Characterization of Field Pennycress (*Thlaspi arvense* L.) Germplasm for Use as a Cover Crop and Biofuel Feedstock

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Dedication

This thesis is dedicated to my farmer grandparents Jerry and Joanne Pettit and Raymond and Marian Altendorf. Thank you for the inspiration.

Abstract

Field pennycress (*Thlaspi arvense* L.) is a winter annual oilseed crop currently under investigation for use as a feedstock for domestic biofuel production. As an undomesticated species, pennycress has a variety of undesirable traits and the species has seen very limited formal selection or genetic improvement. This research seeks to characterize a collection of 42 wild, winter type accessions for morphological and yield component traits (Chapter 2) as well as seed chemistry traits (Chapter 3). This data will be used to guide the breeding and development of improved germplasm and eventual variety release. The germplasm collection was grown in five unique Minnesota environments in 2013/2014 and 2014/2015. Mixed effects models were used to estimate best linear unbiased estimates (BLUEs) for each of the accessions and traits, which were used in subsequent analyses. Within the morphological traits and yield component traits, relationships between traits were assessed using Pearson's correlation coefficients and estimates of heritability were calculated for each trait. Hierarchical clustering was used to identify groups of accessions based on similarity of trait values. Significant variation for accession was detected in 13 of the 19 trait models for morphological and yield component traits at $P < 0.05$, and for 1 of 19 at $P < 0.10$ (Table 2.7). Pairwise differences after adjusting for multiple comparisons using Tukey's Honest Significant Difference (HSD) resulted in more than one grouping in 9 of the 13 models in which accession was significant ($P < 0.05$). For seed chemistry traits, significant variation ($P < 0.05$) for accession was detected for nine of the ten fatty acids detected and oil percentage. Significant variation was observed for many of the traits evaluated, but to make sizable gains in selection for certain traits, additional genetic variation in the form of mutants and additional collections is required.

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Justification

Much of the corn and soybean-dominated agricultural landscape (an estimated 27 million ha) in the Midwestern United States is left without soil cover from the time of harvest in the fall until canopy closure in the early summer (USDA - National Agricultural Statistics Service). During this time the soil is most susceptible to runoff of nitrate (NO_3) and soil loss from wind and water erosion. Excess NO_3 enters waterways and results in decreased surface and subsurface water quality. It is estimated that the artificially drained corn and soybean fields of five upper Midwestern states (IA, IL, OH and MN) supply about 46% of the NO_3 –N load that contributes to hypoxia in the Gulf of Mexico (Alexander et al., 2008; Robertson et al., 2009). It is well supported in the literature that integrating cover crops into these systems can reduce NO_3 losses by expanding the time period in which plants use water and nutrients (Kladivko et al., 2004, 2014; Kaspar et al., 2007, 2008, 2012; Strock et al., 2004). Planting a fall sown cover crop throughout the corn and soybean acres of the Midwest has potential to reduce NO_3 loadings to the Mississippi River by 20% (Kladivko et al. 2014). In addition to reducing NO_3 runoff, cover crops are known to provide protection from soil erosion, increase soil organic matter, suppress weeds, sequester carbon and provide habitat and food resources for pollinators (Lal et al., 1991; Bowman et al., 1998; Sindelar et al., 2015; Eberle et al., 2015).

Despite the well-established benefits of cover crops, many farmers are reluctant to adopt them. The USDA Census of Agriculture estimated that cover crops were planted on less than 2% of all cropland in the United States in 2010-2011(Wade et al., 2015). Low adoption rates are a result of difficulty of establishment, termination, potential interference with cash crops and perceptions of risk and uncertainty (Strock et al., 2004; Snapp et al., 2003; Arbuckle & Roesch-McNally, 2015). Furthermore, low adoption rates may also be a result of the lack of improved germplasm (Runck et al., 2014), as the cover crop cultivars currently available to farmers have not been bred for their performance in these environments or agronomic situations (Brummer et al., 2011). Farmers are more

likely to adopt cover crop systems that reduce operational input costs, boost productivity or improve crop health (Snapp et al., 2003).

In effort to incentivize cover crops, researchers have looked for ways to grow "cash cover crops" that are economically viable and provide environmental services. One area of study is the use of winter annual oilseed crops as double or relay crops in the traditional corn and soybean rotation. Fall planted oilseed crops can cover the soil in the late fall through early spring where they do not displace other cash crops. They can be harvested and sold as a biofuel feedstock and can serve as an additional source of income for farmers. The oilseeds can also help supply a growing demand for alternatively sourced feedstocks for domestic biofuel production.

The demand for alternative, renewable and bio-based fuels is increasing in the United States. In 2005 Congress approved the Renewable Fuel Standard Program, which aims to reduce greenhouse gas (GHG) emissions, expand the renewable fuels sector and reduce reliance on imported oil (Environmental Protection Agency, 2016). The Program mandates an increase in the production of renewable fuels to 36 billion gallons by 2020, or double 2012 production levels (U.S. Congress, House of Representatives, 2007; Enivronmental Protection Agency, 2016). There are four types of fuel included in the Program: 1) biomass-based diesel (biodiesel and renewable diesel) 2) cellulosic biofuels (cellulose, hemicellulose and lignin); 3) advanced biofuels (renewable biomass excluding corn); and 4) renewable fuels (corn starch-derived ethanol) (Environmental Protection Agency, 2016). This thesis focuses on the first component: biomass-based diesel. There are two main types of biomass-based diesel that differ on the basis of their chemistry: biodiesel and renewable diesel.

Biomass-Based Diesel

Biodiesel is defined as mono-alkyl esters of long chain fatty acids that are derived from vegetable oils and animal fats (American Society for Testing and Materials - ASTM). Biodiesel is obtained by a process known as transesterification, which requires an alcohol as a co-reagent (traditionally methanol) and yields a glycerol co-product

(Knothe, 2010). Plant oils are composed of triacylglyceride molecules that contain a three-carbon hydroxycarbon (i.e. a dehydrated glycerol) with three fatty acid R chains attached. Transesterification causes the fatty acids to release from the glycerol backbone and attach to a methanol molecule (Figure 1.2). Key characteristics of biodiesel are that the fatty acid methyl esters (FAMEs) are representative of the fatty acid profile of the parent oil and this composition influences the properties of the biodiesel (Knothe, 2010). For example, the presence of polyunsaturated (two or more carbon-carbon double bonds) fatty acids results in problems with the oxidative stability of the biodiesel and highly saturated (carbon atoms fully saturated with hydrogen atoms) fatty acids can result in poor cold flow ability (Knothe, 2010). Cloud point (CP) is a measure that describes the point at which the first visible crystals form upon cooling and is influenced by the level of saturation in the fatty acid esters. This is an important measure because it can cause fuel filter plugging (Knothe, 2010). Soybean-derived biodiesel has a CP of 0° C, while canola-derived biodiesel is slightly higher (Knothe, 2010). Generally speaking, cold temperature operability and flow is a challenge with 100% biodiesel fuels, as petroleum diesel fuels have much lower CPs.

Biodiesel is not considered a "drop-in" fuel, or a fuel that can be readily used with existing infrastructure, in particular because it cannot be transported using pipelines used for petroleum. Biodiesel-petroleum blends (20/80 a.k.a. "B20" or 50/50 a.k.a. "B50") are popular in the freight trucking industry because they take advantage of the lubrication properties provided by biodiesel and overcome the issue of poor temperature operability in 100% biodiesel (or B100). 5% blends are considered the same as petroleum-derived diesel fuel and are fully compatible with existing engines and infrastructure. In September 2016 there were 95 registered biodiesel production plants in the United States with a total production capacity of 2,107 million gallons per year (EIA, 2016). These plants are operating on the most common biodiesel feedstocks in the U.S., which are soybean, corn, canola, palm, and animal fats (EIA, 2016).

The second form of biomass-based diesel is known as renewable diesel, which is similarly derived from animal or plant lipid feedstock, but is composed of hydrocarbons

as opposed to esters. A process known as hydrodeoxygenation is used to produce renewable diesel. Hydrodeoxygenation is a chemical reaction that takes place at an elevated temperature, pressure and in the presence of a catalyst (Knothe, 2010). The specific catalyst varies, but the is co-reagent is consistently hydrogen. Hydrogen saturates double bonds of the lipids and replaces the oxygen molecules, making the chemical equivalent to fossil-derived fuels (Pearlson et al., 2012). The product is also known as hydroprocessed esters and fatty acids (HFEA) and hydroprocessed renewable jet fuel (HRJ) (Knothe, 2010). Renewable diesel is considered a "drop-in" fuel as it is suitable for use in diesel and jet engines and is certified for commercial aviation and military applications (Pearlson et al., 2012). There are several commercial-scale HEFA production operations around the world including Neste Oil in which operates in Europe and Asia, as well as a joint venture in Louisiana, USA which is led by Syntroleum and Tyson Foods and is known as Dynamic Fuels (Pearlson et al., 2012).

In accordance with the Renewable Fuel Standard Program, major federal regulatory and military agencies have set targets to reduce carbon emissions and increase their use of alternatively sourced fuels. The US Air force aims to fuel 50% or more of their non contingency operations with an alternative fuel blend by 2025, and similarly, the US Navy is set to obtain 50% of their energy from renewable sources by 2020 (U.S. Government Accountability Office, 2015). The aviation industry has similar goals. The Air Transport Action Group seeks to halve 2005 emission levels by 2050 (Air Transport Action Group, 2010) and the Federal Aviation Administration seeks to use one billion gallons of "drop in" renewable jet fuel per year by 2018 (Federal Aviation Administration, 2014).

Biodiesel and renewable diesel have advantages compared to their petroleum counterparts. They can be derived from renewable, domestic feedstocks, have improved lubricity, lower sulfur content, excellent flash point and biodegradability, reduced toxicity and overall lower regulated exhaust emissions (Moser et al., 2009a). Renewable biofuels burn cleaner than petroleum-based fuels and can offset their associated carbon emissions by the carbon the feedstock plants assimilate during growth (Fan et al., 2013).

In general, combustion of biodiesel has 90% fewer unburned hydrocarbons (HC), 75-90% lower polycyclic aromatic hydrocarbons (PAHs), fewer particulates, less carbon monoxide (CO), lower sulfur emissions and lower nitrous oxide (N_2O) when compared to petro diesel (Demirbas, 2007).

