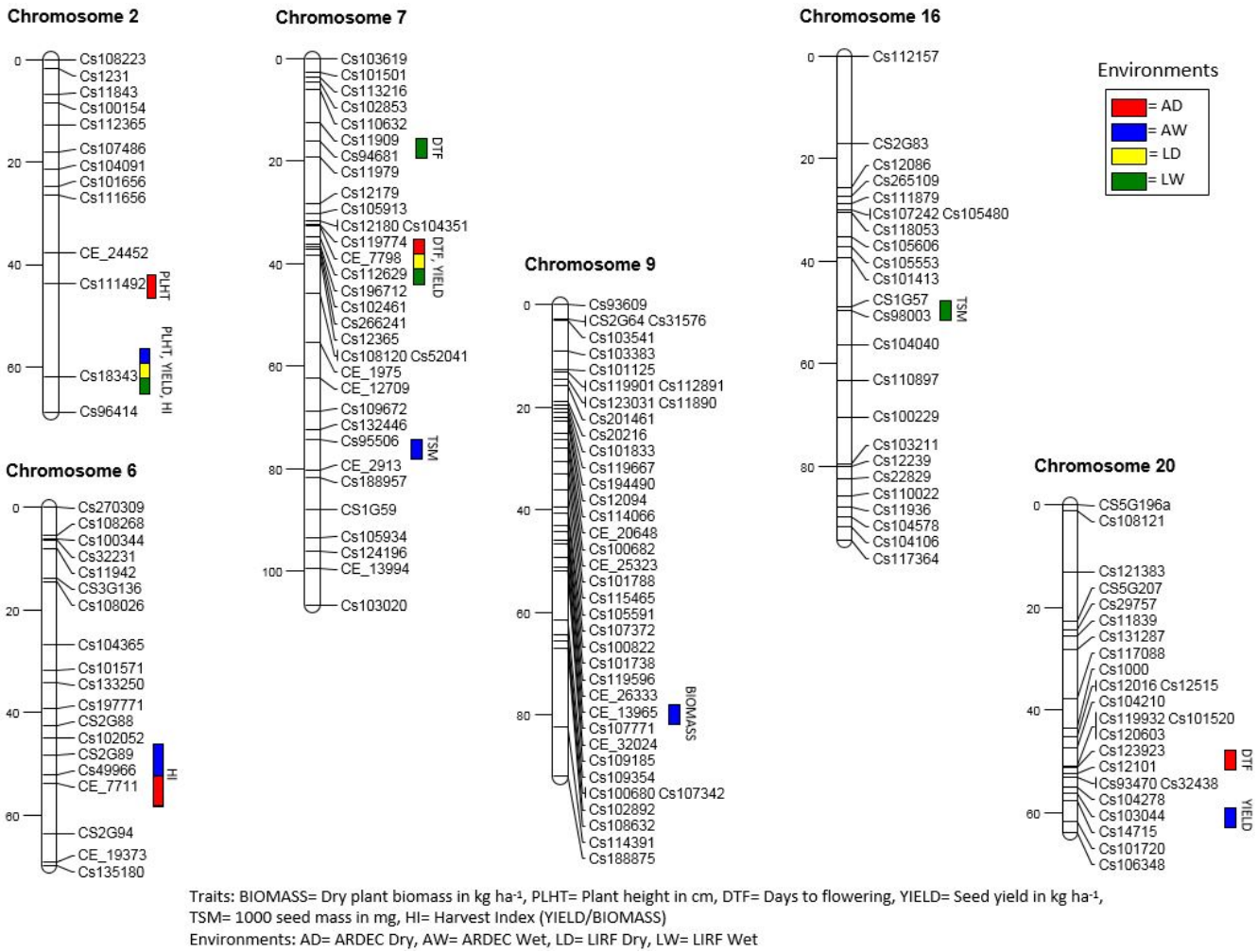

Figure 2.3: Approximate chromosomal locations of QTL detected in a Lindo x Licalla camelina recombinant inbred line (RIL) population during the 2014 growing season at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, CO and at the Limited Irrigation Research Farm (LIRF) in Greeley, CO.