The environmental benefits of renewable fuels as well as the demand and regulatory push for increased production are well established. One major barrier that limits the production of biodiesel and renewable diesel fuels is the sometimes prohibitive cost of feedstock acquisition, or the purchase of the feedstock by the processing plant (Paulson & Ginder, 2007). Of the total cost of producing biodiesel, feedstock acquisition makes up roughly 85% (Paulson & Ginder, 2007). This may be in part due to their competing food-related applications. Alternative feedstock sources with high oil content, acceptable fatty acid profile, low production costs, few agricultural inputs and production during the off-season or on marginal lands are of particular interest to help reduce feedstock prices and increase production (Moser et al., 2009a).

In effort to meet the growing demand for alternative, domestic and affordable feedstocks and economically viable cover crops, there has been increased research into field pennycress (*Thlaspi arvense* L.), a winter annual oilseed crop. Planting a double or relay crop of pennycress may alleviate the aforementioned soil and water quality issues and serve as an additional source of income to farmers to incentivize the planting of cover crops. The potential economic gain is what sets pennycress apart from other cover crops. It can be grown in the traditional corn and soybean rotation without requiring additional land or displacing other food crops, which has proven to be a major concern with other biofuels, ethanol in particular (Boateng et al., 2010; Pimentel et al., 2009). The oil can be used in the production of alternatively sourced, domestic biofuels to help meet demands in this growing industry.

Field Pennycress

Efforts to domesticate pennycress are only recent (Sedbrook et al., 2014) and coincide with the first publication investigating the species as a biofuel feedstock in 2009 (Moser et al., 2009a). There was a brief interest in "fanweed" (pennycress) oil in the 1940s as a replacement for rapeseed oil (Clopton & Triebold, 1944) and in 1993 as an industrial oilseed (Carr, 1993). Prior publications were primarily focused on its persistence as a serious agricultural weed. The economic impact of pennycress as a weed was cited as "detrimental" in Canada in 1975 due to its ability to decrease yield in winter wheat (Best & McIntyre, 1975). For example, pennycress in combination with flixweed (*Descurainia sophia*) can result in 18-32% yield losses in winter wheat (Blackshaw 1990). In 2002, pennycress ranked at or near the top in weed surveys in canola (*Brassica napus* L. and *Brassica rapa* L.) in Saskatchewan and Alberta, Canada (Warwick et al., 2002). There it is known not only to cause yield reductions, but also to contaminate rapeseed oil and seed meal by increasing erucic acid levels and glucosinolate contents (Warwick et al., 2002). Additional reports of yield losses as a result of pennycress exist for forages, safflower and pulse crops in Canada (Warwick et al., 2002).

Life History

Pennycress is part of the Brassicaceae family, tribe Thlaspideae and genus *Brassica* (lineage II) (Best & McIntyre, 1975; Warwick et al., 2002; Franzke et al., 2011). Plants are self-pollinating and diploid (2n=14) with a 1X genome size of 539 Mbp (compared to Arabidopsis 157 Mbp and maize 2,300 Mbp) (Johnston et al., 2005; Best & McIntyre, 1975; Schnable et al., 2009). It is related to canola (*Brassica napus;* a rapeseed variety with double-low erucic acid and glucosinolates), camelina (*Camelina sativa*) and the cruciferous vegetables (domesticates of *Brassica oleracea*) (Franzke et al., 2011). Pennycress has been found on every continent besides Antarctica (Best & McIntyre, 1975; USDA NPGS) and was first reported in North America in an area that is now Detroit, MI in 1818 by English botanist Thomas Nutall (Best & McIntyre, 1975). It is suspected to have established there in 1701 when Detroit became a colony (Best &

McIntyre, 1975). The common name for pennycress is field pennycress, but it has also been referred to as stinkweed, fanweed, frenchweed, bastard cress and pennycress (Best & McIntyre, 1975).

Morphological Characteristics

Pennycress is an annual that can exhibit either spring or winter type habits (Baskin and Baskin, 1989). Existing wild populations include both spring and winter annual types. This trend is observable within the pennycress collection maintained by the United States Department of Agriculture (USDA) Agriculture Research Service (ARS) National Plant Germplasm System (NPGS) (Isbell et al., 2015). The genetic difference between the two growth types was initially investigated by McIntyre and Best who declared growth type to be controlled by a single gene with complete dominance of the winter type allele (McIntyre & Best, 1978; Best & McIntyre, 1972). The genetic control was further elucidated by Dorn (2015) who identified an orthologous gene in the pennycress genome sequence of well-studied genes controlling flowering time in *Arabidopsis thaliana*.

Considering the genetic similarity between pennycress and Arabidopsis, the decades of research into Arabidopsis can be leveraged to help elucidate the underlying genetic mechanisms in pennycress, specifically in this case with regards to flowering time and growth habit. Two negative regulators of flowering time have been described in Arabidopsis: FRIGIDA (FRI) and FLOWERING LOCUS C (FLC). Loss of function mutations in either of these genes can result in rapid flowering spring type (Michaels & Amasino, 2001; Michaels & Amasino, 1999). FRI promotes FLC messenger RNA. Mutations in FRI or deficiencies in its expression can remove the vernalization requirement for flowering (Simpson & Dean, 2002). FLC encodes a MADS-box DNA binding protein that represses flowering by reducing the expression of important floral integrator genes like FLOWERING LOCUS T (FT) (Michaels and Amasino 1999; Sheldon et al., 1999; Helliwell et al., 2006). Cold treatment is known to slow the expression of FLC by increasing repressive histone modifications at FLC chromatin,

allowing the increased expression of FT genes (Bastow et al., 2004). A *fri* null mutant of Arabidopsis was grown in a variety of environments and converted from a winter to spring annual only in a narrow range of conditions, which suggests that a genotype's life cycle in nature can be heavily influenced by the environment (Wilczek et al., 2009). The same genotype may behave as a winter type in one environment and a spring type in another (Wilczek et al., 2009).

Using this prior knowledge, Dorn (2015) investigated spring types of pennycress and found they exhibited a series of five different causative mutations within the orthologous FLC locus. These mutations include a frame shift insertion (c6_7insG), two separate deletions (2385_2841_del and 4.8kb_del), and a base pair substitution (c.100G>T) (Dorn, 2015). c6_7insG was discovered in a selected spring-type of TAMN108, an accession from the University of Minnesota Collection (UMN Collection) that was found to include both spring and winter types (Dorn, 2015). Divergent phenotypes were also identified in TAMN106, another accession from the UMN Collection (Dorn et al., 2015). Some geographic division was observed with regards to the types of spring-type-conferring alleles, with c.100G>T and 4.8kb_del showing up exclusively in spring types from Europe and c6_7insG and 2385_2841_del in spring types from North America (Dorn, 2015; Kevin Dorn, personal communication).

In contrast, winter type pennycress plants require a vernalization period to induce flowering and show extreme cold tolerance (Sharma et al., 2007). Typical vernalization requirements include 21 days below 4° C (Coers & Phippen, 2010) and can be carried out in cold chambers. Winter-type plants remain in the basal rosette phase throughout vernalization with stacked, alternate leaves (Best 1975) (Figure 1.1 B).

In the cases of both spring and winter type pennycress plants, general plant morphology is similar. Stem elongation is associated with flowering and results in withering of the basal leaves. Flowers are white, perfect and in racemes, each featuring four petals and six stamens, a morphology typical to plants in the Brassicaceae family (see Figure 1.1 D) (Best & McIntyre, 1975; Sedbrook et al., 2014). Seed pods are silicles

with two locules containing 5-8 seeds in each and turn yellowish upon maturity (Best 1975) (Figure 1.1 F). Seeds are ovid, compressed and vary from reddish brown to black, often depending on maturity and storage conditions, and feature concentric ridges on the surface (Hume 1984; Best & McIntyre, 1975) (Figure 1.1 A). Single seed weight is 0.80 – 2.39 mg and due to their small size, they germinate best when placed just below or on the soil surface (Sedbrook et al., 2014; Boyd & Van Acker, 2003). Reports of pennycress plant height range from 40 cm (spring types) to 90 cm (Sedbrook et al. 2014), and 29-79 cm (winter types) (Johnson et al. 2015). Best & McIntyre (1975) observed that plant height and branching patterns are heavily influenced by environmental conditions. For example, in conditions of dry, infertile soil, plants may not branch and may remain short. In high fertility and low competition environments, plants may produce multiple lateral flowering branches (Best & McIntyre, 1975).

Pennycress flowers are cleistogamous, meaning that pollination occurs within a closed flower (Warwick et al., 2002). Two highly homogenous populations of a wild accession from the UMN Collection, TAMN106, were found to exist within the same vicinity. These populations were described by Dorn et al. (2015), who suggests that pennycress plants readily self pollinate and do not require isolation in the context of a breeding program. A contradictory alternative – that pennycress is predominately wind pollinated - was proposed by Groeneveld & Klein (2014). Their conclusions are based on lower seed yield, seed weight, and fruit set from self pollinated versus open-pollinated, wind pollinated and assisted self pollination (hand pollination with pollen from other individuals of the same "variety") (Groeneveld & Klein, 2014). This study was conducted on a single plant scale, with a small sample size (10 plant individuals per treatment), had no replication and has yet to be verified or supported by subsequent studies.

Pennycress seeds exhibit a post-harvest dormancy period (Sedbrook et al., 2014), which is a beneficial strategy as a weed but a challenge in the context of an agricultural crop. Two methods are proposed in the literature to overcome the dormancy: 1) seeds can be treated in 0.01 mM Gibberellin A4+A7 (Saini et al., 2016); or 2) stored for several months to allow ripening (Sedbrook et al., 2014). It has been observed that seeds from

field plants are more dormant than greenhouse–grown plants and exhibit overall slower germination rates (Hume, 1984). As pennycress exhibits semi-indeterminate growth, the seeds at the bottom (basal) of the raceme are theoretically more mature than those at the top (apical). However, it was determined that the location of the seed on the stem does not affect seed post-harvest dormancy and germination as much as growth environment (Gesch et al., 2016).

Concerns about the weediness of pennycress are fostered by its expansive spread throughout the world and its ability to survive at a variety of altitudes, indicating that it is not restricted by climatic variability (USDA - APHIS, 2015; USDA - NPGS; Best & Mcintyre, 1975). Pennycress is commonly known to exist in recently disturbed lands as an important species in the principal stages of ecological succession (Best & McIntyre, 1975). Individual plants are high yielding and stands can develop a prolific seed bank that can remain viable in the soil for up to 5-9 years (Toole & Brown 1946; Thompson & Hodgson 1993).

When pennycress exists as a weed, it can typically be controlled effectively with a variety of herbicides in fields of canola, cereals, safflower and forages (Warwick et al., 2002) and can be killed by glyphosate and glufosinate (Sedbrook et al., 2014). Other effective herbicides include sulfonylureas, chlorosulfuron and ethanmetsulfphuron, MCPA, tribenuronmethyl, phenocyacetic acid, flurtamone, 2,4-D, 2,4-D + dicamba, and 2,4-D + picloram (Warwick et al., 2002). However, in 2001 pennycress was reported to have developed herbicide resistance to ALS inhibitors in Canada (Beckie, 2016). Concerns about the weediness of pennycress are based on its potential to develop herbicide resistance, as well as its diverse flowering time and growth habit (Warwick et al., 2002). Its highly competitive root system is known to reduce water and nutrient uptake in neighboring plants (Holm 1997). The aforementioned attributes have moved the USDA Animal and Plant Health Inspection Service (APHIS) to declare its probability of being a “major invader” weed as 95.4% or “High Risk” (USDA - APHIS, 2015). It remains on the list of restricted noxious weeds in the U.S. State of Michigan and the Canadian Province of Manitoba (MDARD; Manitoba Government, 2016). In Canada, its

seeds are regulated under the Seed Act as "Class 3: Secondary Noxious Weed Seeds" (Weed Seeds Order, 2009). Proponents of pennycress as a cultivated oilseed crop suggest it is not invasive, as it only germinates and grows in areas with recent soil disturbance and its potential as a volunteer weed in corn and soybean systems is low (Sedbrook et al., 2014; Sindelar et al., 2015). In 2015, Minnesota seed laws were changed to recognize pennycress as a crop as opposed to a weed when it is planted intentionally as a cover crop or an oilseed crop (M.S. section 21.81, Subd. 3) (Resivor of Statutes - State of Minnesota, 2015).

Additional concerns about growing pennycress on a large scale have stemmed from its reported role as an alternative host to the soybean cyst nematode (SCN) (Venkatesh et al., 2000). SCN is a root pathogen and is the number one cause of yield loss in soybeans in the State of Minnesota (Chen et al., 2011). Pennycress can also be infected by aster yellows (Petrie & Vanterpool, 1965; Katherine Frels, unpublished observation). Aster yellows is caused by a small phytoplasma bacterium and is spread by leafhopper insects. The bacterium causes systematic, irreversible damage to a plant and is known to infect over 350 plants including vegetables, flowers and weeds (Grabowski, 2015).

Agronomics

The winter habit of pennycress allows it to be seeded in late August or early September, meaning it can be grown in any system that allows sowing during that time. Some examples in the upper Midwest include: sweet corn, silage corn and early-harvested sugar beets. The most commonly proposed method includes interseeding into standing corn prior to or immediately following corn harvest in the fall and harvesting before soybean planting in the spring (sequential double cropping system) or harvested over the top of V3 soybean plants (relay cropping system) (Sindelar et al., 2015) (Figure 1.3).

Spring type pennycress may be both planted and harvested in the spring in some areas of the Corn Belt, however this system neglects to provide the full benefits of a

cover crop in the late fall and winter. Pennycress seed yield is reported to be greatest when planted in August or early September (Johnson et al. 2015). Yield estimates reported in the literature are derived from 1 m^2 hand-harvested samples and include: 1345 kg ha^{-1} (Best & McIntyre, 1975) and 1086 – 1387 kg ha^{-1} (Johnson et al., 2015). Seed yield depends heavily on water availability and competition with companion crops (Johnson et al., 2015). Pennycress can be harvested with a traditional combine, and is reported to work most effectively when its seed moisture reaches 12% (Fan et al. 2013). Silicle formation reportedly begins at 25 cm height, which allows sufficient clearance for traditional mechanical combine harvesting equipment (Sedbrook et al. 2014).

Pennycress establishment is dependent on and highly sensitive to planting depth. Drilled plots at 1.0 cm depth had higher stand establishment compared to broadcast/surface seeded plots, likely due to insufficient seed-soil contact (Phippen, Gallant, & Phippen, 2010). Planting seed too deep, however, can be problematic as well. In a test between two planting depths: 1) surface (followed by a pass with tractor-drawn roller) and 2) at a depth of 2 +/- 0.5 cm, the surface seeded (followed by rolling) resulted in the highest stand density (Diaz, 2014).

A high level of phenotypic plasticity is reported in pennycress with regards to plant density. Lower plant density in a stand results in higher seed yield $plant^{-1}$ and shorter plants (Phippen, Gallant, & Phippen, 2010; Matthies, 1990). Higher plant density results in taller plants but not necessarily higher yields (Johnson et al. 2015). A significant decrease in reproductive components (i.e. bud number, proportion of buds:flowers, flowers:fruits, ovule:fruit, ovules:developing seed and the weight of individual seeds) was found as stand density increased (Matthies, 1990).

Relay or double cropping with pennycress creates certain challenges for growers. Due to competition for resources in a double cropping system with pennycress, an 18-20% decrease in soybean yield was, however, the total oilseed yield (soybean and pennycress combined) was 18-28% greater than soybeans alone (Johnson et al., 2015). A similar relationship was also observed for a similar crop of interest, camelina, when grown with soybean (Gesch & Archer, 2013). A contradictory report suggests that

soybean yields may be higher when planted into pennycress residue, due to the increased water storage in the soil (Phippen & Phippen, 2012). One major concern with planting a double crop in a region with a short growing season like the Upper Midwest, is the potential to delay the sowing of the main season crop, as it is important to ensure sufficient time for both crops to reach physiological maturity (Sindelar et al., 2015). The University of Minnesota Extension Service recommends planting soybeans as early as possible in May to maximize yields (Wright et al., 1999), but pennycress harvest occurs in late May or early June in Illinois (Phippen & Phippen, 2012) and Minnesota (Johnson et al., 2015). In a literature review of 17 studies on yield loss as a response to delayed soybean planting, it was found that after planting delays of 19 days, all but 4 site years surveyed showed yield losses ranging from 4 – 28% (Sindelar et al., 2015). It was concluded that an indeterminate soybean variety may be best suited for avoiding significant yield losses in double cropping systems with shorter growing seasons (Sindelar et al., 2015). Alternatively, a relay-cropping system would allow soybean planting at the earliest date, but as mentioned previously, may have impacts on soybean yield due to competition between the plants for resources.

One agronomic benefit of planting pennycress in either a double or relay crop system is its ability to suppress springtime weeds. Weed biomass was reduced by more than 80% in a pennycress system, which may be attributed to the glucosinolates in the plant and/or the competitiveness of its rapid growth in the early spring (Johnson et al., 2015; Sindelar et al., 2015). Pennycress' ability to reduce weed pressure may serve as an integrated weed management strategy that could slow the development of herbicide resistant weeds and increase its value in the cropping system by reducing the need for herbicides (Johnson et al., 2015).

Genetic Resources

Domestication of pennycress began with the initiation of a plant breeding program at Western Illinois State University (WIU) in 2009. The goal of this program is to evaluate wild accessions from the Midwestern United States and select for good stand

establishment (i.e. emergence) and early flowering (Sedbrook et al., 2014). The University of Minnesota initiated a breeding program in 2013 focused on the agronomic, seed chemistry and genotypic evaluation of wild collections from the northern United States as well accessions available through NPGS. A mutation (Ethyl methanesulfonate - EMS, gamma and fast neutron) breeding program and inbred development efforts were also initiated at this time (M. David Marks, personal communication).

The USDA ARS North Central Regional Plant Introduction Station in Ames, Iowa maintains 85 pennycress accessions available for public request (USDA NPGS). There are several pennycress accessions of note to the research community. 'Katelyn' and 'Elizabeth' (PI673443 and PI677360) are USDA releases that are fast germinating and were developed using mass selection (Sedbrook et al., 2014; Isbell et al., 2015). 'Beecher' (AMES29118) is commonly used and cited in agronomy field trials. Two others that are not available from the USDA germplasm collection but are commonly cited in pennycress literature include TAMN106, which is a reliably germinating winter-type collected from Coates, MN and housed in the UMN Collection, and Spring32, a spring-type that is useful for rapid inbreeding and is available from Winthrop Phippen at WIU (Sedbrook et al., 2014).

Pennycress is reported to have high DNA sequence similarity with the well studied, model species *Arabidopsis thaliana* (Brassicaceae lineage I). A functionally annotated *de novo* pennycress transcriptome comprised of 203 million unique Illumina RNA-sequencing reads (average length: 87.6 bp) was published recently (Dorn et al., 2013). This study revealed that over 20,000 transcripts had top BLAST hits to Arabidopsis species and nearly 75% of these were also present within the Brassicaceae family (Dorn et al., 2013). A subtractive expressed sequence tag (EST) library of 646 sequences (average length of 309 bp) from 600 cold-treated pennycress clones had an average of 90% sequence similarity with *A. thaliana (*Sharma et al. 2007). The low level of sequence divergence between the two species was leveraged to validate the gene space in the draft pennycress genome assembly (Dorn et al., 2015). This assembly was generated from TAMN106 and over 47 Gb of sequencing data was collected and aligned

with 87X coverage. This draft represents ~80% of the predicted genome size and a more complete version is under development (Dorn et al., 2015; Kevin Dorn, personal communication). The genome can be downloaded or used to conduct BLAST searches at *pennycress.umn.edu*. A genome comparison with other sequenced Brassicaceae species suggests that pennycress is most closely related with *Eutrema salsugineum* (Dorn et al., 2015). The genome assembly was developed with the intention of enabling the utilization of 30 years of *A. thaliana* and other Brassicaceae species discoveries to assist in the rapid domestication of pennycress (Dorn et al., 2015; Koornneef & Meinke, 2010).