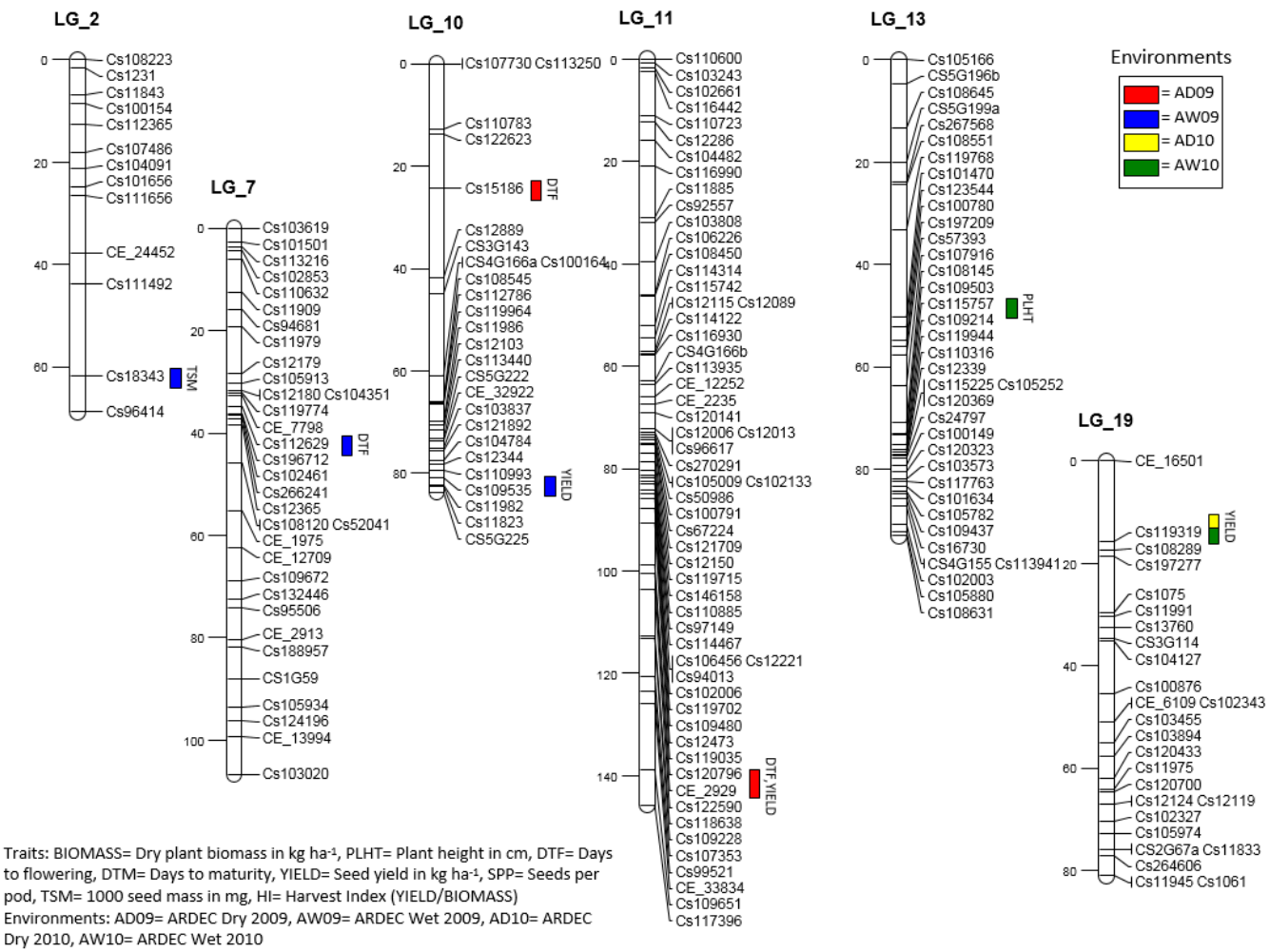

Figure 2.4: Approximate chromosomal locations of QTL detected in a Lindo x Licalla camelina recombinant inbred line (RIL) population, grown at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, Colorado in 2009 and 2010 (Enjalbert, 2011), using a newly available genetic map (Singh et al., 2015).

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CHAPTER 3:

BRASSICA CARINATA AS A POTENTIAL OILSEED BIOFUEL CROP IN COLORADO

SUMMARY

Ethiopian mustard (*Brassica carinata*) has traditionally been cultivated as a food crop in Ethiopia and neighboring countries, seeing little commercial production elsewhere in the world. In recent years, researchers have turned to *B. carinata* as an industrial oilseed with the potential to thrive in more arid conditions than more widely cultivated *Brassica* species. Canadian breeding programs, public and private, have attempted to adapt this crop to North America, with the intention of cultivating the crop in regions that are not favorable for *Brassica napus*. There have been some successes in this arena and there is now a niche *B. carinata* industry in Canada.

Research in Colorado has explored whether *B. carinata* could perform well as a rotation crop in dryland winter wheat (*Triticum aestivum*) cropping systems with potential for on-farm biofuel production. *B. carinata* did not perform as well as other *Brassicaceae* crops *Brassica juncea* and *Camelina sativa* due to susceptibility to flea beetles (*Phyllotreta cruciferae*), later flowering, and lower yields, so research was discontinued on *B. carinata* and more resources were devoted to the higher performing oilseeds. In collaboration with Agrisoma Biosciences (Saskatoon, Saskatchewan, Canada), we have evaluated early-maturing *B. carinata*. Agrisoma Biosciences provided early flowering and early maturing germplasm for us to evaluate under Colorado conditions.

The six lines tested took over 13 days longer to flower than unadapted African accessions that were previously evaluated. Mean yields of the trial across environments

were 669 kg ha^{-1}, which was also lower than the mean yields from previous trials. The commercial check cultivar used in the study, A100, outperformed all of the experimental lines by a wide margin with a mean yield of 1081 kg ha^{-1}. A lack of phenotypic diversity among the six lines did not allow for significant differences to be detected for the majority of traits measured. Although the cultivar A100 performed impressively compared to the other experimental lines of *B. carinata*, its yield and length of its life cycle were not sufficiently attractive compared to other alternative oilseeds to warrant continued research at this time.