Pennycress Oil

Biofuel feedstock is an attractive target end use for pennycress due to its high oil content and acceptable fatty acid profile. Pennycress oil meets the United States American Society for Testing and Materials (ASTM) D6751 regulation for biodiesel production (Moser et al., 2009a). Oil content of cold-press, dried wild pennycress seeds collected near Peoria, IL was 29.0% on a dry weight basis (dwb) (Moser et al. 2009b), which corresponds to previous reports of 24-39% (dwb) and 32.9% (dwb) (Dolya, 1974; Sedbrook et al., 2014; Evangelista et al., 2012). Its oil is composed of 55.6% monounsaturated fatty acids (16:1, 18:1, 20:1, 22:1, 24:1), 38% polyunsaturated fatty acids (18:2, 18:3, 20:2, 20:3, 22:2, 22:3) and 4.6% saturated fatty acids (14:0, 16:0, 18:0, 20:0, 22:0) (Moser et al., 2009b). Erucic, linoleic and linolenic are the primary fatty acids with 32.8, 22.4 and 11.8 wt % respectively (Moser et al., 2009b). Other fatty acids to note are oleic, gondoic and nervonic at 11.1, 8.6 and 2.9 wt %, respectively (Moser et al., 2009b). The oil compares favorably to its petroleum and synthetic (distilled and purified) counterparts for its physical properties and to other plant derived oils (lasquerella, medowfoam and cuphea) for its fatty acid profile, low temperature use, lubricity and oxidative stability (Cermak et al., 2013).

Once the oil is extracted from pennycress, it can be readily converted to biodiesel using transesterification. Pennycress oil can yield 82% wt % biodiesel using this process with methanol and sodium methoxide catalyst at 60° C (Moser et al. 2009a). Pennycress

oil and methyl esters have numerous qualities that make it an acceptable biodiesel feedstock including low sulfur content, high cetane number (CN) and excellent low temperature operability. Despite the high concentration of sulfur containing glucosinolates in its seeds, pennycress oil and methyl esters and have very low sulfur contents (2 ppm and 7 ppm, respectively) likely due to the polar nature of the glucosinolates and their decomposition products (Moser et al., 2009a; Vaughn et al., 2005). Sulfur content in pennycress oil and methyl esters are below the ASTMD6751 maximum of 15 ppm (Moser et al., 2009a; ASTM D6751). A high CN means a shorter ignition delay and longer combustion duration, ultimately resulting in lower particulate emissions (Bart, Palmeri, & Natale Cavallaro, 2010). Pennycress oil methyl esters have a high CN of 59.8, which is well above the minimum of 47 set by ASTM D6751 (Moser et al., 2009; ASTM D6751). The high CN is a function of its high percentage of methyl esters of erucic and gondoic acids (Moser et al., 2009a). In general, biodiesel has poor low temperature operability compared to petro-deisel, but pennycress methyl esters exhibit better low temperature operability compared to soybean oil, which can be attributed to the low level of saturated fatty acids (Moser et al., 2009a; Moser, 2008; Moser et al., 2009a). Overall, Moser et al. (2009a) concludes methyl esters derived from pennycress oil are a suitable substitute to petrodiesel.

Based on the close genetic similarity between pennycress, Arabidopsis and other well-studied Brassicaceae species, it is suggested that the understanding of the genetic control of important traits may be leveraged to rapidly domesticate and improve pennycress (Sedbrook et al., 2014). This is important presently, because there is limited understanding with regards to oil production mechanisms in pennycress specifically. A recent study by Claver et al. (2017) outlined the expression of a well-studied Arabidopsis gene, *wrinkled1* (WRI1) (Focks & Benning, 1998) in pennycress (TaWRI1). WRI1 is known as the master regulator of oil biosynthesis, and in pennycress it is reported to show high expression during the early stages of seed development (Claver et al., 2017). The aforementioned study is the first and only attempt to elucidate the genetic control of oil

accumulation in pennycress. Considering the known genetic similarity, a brief discussion of important genes in related species is warranted.

The genetic control of oil accumulation is well studied in canola. For example, multiple additive effects as opposed to major QTL influence oil accumulation (Delourme et al., 2006), and this trait in particular is reported to have broad and narrow sense heritabilites of 84 and 37% respectively (Wang et al., 2010). Several genes in Arabidopsis and canola have also been identified as targets for manipulation to improve oil quantity. For example, increasing expression the HAIKU2 (IKU2) seed development gene has shown to increase oil accumulation in Arabidopsis seed by 35% (Fatihi et al., 2013). Additionally, *wri-1*-like genes are expressed variably in *Brassica napus* lines with differing oil contents. Overexpression of *Bnwri1* in transgenic Arabidopsis via cauliflower mosaic virus 35S-promoter resulted in a 10-40% increase in seed oil content (Liu et al., 2010). Overexpression of genes related to triacylglycerol biosynthesis including diacylglycerol acyltransferase (DGAT1) in canola as well as increased co-expression of WRI1 and DGAT1 have shown to increase oil content (Taylor et al., 2009; Vanhercke et al., 2013). These important genes, if identified in pennycress, could serve as targets for genetic manipulation.

Erucic Fatty Acid in Pennycress Oil

The fatty acid profile of pennycress oil is diverse in the sense that there is no one highly dominant fatty acid. It is suggested that this may be one reason that additional applications for the oil have not been properly investigated (Isbell et al., 2015). The most concentrated fatty acid is erucic and methods are proposed for increasing it, which may result in additional uses for the oil (Isbell et al., 2015). It has been demonstrated that enrichment strategies can increase erucic acid in pennycress oil content from 36% to greater than 70% using distillation or soap crystallization (Isbell, Evangelista, et al., 2015). As an example of the commercial utility of high erucic acid oil, varieties of oilseed rape that are known as High Erucic Acid Rapeseed (HEAR) that have erucic factions of 45-60% have been developed (Scarth & Tang, 2006). Oil produced from these

varieties has utility as an additive to lubricants, solvents, softeners, and in the manufacturing of polymers, high-fluidity lubricants, surfactants, surface coatings and pharmaceuticals (Leonard, 1994). In order for an oil to be competitive in this marketplace and to limit purification costs, it should have at minimum 45% erucic acid (Sovero, 1993). The demand from these industries has provoked the initial investigation into erucic acid enrichment and biosynthesis in pennycress (Isbell et al., 2015; Claver et al., 2017).

Pennycress has yet to be investigated for its use as oil for human consumption due to its high composition of erucic acid. In animal studies of diets high in erucic acid, myocardial damage can result due to fatty deposits around the heart and kidneys (Eskin et al., 1996) and was found to be damaging to the human heart (Khachatourians et al., 2001). This was one of the motivating factors behind the development of canola. Currently, canola oil is required to have < 2% erucic acid (Fahey et al., 2000), which is a decrease from around 50% or more in its original state (Schmidt & Bancroft, 2011).

Glucosinolates

Pennycress plants and seeds contain glucosinolates (β-thioglucoside-*N*-hydroxysulfates), which are a class of secondary plant compounds. Their hydrolysis products are responsible for the mustard taste and scent of many vegetable species and are thought to play a role in herbivory and microbial defense (Fenwick et al., 1983). Glucosinolates have been identified in 16 different dicotyledonous angiosperm families and exist in at least 120 different forms that are differentiated by their side (R) chains (Fahey et al., 2000). The Brassicaceae family is known to contain the most diverse set of glucosinolates compared to the other 15 angiosperm families (Fahey et al., 2000). Briefly, the biosynthesis of glucosinolates follows the widely accepted model: 1) side chain elongation; b) glucone biosynthesis; and c) side chain modification (Fahey et al., 2000). The biologically active degradation products of glucosinolates (specific products are dependent on the type of glucosinolate and presence of supplementary specifier proteins) are produced when vacuoles are ruptured as a result of tissue damage and glucosinolates

are hydrolyzed by the enzyme myrosinase (Vaughn et al., 2006; Wittstock & Burow, 2007; Wittstock & Burow, 2010). Myrosinase (thioglucoside glucohydrolase) is a glycoprotein; it coexists with glucosinolates but is physically separate (Fahey et al., 2000).

Most glucosinolate-containing species typically contain fewer than one dozen different forms. *Arabidopsis thaliana* is reported to contain 23 different forms (Hogge et al., 1988; Haughn et al., 1991) and rapeseed 6 (Szmigielska et al., 2000). Concentrations and forms of glucosinolates are known to vary in different parts of the plant, throughout different growth stages and are also influenced by environmental factors (Fahey et al., 2000). Sinigrin is the predominant glucosinolate in cruciferous vegetables including cabbage, cauliflower and brussels sprouts (Keck & Finley, 2004). Generally speaking, the hydrolysis products of the glucosinolate sinigrin include: allyl isothiocyanate (AITC), allyl cyanide (AC), 1-cyano-2,3,epithiopropane (CETP) and allyl thiocyanate (ATC) (Shofran et al., 1998). However, the organic thiocyanates products (including ATCs) are not widespread (Wittstock & Burow, 2007).

Sinigrin was identified as the sole glucosinolate in a sample of pennycress seed from Colorado using gas chromatography (GC) of its hydrolysis products (Daxenbichler et al., 1991). A similar conclusion was reached for wild collected pennycress seed from Peoria County, IL (Vaughn et al., 2006). Sinigrin was found, in addition to traces of two other glucosinolates: 3-methylsulfinylpropyl-glucosinolate and benzylglucosinolate, in pennycress leaves from Colorado (Rodman & Chew, 1980). Pennycress seed is reported to have a sinigrin content between 36.71 – 44.91 mg sinigrin g^{-1} sample (92.36 – 112.99 μmol g^{-1} sample) (Vaughn et al., 2005; Daxenbichler et al., 1991; Hojilla-Evangelista et al., 2015).

The hydrolysis products of sinigrin in pennycress are unique in that they include allyl thiocyanate (ATCs) (Gmelin & Virtanen, 1959). TaTFP, a thiocyanate-forming protein (TFP), was identified and found to exist in all plant organs of pennycress. The protein promotes the formation of ATCs and epithionitrile (Kuchernig et al., 2011).

ATCs are responsible for the garlic scent in pennycress, and exist at concentration of 49.5 +/- 3.0 nmol/gdw. The second hydrolysis product of sinigrin in pennycress is AITCs, which are responsible for the mustard scent, and exist at a concentration of 5.0 +/- 1.0 nmol/gdw (Vaughn et al., 2006; Warwick et al., 2002). AITCs often serve as attractants for crucifer-host seeking insect species and the change in chemistry may reduce pennycress' chances of discovery by herbivorous insects (Feeny, 1977). The levels of ATC and AITC in pennycress have been reported to vary between plant parts and plants themselves. Rosette leaves have lower ATC levels (40-93 mg 100 g^{-1} sample) compared to mature seeds (855-1165 mg 110 g^{-1} sample) (Majak et al., 1991). Hydrolysis of sinigrin in a sample of pennycress leaves from Colorado produced more AITC than ATC, which indicates that geographic variation may exist for ATC formation (Rodman & Chew, 1980).