INTRODUCTION

Ethiopian mustard (*Brassica carinata*), a.k.a. Abyssinian mustard, or gomenzer, has traditionally been cultivated as a food crop in the highlands of Ethiopia for its edible leaves and oil. Despite high levels of glucosinolate and erucic acid, locals value the crop for its high yields compared to other oilseed crops that are amenable to cultivation in the same ecological niche (Teklewold and Becker, 2005). Commercial production of *B. carinata* is limited outside of Ethiopia and neighboring countries, but an inherent tolerance to abiotic and biotic stresses under semi-arid conditions, combined with potential for biofuel production, phytoremediation, food and animal feed, medicine, and specialty fatty acids and chemicals, have given researchers worldwide reason to critically study this species (Taylor et al., 2010; Teklewold and Becker, 2005; Chen et al., 2011).

In Colorado, dryland winter wheat (*Triticum aestivum*) cropping systems cover more than 2.3 million acres (Clark, 2013), mainly on the semi-arid eastern plains. Typical dryland wheat production uses a wheat-fallow rotation, where wheat is planted in the fall

and harvested the following mid-summer. This would be followed by a year of fallow and planted the next fall, harvesting one crop every other year. A common alternative uses a wheat – spring crop – fallow rotation, where Colorado farmers add a spring-planted crop to the rotation, such as maize (*Zea mays*), sunflower (*Helianthus annus*), or proso millet (*Panicum miliaceum*). This increases water use effieciency over a traditional wheat-fallow rotation. *Brassicaceae* oilseeds have been identified as having potential to intensify these crop rotations by replacing the fallow portion of the rotation, further improving water use efficiency and creating a sustainable source of biofuel (Enjalbert et al., 2013, Peterson and Westfall, 2004). Adding an oilseed crop into a rotation during what would typically be a fallow period lowers production costs while eliminating competition with food crops (Keske, 2013). An assessment of various *Brassicaceae* oilseeds in Colorado praised *B. carinata* for its large seed size, but ultimately found it to be unsuitable for dryland production due to susceptibility to flea beetles (*Phyllotreta cruciferae*), later flowering, and lower yields than other alternative oilseeds (Enjalbert et al., 2013). After this study, research on *B. carinata* was suspended while other oilseed research continued.

In 2013, collaboration with Agrisoma Biosciences (Saskatoon, Saskatchewan, Canada) began with a common interest in advancing sustainable biofuel research. After providing oil for a study on engine performance and emissions of industrial oilseeds (Drenth et al., 2014), a dialogue began about new, early flowering and drought resistant lines of *B. carinata* that Agrisoma Biosciences was developing. From this relationship, new interest in *B. carinata* as an alternative oilseed emerged and it was agreed that a two-year trial would assess the adaptability of short-season *B. carinata* in Colorado.

The aims of this research are to (1) assess the field performance of six lines of *B. carinata* in Colorado's semi-arid environment under limited and full irrigation; (2) determine if any particular lines perform well and have potential for local production; and (3) examine relationships among various yield components and physiological traits to identify characteristics correlated with favorable yield and/or drought tolerance.

MATERIALS AND METHODS

Plant Materials

Agrisoma Biosciences provided all seed for this trial. Five experimental lines were provided that had been previously identified as having early flowering and early maturing qualities in Canada. Since Agrisoma Biosciences is a private company, pedigree information was not provided and molecular analysis was not performed to respect the company's intellectual property. The five lines provided were as follows: 080814EM, 110994EM, 110999EM, 111010EM, and 110996EM. In addition, a commercial cultivar developed by Agrisoma Biosciences, A100, was provided as a check.

Environmental Conditions

During the 2013 and 2014 growing seasons, trials were conducted at the Agricultural Research Development and Education Center (ARDEC) in Fort Collins, Colorado. This facility is at latitude 40.65 and longitude -105.00, with an elevation of 1,557 m and average annual precipitation of 408 mm. The soil texture at this location is a sandy clay loam. ARDEC is equipped with an overhead linear sprinkler irrigation system, which was used throughout the study. Weather data for ARDEC and Fort Collins were obtained

from the Colorado Agricultural Meteorological network (COAGMET) and the National Oceanic and Atmospheric Administration (NOAA) National Climatic Data Center (NCDC).

Seedbed preparation included disking the soil and incorporating approximately 39 kg ha^{-1} of nitrogen in the form of urea and 2.34 L ha^{-1} of the herbicide Sonolan (ethafluralin) with a roller harrow. Agrisoma Biosciences' production manual (2014) recommends a target plant density from 86 to 183 plants per square meter, with the high end recommended for challenging conditions. So the high rate was used to calculate seeding rate, overseeding by approximately 45% to account for any issues with germination or seedling mortality. This led to a final seeding rate of approximately 325 seeds per square meter or 3,253,392 seeds per hectare. During the 2013 season, Sevin XLR Plus (Carbaryl) was applied twice, at a rate of 0.85 L ha^{-1}, to control flea beetles. For the 2014 season, seed was treated with Prosper (Clothianidin (21.75%), Carboxin (3.81%), Trifloxystrobin (0.544%), Metalaxyl (0.408%), 1,2-Propanediol, Titanium dioxide), which was sufficient to control flea beetle damage.

A second trial location was planted in 2014 in Rocky Ford, Colorado (latitude 38.05, longitude -103.72). Unfortunately, crop failure was experienced due to a major rain and hailstorm, so no data was collected from this location.

Experimental Design

This study utilized a Randomized Complete Block (RCB) design with two treatments, limited irrigation (Dry) and full irrigation (Wet). Seven row plots were machine planted with a wheat drill and each plot was 4.57 m long and 1.89 m wide. Spacing between ranges was 1.52 m, while spacing between adjacent plots was 0.30 m. Each

genotype was replicated three times in each treatment for a total of six blocks, three with limited irrigation and three fully irrigated. The fully irrigated treatments received 25.4 mm of irrigation weekly, unless there was 25.4 mm or greater rainfall in a given week. The limited irrigated treatments were mostly rainfed. If the plants were visibly drought stressed, i.e., wilting, a half-rate (12.7 mm) irrigation was applied as "rescue irrigation".

Data Collected

Weather Data

Weather data was collected for the growing season from COAGMET including precipitation, maximum daily temperature and minimum daily temperature. From these data several other parameters were calculated, including number of heat stress days (HSD, days during the growing season where maximum temperatures exceeded 29.5°C (Morrison and Stewart, 2002)), %HSD (number of HSD/total number of days in season) and total growing degree-days (GDD), calculated as $((T_{max}+T_{min})/2)-T_{base}$ where T_{base}=4.4°C (McMaster and Wilhelm, 1997) for each day and summed over the season.

More resources were available during the 2013 growing season, so more extensive collection of phenotype data on subsampled plants was performed in addition to the plot level traits. Only plot level traits were measured during the 2014 growing season.

Plot Level Traits

Days to flowering (DTF) were calculated as the number of days from the date of planting to the date when 50% of the plants in a given plot showed at least one open flower. Days to maturity (DTM) were similarly calculated as the number of days between

the date of planting and the date when 50% of the plants in a given plot reached physiological maturity. A plant was considered to be physiologically mature when the main stem of the plant changed from green to brown.

At maturity, the height of the first branch (BRHT) was measured, from the soil surface to the base of the first branch off of the main stem, on five random plants per plots and the mean of these measurements represent height of first branch. Plots were direct harvested with a Wintersteiger Nurserymaster Elite (Ried im Innkries, Austria) small plot combine and the seed was collected, cleaned, and weighed to determine seed yield. Thousand seed mass (TSM) was also measured, by weighing 1,000 randomly sampled seeds from each plot.

Subsampled Plant Traits

In 2013, five plants per plot were chosen at random, when a plot was scored for flowering and tagged for more extensive data collection. For each of these plants, height (PLHT) was measured from the soil surface to the highest point of the plant, during flowering, to the closest centimeter. A mean of these measurements was calculated to represent plant height of each plot. Number of branches (NOBR) and number of leaves (NOLV) were counted for these subsampled plants as well. Five flowers were collected from each plot and dissected in the lab. Ovule counts were performed manually under a microscope and pollen viability was determined via imaging techniques as described in Mudd and Arathi (2012).

During late flowering to early seed fill, number of total flowers and pods was counted (FLPD), along with number of total aborted flowers and pods (ABORT). A

calculated trait was derived from these floral counts to estimate reproductive success (RS), calculated as a percentage, (FLPD/FLPD+ABORT)*100. The tagged plants were then harvested by hand and dried in an oven at 60°C for five days. The dried plants were weighed to obtain an estimate of dry biomass (PLBM). Two pods from each of the five subsampled plants, for a total of 10 pods per plot, were opened and the seeds were counted manually. Mean number of seeds per pod (SPP) was calculated from the manual seed counts by plot. The plants were then threshed by hand to determine individual plant seed yield (PLYLD) and a harvest index (HI) ratio was calculated from these terms as (PLYLD/PLBM).

Statistical Analyses

Phenotypic data were analyzed using the statistical web-based program SAS Studio (SAS Institute, Inc., Cary, NC). The PROC MIXED function of SAS was used to create a mixed model, with environment and replication as fixed effects and genotype as random effects. Least squares means were obtained for the traits from these models for each trait using the LSMEANS option. Pearson correlation coefficients among least squares means for all traits were calculated using the PROC CORR function of SAS. Broad-sense heritability on a plot-mean basis combined over environments was calculated by hand for all traits, using variance components from the SAS function PROC VARCOMP with the 'method=REML' option, according to a formula provided by Clay Sneller from the Ohio State University (personal communication) adapted from Hallauer et al. (1988) for plot-mean data. The broad-sense heritability formula is as follows:

$$\text{Heritability} = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma^2}{re} + \frac{\sigma_{GE}^2}{e}}$$

In this formula, σ^2_G represents genotypic variance, σ^2 represents error variance, σ^2_{GE} represents genotype x environment variance, *r* represents replications, and *e* represents number of environments.

RESULTS

Environmental Conditions

The weather during the 2013 and 2014 growing seasons was substantially different, with a more intense level of drought experienced in 2013. The 2014 growing season was wetter, with much of the rainfall late in the season. Monthly precipitation was nearly double in July and August compared to 2013. A summary of precipitation, maximum temperatures, and total growing degree days is presented in Table 3.1 along with a breakdown of precipitation by month in Table 3.2. In 2014, the abundance of late season rain triggered an indeterminate flowering response in all of the *B. carinata* lines, resulting in a second round of flowering as the first set of pods matured. Figure 3.1 shows an example of ripe pods juxtaposed with new flowers and young seed pods on the same plant. This response was nearly uniform across both treatments and all genotypes. Because of this second round of flowering, full maturity was never reached in the 2014 season. When the first round of seed pods began to shatter, the decision was made to harvest the study before major seed loss occurred. This was in contrast to the 2013 season, where comparatively dry late-season weather allowed the crop to dry down properly and harvesting was performed at an appropriate level of maturity. Pest pressure was also a

factor during both years. Flea beetles caused some damage during the early stages of growth and pesticides (Prosper and Sevin XLR Plus by Bayer CropScience (North Carolina, USA)) were utilized to limit this damage. Near maturity, false cinch bugs (*Nysius raphanus*) were present in all plots during both years as well, causing visible damage to siliques. No pesticide was applied to control the false cinch bugs since the infestation happened late in the season.