Isothiocyanates (ITCs) are the most commonly studied hydrolysis products of glucosinolates in general. They are highly reactive and have shown to be toxic to nematodes, bacteria, insects and fungi and have also generated significant interest for their chemo preventative abilities (Traka & Mithen, 2009; Wittstock et al., 2003). ITCs, however, are not to be confused with ATCs, which are known to be highly toxic to animals (Warwick et al., 2002). ATCs cause gastric distress, abortion, and death, among other symptoms when consumed by cattle (Smith & Crowe, 1987). Milk and flesh products from livestock that consume it may exhibit an unpalatable flavor (Warwick et al., 2002).

Biomass presscakes (the byproduct of oil extraction) of oilseed species like soybean and palm fruit can serve as valuable sources of protein for animal feed (Boateng et al. 2010). However due to the potential toxicity of the glucosinolate hydrolysis products in pennycress, presscakes they cannot be used for this purpose. The glucosinolate-rich presscakes may have utility for use as a biofumigant for reducing weed pressure in high-value horticultural, conventional and organic agricultural systems (Vaughn et al., 2005). In a study of seed germination, the active components in defatted pennycress seed meal, AITCs and ATCs, inhibited germination of wheat and arugula

seeds (Vaughn et al., 2005). The defatted seed meal also significantly reduced plant biomass compared to the control (Vaughn et al., 2005). AITC is a biocide that may have allelopathic properties and could possibly play a role in suppressing springtime weeds, specifically in post-harvest situations (Vaughn & Berhow, 1999).

Alternative End Uses

In effort to identify alternative uses for the presscakes, researchers have quantified the protein in the seed, which ranges from 23 – 50% and the presscake, which ranges from 31 – 83%, depending on extraction technique (Selling et al., 2013). The major classes of proteins in pennycress seed are water and NaCl-soluble and in general show promise in their emulsification and foaming properties which may prove desirable in the production of pressurized foams and whipped products/emulsions (Hojilla-Evangelista et al., 2013). This indicates that pennycress protein derived from the seedmeal may have utility in industrial applications.

The presscakes have also been studied for their efficacy in producing pyrolysis oil, which is another feedstock for producing biomass-based hydrocarbon fuels. Pyrolysis is a thermochemical technique that involves the decomposition of organic matter in the absence of oxygen, and yields char, gas and pyrolysis oil. Pyrolysis oil contains high concentrations of oxygenated hydrocarbons and can be converted to biomass-based hydrocarbon fuels like gasoline and diesel using traditional petroleum-refining techniques (Boateng et al., 2010). Pennycress pyrolysis oil specifically is high-carbon, high energy and "unusually stable" (stability is characterized by its ability to reduce changes in viscosity with storage, a function of the oxygen level), which is typically a major barrier for storing, transporting and producing hydrocarbon fuel (Boateng et al., 2010).

Improvement Potential: Canola as an Example

The history of oilseed rape (*Brassica napus*) provides an example of how breeding can improve the fatty acid composition and reduce glucosinolate content in an oilseed crop. The events associated with oilseed rape improvement are remembered as

"remarkable quality improvement achievements" (Bell, 1982). *Brassica napus* L. var *oleifera* (2n = 4x = 38) resulted from the spontaneous hybridization of *B. rapa* and *B. oleracea* and traditionally exhibits high levels of erucic acid and high concentrations of glucosinolates (Kondra & Stefansson, 1970). Aside from its early use as an annual forage crop, *B. napus* was initially cultivated in Canada for its utility as a marine lubricant (a property later attributed to its high erucic acid content) (Bell, 1982). Reduced access to imports from Europe and Asia during World War II provoked the Canada Department of Agriculture to initiate domestic production and breeding in 1942 (Khachatourians et al., 2001). Research after WWII focused on meal for animal use, oil for human use and general plant breeding (Bell, 1982). It was determined that the high concentration of erucic fatty acid in rapeseed oil was damaging to the human heart, and the glucosinolates in the seed meal were found to be harmful when fed to livestock (Khachatourians et al., 2001). 'Bronowski,' a spring varietal released in Poland in 1955, was grown in Canada in 1967 and found to be low in glucosinolates. Some plants produced seeds that were "practically free" of glucosinolates (Bell, 1982; Kondra & Stefansson, 1970). A cultivar of feed rape with low erucic acid content was identified and named "LIHO" in the 1960s (Downey & Craig, 1964). These parents were used to develop lines with low erucic acid (<2% in the oil) and low glucosinolates (<30 μmol g^{-1} sample in the meals) and were referred to as "double low" and marketed as CanOLA (Canadian Oil Low Acid). The molecular mechanisms behind these traits were later elucidated. Low erucic acid is a result of a 4 bp deletion in the coding region of Fatty Acid Elongase 1 (FAE1), which causes a frame shift mutation and premature stop codon (Wu et al., 2008). Rapeseed with low erucic acid results in a corresponding increase in oleic acid without any overall decrease in oil content (Downey & Craig, 1964). High oleic (HO) and low linoleic (LL) acid oils are more thermally stable and are used in cooking and frying and diets high in oleic fatty acid are known to reduce low-density lipoprotein cholesterol in plasma (Grundy, 1986).

Canola is an edible oil and has a desirable nutritional profile: low in saturated fats, high in monosaturated fats and high in omega-3 fatty acids (Schmidt & Bancroft, 2011).

Canola has oil content of 40-42% (dwb) and its residual seed meal contains 38-42% protein and is commonly used for livestock, poultry or fish feed (Khachatourians et al., 2001). By regulation canola must contain < 2% erucic acid and must also have < 30 μmol of glucosinolates $gram^{-1}$ seed meal (Fahey et al., 2000). Canola is bred for hybrid seed production as it exhibits high levels of heterosis (Schmidt & Bancroft, 2011). As a result of tremendous breeding success, today canola is the second most widely grown oilseed crop in the world after soybean (Schmidt & Bancroft, 2011).

Figures

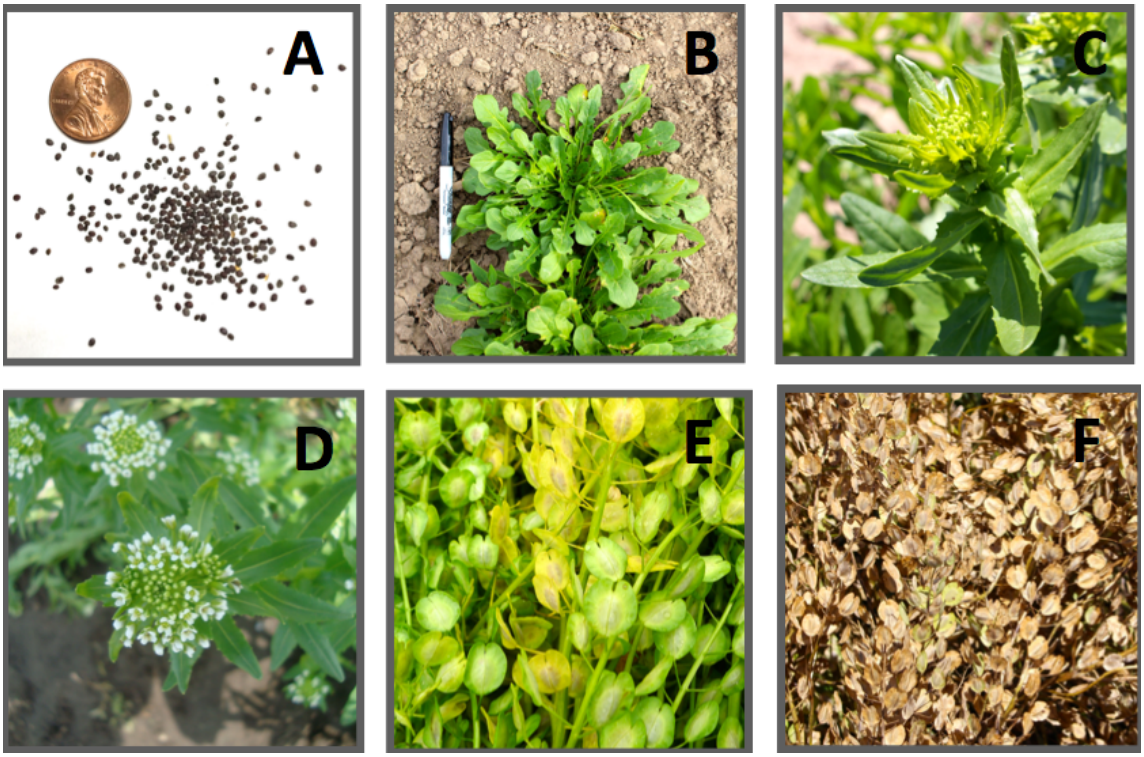

Figure 1.1. Depictions of the various growth stages of pennycress; (A) seeds with a penny for size comparison; (B) a plant in its basal rosette form prior to vernalization; (C) stem elongation just before flowering; (D) flowers; (E) immature silicles; (F) mature silicles.

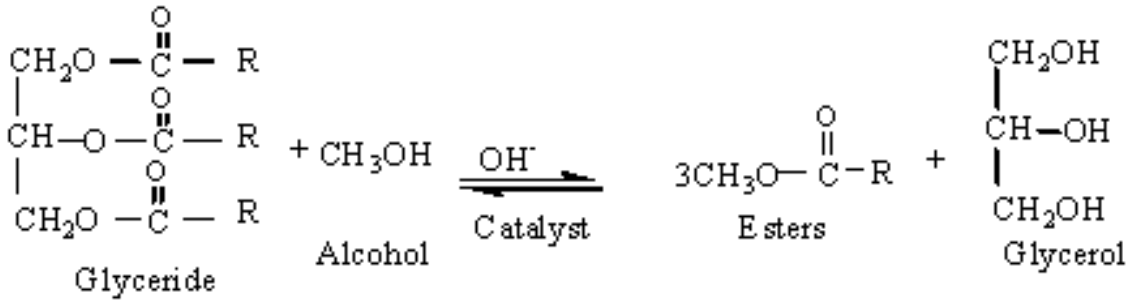

Figure 1.2. Depiction of triacylglyceride molecule and release of fatty acid methyl esters from the glycerol backbone. Figure from: http://www.esru.strath.ac.uk/EandE/Web_sites/02-03/biofuels/what_biodiesel.htm

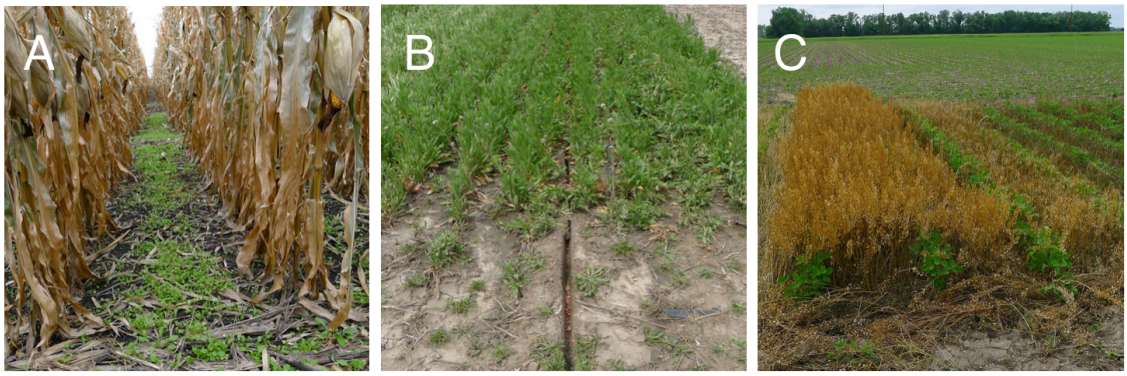

Figure 1.3. Depiction of pennycress agronomic relay systems. (A) Pennycress seedlings in standing corn on early October 2014 in Rosemount, MN. (B) No till planted soybean seeds in standing Pennycress on May 6th, 2014 in St. Paul, MN. (C) Pennycress harvest over V3 soybeans on June 27th in Rosemount, MN. Photos are courtesy of Kevin Anderson.

Chapter 2: Morphological and Yield Component Traits of Winter Annual Pennycress Germplasm

Introduction

Much of the corn and soybean agricultural landscape in the Midwestern United States is left without soil cover from the time of harvest in the fall until canopy closure in the early summer. During this time the soil is most susceptible to nutrient runoff and soil erosion. Increasing the time period in which plants take up water and nutrients by planting cover crops can reduce NO_3 losses and soil erosion, increase organic matter, suppress weeds, sequester carbon and provide habitat and food resources for pollinators (Kladivko et al., 2004, 2014; Kaspar et al., 2007, 2008, 2012; Strock et al., 2004; Lal et al., 1991; Bowman et al., 1998; Sindelar et al., 2015; Eberle et al., 2015). Despite their benefits, the USDA Census of Agriculture estimated that cover crops were planted on less than 2% of all cropland in the U.S. in 2010-2011 (Wade et al., 2015). Low adoption rates are a result of difficulty of establishment, termination, potential interference with cash crops, and perceptions of risk and uncertainty (Strock et al., 2004; Snapp et al., 2003; Arbuckle & Roesch-McNally, 2015). The low adoption rates of winter annual covers may also be a result of the lack of improved germplasm as cultivars currently available have not been bred for their performance in these environments or agronomic situations (Runck et al., 2014; Brummer et al., 2011). The adoption of cover crops is typically a long-term investment with little to no immediate realized gains. Recently, there has been an increased interest in developing second generation biofuel crops: oilseed crops that may provide the environmental services of a cover crop while increasing crop diversity and serving as an additional income opportunity in the form of a biodiesel feedstock.

Pennycress (*Thlaspi arvense* L.) is a self-pollinating, diploid (2n = 14) winter annual oilseed crop belonging to the Brassicaceae family. Pennycress is native to Eurasia but is highly adapted throughout North America (Best & McIntyre, 1975). Seed oil content on a dry weight basis (dwb) ranges from 13.5 – 39% (Sedbrook et al., 2014; Moser et al. 2009b; Dolya, 1974) and yield from 1086 – 2240 kg ha^{-1} (Best & McIntyre,

1975; Johnson et al., 2015). The winter habit of pennycress allows it to be grown as a sequential crop (no overlap) or a relay crop (overlap) in conventional rotations without displacing cash crops.

Pennycress oil is an acceptable feedstock for biodiesel production and meets the United States American Society for Testing Materials (ASTM) D6751 (Moser et al., 2009a). Its oil is composed of 55.6% monounsaturated fatty acids, 38% polyunsaturated fatty acids, and 4.6% saturated fatty acids with erucic, linoleic and linolenic being the primary fatty acids with 32.8, 22.4 and 11.8%, respectively (Moser et al., 2009b). Pennycress oil is reported to have excellent low temperature fluidity and lubrication properties (Moser et al., 2009a; Cermak et al. 2013) and a standard transesterification process with methanol and sodium methoxide can yield 82% wt biodiesel (Moser et al. 2009a). Defatted pennycress seed meal has a protein content of 33.44 ± 1.52 and can be 97.1 ± 3.4 and 90.4 ± 1.2% (db) when using high recovery protein methods known as saline extraction and acid precipitation, respectively (Hojilla-Evangelista et al., 2014). Protein from seed presscakes remaining after oil extraction may have use in the production of pressurized foams and whipped products/emulsions (Hojilla-Evangelista et al., 2013).

Glucosinolates in pennycress seed prevent its use as a feed or food source. Sinigrin is the sole glucosinolate in pennycress (Daxenbichler et al., 1991; Vaughn et al., 2006). Upon hydrolysis by the enzyme myrosinase, sinigrin in pennycress yields allyl thiocyanate (ATC) in the presence of TaTFP, a thiocyanate-forming protein (TFP) (Kuchernig et al., 2011). ATCs are toxic to animals (Warwick et al., 2002), cause gastric distress in cattle, among other symptoms (Smith & Crowe, 1987), and result in an unpalatable flavor in products derived from livestock who consume it (Warwick et al., 2002). Glucosinolate content in defatted seedmeal is reported to be 36.71 ± 0.41 mg gram^{-1} sample (92.36 ± 1.03 μmol g^{-1} sample) (Hojilla-Evangelista et al., 2015). Lowering or nearly eliminating the sinigrin content in pennycress seed could improve its use as a biofuel feedstock as it would increase the profitability and utility of the presscakes co-product as livestock feed.

Pennycress is currently an undomesticated species and maintains several undesirable traits including seed dormancy, inconsistent stand development, shattering and non uniformity of flowering and maturity (Sedbrook et al., 2014). Improvements to seed size, glucosinolate content, fatty acid profile and maturity are also of interest (Sedbrook et al., 2014). Efforts to improve pennycress are recent but multifaceted and include germplasm characterization, mass selection for germination improvement (Isbell et al., 2015), a mutation breeding program (M. Marks, personal communication), targeted genome editing (Sedbrook et al., 2014) and Targeted Induced Local Lesions in Genomes (TILLING) of ethyl methanesulfonate (EMS) mutants (Sedbrook et al., 2014). The genetic similarity of pennycress to the well-studied model species *Arabidopsis thaliana* and the recently sequenced transcriptome and genome will greatly aid and expedite the improvement process (Dorn et al., 2013; Dorn et al., 2015).

Germplasm collection, screening and early breeding efforts of pennycress was initiated at the University of Minnesota in 2013. With the understanding that sufficient variation for traits of interest is required to carry out successful selection and improvement (Fehr, 1991), we sought to assess a collection of forty-two accessions for a variety of morphological and yield component traits. This served as the initial stage in the development of our breeding program. Here we report the characterization of these accessions and address the following objectives:

1) Assess the phenotypic variation of morphological traits in the collection of 42 winter type pennycress accessions.

2) Assess the phenotypic variation of seed yield component traits in the collection of 42 winter type pennycress accessions.

3) Calculate best linear unbiased estimates (BLUEs) as trait values for each accession and trait combination, estimate broad sense heritability, explore correlations between traits and identify clusters of accessions that display similar phenotypes.

Materials and Methods

Pennycress accessions

An original collection of 69 wild *Thlaspi arvense* accessions from the USDA Agriculture Research Service (ARS) National Plant Germplasm System (NPGS) and the University of Minnesota Collection was sown and phenotyped in two environments (1 rep each; due to seed limitations) in St. Paul, Minnesota in 2014. Accessions that behaved as spring types (i.e. flowered, or showed signs of floral initiation in the fall before vernalization (Figure 2.1) were culled from the experiment (Table 2.1). The final collection consisted of forty-two winter-type pennycress accessions of diverse origin (Table 2.2; Figure 2.2). This collection was planted again in Fall 2015 at three locations, 2 replications each, in a randomized complete block design (Table 2.3).

Field Evaluations

The soil and weather conditions at the five environments are described in Table 2.4. The 2013/2014 plots were seeded with a single row cone seeder, and 2014/2015 plots Wintersteiger TRM 2200 4-row seeder in St. Paul and a Hefty G plot drill in Waseca and Rosemount. Alleys in all fields were 1.5 m wide except for the St. Paul environments with 0.61 m, and planting depth was 0.635 cm. Plot size, seeding rate, and preceding crop information is shown in Table 2.3. Seed was pretreated before planting by soaking overnight in a 2% solution of Gibberellic Acid and surface dried prior to planting (GA; ProGibb) at all sites in 2013/2014 and at St. Paul in 2014/2015 (Table 2.3). Plots were hand weeded and alleys cultivated as necessary. All St. Paul environments (2013/2014 & 2014/2015) received supplemental overhead irrigation to enhance seed germination. No supplemental fertilizer was applied.