Phenotypic Analyses

Plot Level Traits

An analysis of variance (ANOVA) was performed across all environments to determine significance of the variables in the mixed model. Table 3.3 contains the results of the ANOVA, as well as the coefficient of variation (CV%), and estimates of broad sense heritability for the traits in common over both years of the study. There was a highly significant effect of environment, at the 0.001 probability level, for all plot level traits. There was also a highly significant genotype effect (0.001 probability for DTF, DTM, and YIELD and 0.01 probability for TSM) for all of these traits except BRHT. This means that variation in BRHT was not attributable to genotype. Heritability for BRHT was calculated to be 0.00, indicating that this is not a heritable trait based on the sample size and lines evaluated. Genotype by environment interaction was not detected for any of the plot level traits measured.

Since genotype was highly significant in the yield model and this parameter is important in assessing overall performance of the individual lines, pairwise comparisons of mean yields were performed. The results of these comparisons are presented in Table 3.4.

Only significant comparisons were presented in this table. Interestingly, the only significant differences detected among lines were when comparing A100 to the other five lines. The genotype A100 exhibited the highest mean yield across environments, at 1081 kg ha^{-1}, ranging from 399 to 580 kg ha^{-1} greater than the other lines. A100 also had the highest yield when data was sorted by irrigation treatment. Mean yields for A100 under limited irrigation and full irrigation were 917 kg ha^{-1} and 1218 kg ha^{-1}, respectively.

The mean yield of all lines across environments was 669 kg ha^{-1}, with a heritability of 0.77. Overall mean yield by treatment was 495 kg ha^{-1} under limited irrigation and 837 kg ha^{-1} under full irrigation. Days to flowering and DTM were the most highly heritable traits at 0.85 and 0.90, respectively. Across all environments and genotypes, it took an average of 73.59 days to flower and 134.14 days to reach maturity. Although pairwise comparisons were not significant at the 0.05 probability level across environments for DTF, A100 had the lowest mean in this category across environments of 71.45 days. Thousand seed mass had an overall mean of 3.92 g and a heritability of 0.59. Additional information about these traits is available in Table 3.5, which shows means, standard deviation, and maximum and minimum values for these traits by environment and year. As was previously mentioned, data for DTM was not available in the 2014 season due to indeterminate flowering.

Pearson correlation coefficients and significance levels for these traits, across all environments, are provided in Table 3.6. Yield was significantly negatively correlated with DTF (r=-0.33, p<0.01). Yield also shared a strong positive correlation with TSM (r=0.52, p<0.001). This means that yield was higher in lines that flowered earlier and had larger seed size. Height of first branch was significantly positively correlated with DTF (r=0.74,

p<0.001), indicating that lines that flowered later had higher first branches. Days to maturity shared a very strong negative correlation with thousand seed mass (r=-0.89, p<0.001), showing that late maturing lines exhibited reduced seed size.

Subsample Level Traits

An ANOVA was also performed across treatments for the 2013 subsample level traits, the results of which can be found in Table 3.7. The only traits for which a significant genotype effect was detected were HI, PLHT, and SPP. Irrigation treatment effect was significant for all three of these traits as well, with significant genotype by environment interaction for HI and PLHT. Mean HI was 0.18, with a calculated heritability of 0.84. Mean PLHT across treatments was 83.99 cm, with a lower heritability of 0.33. Average number of seeds per pod was 14.27 and heritability for the trait was 0.76.

Subsample level traits were combined with plot level traits from 2013 to determine relationships among primary and secondary traits. The Pearson correlation coefficients and significance level of these relationships can be found in Table 3.8. A strong positive correlation was detected between PLHT and DTM, (r=0.75, p<0.001), showing that taller plants took longer to mature. Days to maturity was also strongly negatively correlated with HI and TSM (r=-0.82, p<0.001; r=-0.89, p<0.001), indicating a longer time to maturity was correlated with a lower yield to biomass ratio and smaller seed size. Harvest index and TSM were highly correlated (r=0.81, p<0.001) to each other, with larger seed size corresponding to more favorable harvest indices. Thousand seed mass also had a strong negative correlation with PLHT (r=-0.80, p<0.001), where taller plants exhibited smaller seed size and vice versa. Another strong correlation was detected between NOLV and FLPD

(r=0.71, p<0.001). This correlation, however, is an artifact of randomly selected larger plants with more leaves also having more flower sites. Seeds per pod, POLVIA and OVCNT were not significantly correlated with any traits measured.

DISCUSSION

Previous research at Colorado State University determined that *B. carinata* was inferior to other alternative oilseed crops due to a susceptibility to flea beetles, later flowering, and lower yields (Enjalbert et al., 2013). In a related study, Enjalbert (2011) evaluated a diverse set of 30 accessions of *B. carinata*, 94 accessions of *B. juncea*, and 81 of *Camelina sativa* in three environments, two dryland and one irrigated. This study reported mean yields of 795 kg ha^{-1} for *B. carinata*, 973 kg ha^{-1} for *B. juncea* and 1,383 kg ha^{-1} for *C. sativa*. The mean yield of the current study over four environments (two under limited irrigation and two fully irrigated) was 669 kg ha^{-1}, which is lower than the average of the 30 unadapted accessions tested by Enjalbert (2011). For comparison, a concurrent study of 186 recombinant inbred lines of *C. sativa*, conducted at the same location in 2014, had a mean yield of 1,291 kg ha^{-1} under nearly identical conditions. Enjalbert (2011) also reported mean days to flowering for *B. carinata* under dryland and irrigated conditions as 59.88 and 60.13 days, respectively. In the current study, mean DTF was 73.30 days under limited irrigation and 73.53 days under full irrigation over two years. These lines took over 13 days longer on average to flower than previously evaluated *B. carinata* lines, which were deemed to have unacceptably long flowering times. In short, the early flowering lines of *B. carinata* from Agrisoma Biosciences do not appear to be better adapted to the climate of Colorado than previously tested accessions.

A secondary objective was to identify any promising lines that have potential for local production. Among the six lines that were tested, there was a clear frontrunner. The commercial check variety, A100, significantly outyielded all of the experimental lines. With a mean yield of 1081 kg ha^{-1}, A100 yielded 399 kg ha^{-1} more on average than the closest competing line. A100 also exhibited the earliest flowering with a mean of 71.45 days to flowering, although this number was not statistically significantly different from the other lines in the study. None of the five experimental lines stood out as better than the others, with few detectable differences in the primary traits. Although A100 was clearly the best genotype in the trial, it did not perform well enough to recommend for on-farm testing over other alternative oilseed crops, such as *C. sativa*.

Lastly, relationships among a variety of secondary traits were examined to determine if they played a role in increasing yield by heat or drought tolerance or by other means. The only meaningful correlation that was found with yield was a negative correlation with days to flowering. The Pearson correlation coefficient for this relationship was -0.50 in 2013 and -0.33 overall. This relationship was not surprising considering that escape is one of the most common heat and drought tolerance strategies. Lines that took longer to flower were more likely to experience heat and drought stress, which appears to be the case for this trial. Heat stress can also cause pollen sterility and ovule damage (Morrison and Stewart, 2002), but neither pollen viability nor counts of ovules were statistically different among genotypes or significantly correlated with any traits. It can be inferred from this that either the stress was not intense enough in the 2013 season to make either of these traits a limiting factor, or there was a roughly similar response to stress across the small number of genotypes studied. Mean pollen viability was 0.89, so it is likely

that fertile pollen was abundant despite any stress experienced. The reproductive success trait ((healthy flowers/all flower sites)*100) was not any more informative, with no significant differences among genotypes. The secondary trait of interest to Agrisoma Biosciences, height of first branch, did not differ significantly among genotypes. Additionally, it was negatively correlated, -0.39 at the 0.05 probability level, with days to flowering. This means that higher first branches are correlated with later flowering. Combined with a heritability of 0.00, this is a trait that will be very difficult to improve.

Agrisoma Biosciences reports mean yield of A100 from 2011 to 2013 as approximately 2,330 kg ha^{-1} (Agrisoma, 2014). While it may be possible to achieve consistent yields in this range in the Canadian prairie, the lines tested in this trial indicate that the genetics are significantly less adapted to growing in the more southern, semi-arid environment of Colorado's eastern plains. There was not a great deal of genetic variation present among the lines for the traits measured, with only six of the 17 traits measured showing any type of significant effect of genotype. If research on *Brassica carinata* as a dryland oilseed crop for production in Colorado is to continue in the future, germplasm that has proven to yield consistently well in semi-arid environments should be a requisite starting point. In the meantime, oilseed research at Colorado State University will likely continue to focus on more promising members of the diverse *Brassicaceae* family.

Table 3.1: Environmental conditions for the 2013 and 2014 growing seasons in Fort Collins, CO.

Environment	Date of Planting	Date of Harvest	Growing Season Precipitation[1] (mm)	Avg. Max. Temperature[2] (°C)	%HSD[3]	GDD[4](°C)
ARDEC Dry 2013	3/29/13	8/2/13	143.26	23.14	28.57	1338.73
ARDEC Wet 2013	3/29/13	8/14/13	269.75	23.50	26.81	1519.68
ARDEC Dry 2014	4/11/14	9/3/14	251.08	24.87	23.45	1817.91
ARDEC Wet 2014	4/11/14	9/4/14	330.45	24.86	23.29	1831.06

[1]Growing Season Precipitation includes rainfall and irrigation from date of planting to date of harvest

[2]Average Maximum Temperature is the mean of daily maximum temperatures from planting to harvest

[3]Percentage of Heat Stress Days during the growing season (where maximum temperatures exceeded 29.5°C)

[4]GDD=Growing degree days $(T_{max}+T_{min})/2-T_{base}$. T_{base}=4.4°C (McMaster and Wilhelm, 1997)

Table 3.2: Comparison of 2013 and 2014 growing season precipitation with long-term averages in Fort Collins, CO.