A total of 19 morphological and yield component traits were evaluated (Table 2.5). Emergence was measured as the number of plants one-meter^{-1} in a single row and was recorded in the fall 4 weeks after planting. Maturity was recorded on 4-6 occasions throughout the growing season using an adapted BBCH maturity scale from 0-100 for

camelina by Martinelli & Galasso (2011) (Table 2.6; Figure 2.3). In 2014 plots were assessed for maturity on May 2 (Julian Day 122), May 9 (129), May 20 (140), May 28 (148) and June 4 (155). Plots in St. Paul in 2015 were measured on April 16 (106), April 24 (114), May 1 (121) and May 18 (138). Plots in Rosemount were measured on April 17 (107), April 25 (115), May 1 (121), May 8 (128), June 1 (152) and June 10 (161). Plots in Waseca were measured on April 17 (107), April 25 (115), May 1 (121), May 8 (128), June 2 (153) and June 10 (161). Five randomly selected plants in each plot were measured at growth stage (GS) 50 for basal width before flowering initiation in the spring. Six stems were measured for thickness using a digital caliper just below the floral head at GS 67-71. Five plants were measured for height at two growth stages, 67-71 (pre-maturity – 2015 only) and 79 (post-maturity). Measurements of post-maturity height also included a measure of raceme length, or the length of the plant from the most basal pod to the most apical pod. Plant stature was scored at GS 63 on a scale from 1-4, with 1 being the completely erect and 4 completely prostrate. Plant vigor was recorded four times throughout the growing season on a scale from 1-9, 9 being the most vigorous. Lodging was recorded as a percentage of plants standing just prior to harvest (GS 89). Shattering was also recorded prior to harvest on a 1-5 scale where 1 = low shattering (≤10% loss); 2 = ~20% loss; 3 = ~30% loss; 4 = ~40% loss, 5 = high shattering (≥ 50% yield loss). Two representative plants were removed from each plot at harvest ripeness and stems plant^{-1}, pods plant^{-1} and seeds pod^{-1} (random subsample size of 5 plant^{-1}) were recorded. In 2015, a one-meter subsample was hand-harvested from the outer row of each plot and stems meter^{-1}, and yield stem^{-1} were recorded. All St. Paul (2013/2014 and 2014/2015) plots were single row and were hand harvested. Rosemount and Waseca plots were combine-harvested using a Wintersteiger Nurserymaster Elite plot combine in 2014/2015 or a Massey Ferguson/Kinkaid 8XP research plot combine, respectively.

Seed Yield Indices

To further investigate the importance of seed weight, seeds pod^{-1} and pods plant^{-1}, two indices were utilized. First, the total weight of seeds pod^{-1} was estimated as:

Index 1: (100 seed weight (mg) / 100) * Average No. Seeds Pod^{-1} = Weight of Seeds Pod^{-1}

A second index was calculated to measure the total weight of seeds plant^{-1} and was estimated as:

Index 2: Weight of Seeds/Pod * Average No. Pods Plant^{-1} = Total Weight of Seeds Plant^{-}

Statistical Analysis

A linear mixed effects modeling approach was used to account for the unbalanced nature of the dataset and to allow for random effects in the model (Vargas et al., 2013). Imbalance in the dataset resulted from the addition of new accessions to the collection in the 2014/2015 field season, unequal number of replications in 2014/2014 and 2014/2015, culling of spring types as well as missing data. Environments are defined as a unique location*year combinations. Accession was treated as a fixed effect in all cases because: 1) comparisons among accessions are of interest and; 2) accessions were selected and did not represent a random sample of all wild pennycress (Piepho & Bu, 2003). Environment was treated as a random effect because locations were not intentionally chosen specifically for inference and served primarily as replication (Piepho & Bu, 2003; Bernardo, 2002). Replication was nested within environment and accession-by-environment interaction were also both treated as random because both terms contain random factors (Piepho & Bu, 2003). A random term for seeding rate was included to account for differences across environments. Seeding rate was treated as random because the values used represent a random sample from a set of possible values (Bates, 2010) and were chosen arbitrarily (and not for inference) based on seed availability. Restricted Maximum Likelihood (REML; Patterson & Thompson, 1971) was used to estimate accession as a fixed effect for each of the traits. Estimates for each accession and trait combination are known as Best Linear Unbiased Estimation (BLUEs). Square root, log and natural log transformations were used as necessary to fulfill linear mixed effects model assumptions.

All mixed effect model analyses were performed in Program R version 3.2.3 (R Core Team, 2015) using the “lme4”, “lmerTest” and “lsmeans” packages (Bates et al., 2014; Kuznetsova et al., 2016; Lenth, 2016). Linear mixed effect models were fit

separately for each trait using backward elimination of terms using the following starting REML equation:

$$y_{ijk} = \mu + acc_i + env_j + rep(env)_{kj} + (acc * env)_{ij} + seeding\_rate_l + e_{ijkl} \quad \text{Equation 1}$$

Where y_{ijk} is the observed trait value of the i^{th} accession in the k^{th} replication of the j^{th} environment, μ is the fixed effect of the overall mean, acc_i is the fixed effect of the i^{th} accession, env_j is the random effect of the j^{th} environment, $rep(env)_{kj}$ is the random effect of the k^{th} replicate nested within the j^{th} environment, $(acc * env)_{ij}$ is the random interaction effect between the i^{th} accession and j^{th} environment, $seeding\_rate_l$ is a partially crossed random effect with environment and e_{ijkl} is the random error term.

Each model term was sequentially removed and tested against the full model using a likelihood ratio test and compared to a Chi-squared (χ^2) distribution with one degree of freedom (Tiede et al., 2015). Terms with a p-value exceeding $\alpha = 0.10$ were eliminated. The α significance threshold of 0.10 was used to mimic the default set by the lmerTest package in R for fitting random effects (Kuznetsova et al., 2016). The variance contribution and standard deviation of each remaining random term was estimated using the R "summary()" function. The significance of the fixed effect's (accession) contribution to the model was estimated using the lmerTest "anova()" function and a Satterthwaite approximation for degrees of freedom (Kuznetsova et al., 2016; Satterthwaite, 1946).

Fixed effects were estimated as BLUEs using the "lsmeans" package and were used as the phenotypes in all subsequent analyses. Trait summary statistics including minimum, mean, maximum, standard deviation, standard error and coefficient of variation (standard deviation/mean; C.V.) were recorded. Pairwise differences were calculated and adjusted for multiple comparisons using Tukey's Honest Significant Difference (HSD) using the compact letter display ("cld") function in "lsmeans" (Lenth, 2016).

Heritability

Most heritability equations assume balanced data but it is common for plant breeding trials exhibit some imbalance (Piepho & Möhring, 2007). To account for the imbalanced dataset, an *ad-hoc* method utilizing lsmeans described in Holland et al. (2003; p. 64) was used:

$$\overline{\mathrm{H}}^2 = \frac{\sigma_{\mathrm{g}}^2}{\sigma_{\mathrm{g}}^2 + \overline{v} / 2} \qquad \text{Equation 2}$$

Where $\overline{\mathrm{H}}^2$ is the broad sense heritability, σ_{g}^2 is the variance component due to genotype (i.e. accession) and $\overline{v}$ is the average variance of a difference of lsmeans pairwise comparisons. The average variance of a difference between lsmeans was calculated using the R function "difflsmeans" in the package "lmerTest" (Kuznetsova et al., 2016). The fixed effect accession was changed to a random effect in all of the final, fitted models and random effect summaries were generated to estimate the genetic variance component (σ_{g}^2).

Correlations

Pearson's correlation coefficients and *P*-values for relationships between BLUEs for morphological and yield component traits were estimated using the "Hmisc" R package's "rcorr" function (Harrel & Dupont, 2015).

Hierarchical Clustering

Hierarchical clustering was used to assess the presence of subgroups within the pennycress collection using a transformed dataset. BLUEs for all traits were subjected to a "unit.sd" transformation function from the "fields" package in R, which subtracts the mean from each unique accession trait value and divides by the standard deviation (Nychka et al., 2015). Using this dataset, a Euclidian distance matrix was calculated and between cluster distance was approximated using Ward's (Ward, 1963) linkage method via the "hclust" function in R. The function "hclust" utilizes an iterative process that begins with each unique object (i.e. accession) assigned to its own cluster and joins the two clusters that are most similar until a single cluster remains (R Core Team, 2016).

Maturity Ratings

BBCH maturity ratings were fit in a linear mixed effects model as described previously, but in this case, a covariate Julian days was added. In order to obtain tangible values for estimates of days to flowering and maturity (rather than slopes or average maturity ratings), the number of days to 50% flowering (GS 65) and maturity (GS 89) were predicted for each of the accessions based the fitted mixed effects model by adjusting the reference grid in the "lsmeans" package (Lenth, 2016). The predicted values were used as phenotypes in subsequent analyses.

Results

Mixed Modeling

Significant variation for accession was detected in 13 of the 19 trait models at $P < 0.05$, and for 1 of 19 at $0.05 < P < 0.10$ (Table 2.7). Pairwise differences after adjusting for multiple comparisons using Tukey's HSD resulted in more than one grouping in 9 of the 13 models in which accession was significant ($P < 0.05$). Pairwise differences were found for the following morphological traits: emergence, basal width, stem width, plant habit and height, and the following yield component traits: single row hand harvest yield, hundred seed weight, seeds pod^{-1}, and plot combine yield. The accession term was not significant ($P > 0.10$) in the models for prematurity height, lodging, pods plant^{-1}, raceme length, and pod shattering (Table 2.7).

The random effect for seeding rate was insignificant ($\alpha = 0.10$) and therefore excluded from every model besides single row hand harvest yield where it was significant (Table 2.9). This is not surprising as this term was partially confounded with environment, suggesting that the majority of the variance was accounted for within the random environment term. The random accession*environment (A*E) interaction term was moderately significant (using the default threshold of $P < 0.10$) for ten traits including basal width, stem width, height, lodging, raceme length, vigor, hundred seed weight, prematurity height, yield and seeds pod^{-1} (Table 2.9), indicating the presence of

genotype by environment interaction for these traits. All random effects were insignificant in the model on the basis of a log likelihood test for pod shattering and combine yield, which resulted in a simple linear model with no random effects and accession as a fixed effect (Table 2.9). In the mixed effect models, the components environment and residual explained the majority of the variance for the traits with an average of 38.2% and 51.7% respectively. A significant environment term suggests that the accessions displayed a high degree phenotypic plasticity across environments for these traits. A*E and R/E were minor contributors to the total model variance in models in which they were significant, and explained an average of 16.3% and 4% of the variance, respectively (Table 2.9).

Growth Habit

Spring types were classified as plants that initiated stem elongation and/or flowering in the fall prior to vernalization. Spring type plants either died over the winter or survived and re-initiated flowering in the spring. Spring types that survived exhibited unfavorable phenotypes including severe lodging and brittle stems (Figure 2.1). Accessions that behaved as spring types were culled from the collection following the 2013/2014 season (a total of 27 accessions). In addition, four accessions that displayed poor emergence (< 5 plants per plot) were removed. These accessions included: TAMN113 (St. Louis, MO), TAMN119 (Roseau, MN), TAMN122 (Roseau, MN) and Ames32239 (Colorado). Accessions TAMN112 and TAMN120 displayed winter-type growth habits in 2013/2014 but exhibited segregation for spring/winter habit in 2014/2015 in St. Paul but not at Waseca or Rosemount. Data from these plots at St. Paul specifically were removed from the analysis on the basis of their deviant phenotypes and resulting incomplete and uneven stands.