Precipitation by Month								
ARDEC 2013 Growing Season								
	March	April	May	June	July	August	September	Season Total
Rainfall (mm)	5.33	30.23	35.81	11.94	38.86	12.70	.	134.87
Monthly avg. (mm)	36.07	53.09	66.04	50.55	47.50	35.56	.	288.80
% of avg.	14.79	56.94	54.23	23.62	81.82	35.71	.	46.70
ARDEC 2014 Growing Season								
	March	April	May	June	July	August	September	Season Total
Rainfall (mm)	.	3.56	78.74	39.12	68.83	26.42	23.11	239.78
Monthly avg. (mm)	.	53.09	66.04	50.55	47.50	35.56	35.05	287.78
% of avg.	.	6.70	119.23	77.39	144.92	74.29	65.94	83.32

Table 3.3: ANOVA results, coefficient of variation, and heritability estimates across environments for six *B. carinata* lines grown in Fort Collins, CO in 2013 and 2014.

		Trait[1]				
Effect	**DF**	**DTF**	**DTM**	**BRHT**	**YIELD**	**TSM**
Environment	3	***	***	***	***	***
Rep(Environment)	4	ns	*	ns	ns	ns
Genotype	5	***	***	ns	***	**
Genotype*Environment	15	ns	ns	ns	ns	ns
CV%		2.11	1.58	40.83	34.10	12.01
Mean		73.59	134.14	11.03	668.84	3917.06
Heritability		0.85	0.90	0.00	0.77	0.59

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: DTF=Days to flowering, DTM=Days to maturity, BRHT=Height of first branch, YIELD=seed yield, TSM= 1000 seed mass

Table 3.4: Mean yield pairwise comparisons of significantly different genotypes for six *B. carinata* lines grown in Fort Collins, CO in 2013 and 2014.

ENTRY Comparison	Difference Between Means (kg ha^{-1})	95% Confidence Limits		Significance Level
A100 - 110999EM	399.30	64.30	734.30	***
A100 - 110996EM	445.90	110.90	780.90	***
A100 - 110994EM	460.10	125.10	795.10	***
A100 - 080814EM	566.70	231.70	901.70	***
A100 - 111010EM	580.20	252.20	908.10	***

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

Table 3.5: Descriptive statistics for plot level traits by environment for six *B. carinata* lines grown at Fort Collins, CO in 2013 and 2014.

			Limited Irrigation				Full Irrigation			
Trait	**Environment**	**Unit**	**Mean**	**Std Dev**	**Min**	**Max**	**Mean**	**Std Dev**	**Min**	**Max**
Days to flowering	ARDEC 2013	days	77.39	1.82	73.00	82.00	77.28	1.56	73.00	80.00
	ARDEC 2014		69.20	2.70	66.00	72.00	69.78	2.16	66.00	72.00
Days to maturity	ARDEC 2013	days	126.33	3.25	116.00	130.00	141.94	2.65	136.00	145.00
	ARDEC 2014		-	-	-	-	-	-	-	-
Height of first branch	ARDEC 2013	cm	17.86	6.04	6.60	25.66	20.56	6.43	11.40	34.40
	ARDEC 2014		2.60	0.90	1.60	4.70	1.69	0.48	0.88	2.64
Yield	ARDEC 2013	kg ha^{-1}	580.97	276.84	294.91	1247.00	507.46	470.88	72.34	1541.00
	ARDEC 2014		392.29	181.97	102.24	691.90	1131.00	244.81	756.15	1577.00
1000 Seed Mass	ARDEC 2013	mg	4230.00	263.53	3868.00	4817.00	2453.00	477.29	1933.00	3588.00
	ARDEC 2014		4130.00	708.46	2533.00	5384.00	4728.00	628.18	3352.00	5740.00

Table 3.6 Trait correlations for plot level traits across environments for six *B. carinata* lines (*n*=72, except for DTM (*n*=36)) grown at Fort Collins, CO in 2013 and 2014.

Trait[1]	DTF	DTM	BRHT	YIELD	TSM
DTF	-	0.10 ns	0.74 ***	-0.33 **	-0.44 ***
DTM	-	-	0.08 ns	-0.32 ns	-0.89 ***
BRHT	-	-	-	-0.21 ns	-0.56 ***
YIELD	-	-	-	-	0.52 ***
TSM	-	-	-	-	-

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: DTF=Days to flowering, DTM=Days to maturity, BRHT=Height of first branch, YIELD=seed yield, TSM= 1000 seed mass

Table 3.7: ANOVA results, coefficient of variation, and heritability estimates across treatments for subsample level traits of six *B. carinata* lines grown in Fort Collins, CO in 2013 and 2014.

	Trait[1]											
Effect	**NOBR**	**NOLV**	**FLPD**	**ABORT**	**PLBM**	**PLYLD**	**RS**	**HI**	**PLHT**	**SPP**	**POLVIA**	**OVCNT**
Treatment	***	*	**	ns	**	ns	**	***	***	*	ns	ns
Rep(Treatment)	ns	ns	ns	ns	ns	ns	***	ns	*	ns	ns	ns
Genotype	ns	ns	ns	ns	ns	ns	ns	***	**	*	ns	ns
Genotype*Environment	ns	ns	ns	ns	ns	ns	ns	**	**	ns	ns	ns
CV%	16.92	35.11	46.20	46.42	75.05	63.07	11.88	17.16	5.31	12.72	8.10	7.99
Mean	9.48	53.28	125.93	82.73	16.28	2.52	58.26	0.18	83.99	14.27	0.89	17.38
Heritability	0.11	0.00	0.20	0.28	0.00	0.09	0.59	0.84	0.33	0.76	0.55	0.62

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: DTF=days to flowering, DTM= Days to maturity, BRHT=height of first branch, YIELD=seed yield, TSM=1000 seed mass, NOBR=number of branches, NOLV=number of leaves, FLPD=total number of flowers and pods, ABORT=aborted flowers and pods, PLBM=dry plant biomass, PLYLD=plant yield, RS=reproductive success %HI=harvest index, PLHT=plant height, SPP=seeds per pod, YIELD=total seed yield (kg ha-1), POLVIA=pollen viability ratio,OVCNT=ovule count

Table 3.8: Trait correlations and significance levels of all measured traits of six *B. carinata* lines (*n*=72, except for DTM and subsample traits (*n*=36)) grown in Fort Collins, CO in 2013 and 2014.

Traits[1]	DTM	BRHT	YIELD	TSM	NOBR	NOLV	FLPD	ABORT	PLBM	PLYLD	RS	HI	PLHT	SPP	POLVIA	OVCNT
DTF	0.10 ns	-0.39 *	-0.50 **	0.06 ns	-0.03 ns	0.08 ns	0.04 ns	0.18 ns	-0.02 ns	-0.17 ns	-0.28 ns	-0.29 ns	-0.14 ns	-0.31 ns	0.23 ns	-0.04 ns
DTM	-	0.08 ns	-0.32 ns	-0.89 ***	0.60 ***	0.43 **	0.58 ***	0.33 *	0.45 **	-0.13 ns	0.32 ns	-0.82 ***	0.75 ***	0.31 ns	-0.10 ns	-0.22 ns
BRHT	-	-	0.27 ns	-0.26 ns	0.33 ns	-0.21 ns	-0.18 ns	-0.41 *	-0.01 ns	-0.15 ns	0.30 ns	-0.04 ns	0.38 *	0.08 ns	-0.12 ns	-0.28 ns
YIELD	-	-	-	0.32 ns	-0.06 ns	-0.21 ns	-0.11 ns	-0.27 ns	0.14 ns	0.54 ***	0.21 ns	0.61 ***	0.19 ns	0.12 ns	-0.09 ns	-0.09 ns
TSM	-	-	-	-	-0.65 ***	-0.46 **	-0.54 **	-0.22 ns	-0.44 **	0.17 ns	-0.41 *	0.81 ***	-0.80 ***	-0.47 **	0.00 ns	0.21 ns
NOBR	-	-	-	-	-	0.60 ***	0.45 **	0.03 ns	0.48 **	0.02 ns	0.51 **	-0.48 **	0.69 ***	0.33 ns	-0.06 ns	-0.09 ns
NOLV	-	-	-	-	-	-	0.71 ***	0.54 ***	0.57 ***	0.21 ns	0.28 ns	-0.35 *	0.43 **	0.24 ns	0.03 ns	0.12 ns
FLPD	-	-	-	-	-	-	-	0.56 ***	0.60 ***	0.36 *	0.53 ***	-0.32 ns	0.58 ***	0.38 *	0.05 ns	-0.06 ns
ABORT	-	-	-	-	-	-	-	-	0.41 *	0.16 ns	-0.32 ns	-0.32 ns	0.13 ns	0.04 ns	0.07 ns	0.20 ns
PLBM	-	-	-	-	-	-	-	-	-	0.48 **	0.25 ns	-0.34 *	0.56 ***	0.38 *	0.08 ns	-0.08 ns
PLYLD	-	-	-	-	-	-	-	-	-	-	0.18 ns	0.51 **	0.13 ns	0.35 *	-0.11 ns	0.09 ns
RS	-	-	-	-	-	-	-	-	-	-	-	-0.07 ns	0.59 ***	0.50 **	-0.05 ns	-0.17 ns
HI	-	-	-	-	-	-	-	-	-	-	-	-	-0.49 **	-0.06 ns	-0.10 ns	0.26 ns
PLHT	-	-	-	-	-	-	-	-	-	-	-	-	-	0.48 **	-0.04 ns	-0.20 ns
SPP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-0.02 ns	0.16 ns
POLVIA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.15 ns

"ns" non significant,* Significant at the 0.05 probability level; ** Significant at the 0.01 probability level; *** Significant at the 0.001 probability level

[1]Traits: DTF=days to flowering, DTM= Days to maturity, BRHT=height of first branch, YIELD=seed yield, TSM=1000 seed mass, NOBR=number of branches, NOLV=number of leaves, FLPD=total number of flowers and pods, ABORT=aborted flowers and pods, PLBM=dry plant biomass, PLYLD=plant yield, RS=reproductive success %HI=harvest index, PLHT=plant height, SPP=seeds per pod, YIELD=total seed yield (kg ha-1), POLVIA=pollen viability ratio,OVCNT=ovule count

Figure 3.1: *Brassica carinata* displaying late-season indeterminate flowering (Photo taken by Brian Campbell, ARDEC 2014)

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