Morphological Traits

Summary statistics for each of the trait values calculated as BLUEs are shown in Table 2.8 and correlation between traits in Table 2.10. Emergence was the most variable

of the morphological traits with a range of 16.1 – 66.3 and C.V. of 35%. Emergence had a significant ($P < 0.05$) negative correlation with stem width ($r = -0.61$) and positive correlations with prematurity height ($r = 0.57$), stems meter^{-1} and vigor ($r = 0.40$). Therefore, greater plant density in the fall resulted in narrower stems, taller, more vigorous plants and more stems meter^{-1}. Narrower stems were correlated with a less erect phenotype ($P < 0.001$; $r = 0.63$). Basal width had significant ($P < 0.05$) positive correlations with height ($r = 0.68$) and vigor ($r = 0.47$). Prematurity height was positively correlated with height at maturity ($r = 0.38$), vigor (0.68), and negatively correlated with days to flowering and days to maturity ($r = -0.59$).

In the analysis of maturity, accession was not significant ($P = 0.13$), and there were no pairwise comparisons. In the predicted values from the linear mixed effects model using "lsmeans," for GS 65 (full flowering) and 89 (maturity), the average Julian days was 132.7 and 165.0, respectively. The range between the earliest and latest accessions was 3 Julian days.

Yield Component Traits

Summary statistics for each of the yield component trait values calculated as BLUEs are shown in Table 2.8 and correlation between traits in Table 2.11. Yield was estimated in two different ways: plot combine yield, which was a mechanical harvest, and single row hand harvested yield. The two were kept separate due to the differences in plot size and harvesting method as hand harvesting resulted in less seed loss from shattering. There was a weak ($r = -0.04$) and insignificant ($P = 0.81$) correlation between the two yield estimates. Single row hand harvest yield showed a significant ($P < 0.05$) but moderate positive correlation with one hundred seed weight ($r = 0.32$) and yield stem^{-1} ($r = 0.44$), and a negative correlation with stems plant^{-1} (-0.38). As the number of pods plant^{-1} increases, one hundred seed weight decreased ($r = -0.54$), however this relationship did not hold true for seeds pod^{-1} and one hundred seed weight ($r = -0.21$, $P = 0.18$). Yield component indices, seed weight pod^{-1} (Index 1) and seed weight plant^{-1}

(Index 2) were not correlated with plot combine yield or single row hand harvest yield (Table 2.10).

Heritability

All heritability estimates are shown in Table 2.7. Estimates for the morphological traits ranged from 0.21 for lodging to 0.84 for plant habit. In the yield component traits, estimates ranged from 0.20 for pods plant^{-1}, and 0.68 for one hundred seed weight.

Hierarchical Clustering

Hierarchical clustering revealed four clusters: A1, A2, B1 and B2 (Figure 2.4; Table 2.12). Fourteen accessions clustered in 1A and in general had high emergence, fewest days to flowering and maturity, highest combine plot yield and the least number of stems plant^{-1}. Cluster A2 had three accessions and had low emergence, low basal width and most days to flowering and maturity. Cluster B1 had 4 accessions and the plants were on average larger for traits including basal width, stem width, height, raceme length, one hundred seed weight, most shattering resistance and had the highest hand harvest yield. Cluster B2 had twenty-one accessions that had the lowest hand harvest yield, most seeds per pod and generally ranked in the middle for most traits.

Discussion

Growth Habit

For successful adoption in an agricultural system, pennycress must show reliable winter survival. The winters of 2013/2014 and 2014/2015 in Minnesota posed different challenges for overwintering plants. The 2013/2014 winter had lower average temperatures, greater snow coverage and a later spring than 2014/2015, which was much more mild with higher average temperatures and less snow cover (Table 2.3). None of the winter type pennycress accessions in this collection suffered any noticeable winterkill, demonstrating potential for high survivability in northern climates. Excellent winter

survivability (min. temperature of -30 deg C) was also reported for a collection of 100 pennycress accessions (some of them overlapping with the present collection) in Macomb, IL (Sedbrook et al., 2014).

The divergent growth habits observed within an accession in the fall of 2014 (experimental year 2014/2015) in St. Paul may have resulted from the early planting date (compared to the other locations) and the lengthened opportunity to initiate flowering prior to vernalization (Table 2.3). These 24 accessions, in addition to the two discovered in 2014/2015 in St. Paul (TAMN112 and TAMN120), were all derived from wild collections and may be segregating at Flowering Locus C (FLC). Flowering Locus C is one of the central negative regulators of flowering in Arabidopsis and contains an orthologous gene in pennycress. Five separate alleles of FLC of pennycress that result in the spring type habit have been described (Dorn, 2015). Two of the accessions featured in this study, TAMN106 and TAMN108 have been genetically proven to contain both winter type and spring type individuals (Dorn et al., 2014; Dorn, 2015). TAMN108 contains a single base pair insertion, which causes a frame shift in FLC (c.6_7insG) (Dorn, 2015). The mutated alleles are recessive, while the wild type allele, which results in the winter growth type, is dominant (Dorn, 2015; Sedbrook et al., 2014; McIntyre & Best, 1978).

The accessions containing divergent spring and winter phenotypes may be a result of insufficiently inbred accessions, and also likely due to the fact that many of the accessions were not derived from a single plant. The USDA germplasm collection method for collection is to sample multiple individuals in an area, but as many of the accessions were donated, exact collection methods are unknown (Laura Marek, personal communication). A recent observation by Isbell et al. (2015) indicates that both spring and winter types exist within single NPGS accessions. This is likely in effort to maximize reproductive success. The early collections within the UMN Collection that were made by Dr. Donald Wyse were also derived from multiple individuals in a population (Donald Wyse, personal communication).

Morphological Traits

It was suggested by Sedbrook et al. (2014) that stand establishment and germination are two of the greatest barriers to successful adoption of pennycress as a crop. Emergence (plants meter^{-1}) was used as a measure for stand establishment, and shows a moderately high heritability ($H^2 = 0.59$) and variability among accessions, suggesting that breeding could improve this trait. An accession of pennycress 'Katelyn' was publically released to NPGS in 2015 (Isbell et al., 2015). 'Katelyn' was developed from two generations of mass-selection for improved germination response of freshly harvested seeds from the spring type accession 'Beecher' (PI 672505), a wild collection from Hannah City, IL (Isbell et al., 2015). 'Katelyn' (S_2) seed displays a 91% post-harvest germination rate compared to 'Beecher' at 7% under 12 hr light/dark cycles at 27.5° and 11.5° C, respectively (Isbell et al., 2015). The significant improvement observed with only two cycles of selection suggests the potential for improving germination rates and stand establishment of the winter-type accessions within the present collection.

Pod shatter is a method for seed dispersal for plants in the wild, however it is a disadvantage in cultivated settings. Pod shatter is also a trait typically associated with domestication, but has yet to be addressed in pennycress breeding (Sedbrook et al., 2014). Shattering was observed to be a major challenge especially with the mechanical harvesting of pennycress in this study. Timely harvest is essential to prevent major yield loss as the transition from mature, harvestable material to over-ripe, shattered material is rapid and can happen within a matter of days. No significant variation ($P = 0.99$) was observed for shattering resistance within the accessions in the present collection. It is possible that the qualitative resistance scale used in this experiment was not sensitive enough to differentiate between accessions and that a more quantitative measure is needed. Furthermore, it may also suggest that breeders need to look elsewhere for resistance. It was observed by Sedbrook et al. (2014) that the morphology of the dehiscence zone in pennycress is very similar to that of Arabidopsis. The morphological and genetic mechanisms underlying shatter resistance in Arabidopsis have been well

studied. Shattering is caused by the breakdown and lignification of cells near the dehiscence zone and a layer of cells in an internal valve (Spence et al., 1996). SHATTERPROOF (SHP) and SHATTERPROOF2 (SHP2) are functionally redundant MADS-box (transcriptional regulators associated with plant development) genes that are necessary for seed dehiscence. Double mutants (*shp* and *shp2*) show less constriction in valve margins, lack of dehiscence zone formation, and a reduction in lignification (Liljegren et al., 2000). FRUITFULL (FUL), another MADS-box gene, is a negative regulator of SHATTERPROOF expression and that gain-of-function in Arabidopsis was sufficient for producing indehiscent fruit (Ferrándiz et al., 2000). Orthologs of these genes and others in pennycress are a potential focus for targeted genome editing (Sedbrook et al., 2014). Another potential source of shattering resistance includes the large-scale pennycress mutagenesis populations developed using fast neutron, gamma and ethyl methanesulfonate (EMS) at the University of Minnesota in 2013. Several mutants have been identified for their increased resistance to shattering (M. David Marks, personal communication).

In the present study, there was no significant difference in the number of days to flowering and maturity across accessions ($P = 0.13$) and no pairwise differences were detected. Minor variation was found among the accessions assessed in Sedbrook et al. (2014), but no specific data or estimate is given on the type of variation observed. A report on the flowering time of pennycress suggests that as a long-day plant, time to flowering is reduced under long day conditions and increased under short day conditions, an effect that is exacerbated by low nitrogen levels (Best & McIntyre, 1972). Earlier flowering time and maturity is desired to reduce potential yield-reducing delays in soybean planting. Despite our efforts to sample pennycress accessions from a wide variety of collection sites (Figure 2.2), once planted in a common garden experiment they all reached the same stage of flowering and maturity within 3 d of each other. As a comparison, a flowering time phenology study for *Brassica napus* L. revealed a 34 – 83 d range from planting to 50% flowering (difference of 49 d), depending on the environment (Cruz et al., 2007). In a study that sought to compare late and early flowering strains

(spring and winter types) of pennycress, seeds from natural populations were collected from within the Regina Research Station in Regina, Saskatchewan, Canada. When planted together, their range for days to flowering were 105 to greater than 120 days starting from emergence time (difference of 15 d) (McIntyre & Best, 1978). It was not feasible to visit all our locations on a daily basis, so BBCH ratings were taken on a weekly basis. It is possible that greater resolution and more sampling may have been able to capture the more subtle variations for this trait.

Yield Component Traits

Two different methods were utilized for planting and harvest: single rows were harvested by hand and plots were mechanically harvested using a combine. There is a lack of correlation between these estimates, as well as rank changes in the accessions across the two traits. This result may be confounded both by the orientation of the plot the harvest method used and shattering in the combine. The fact that plants in general respond differently to differences in row spacing is well documented in the literature, especially with regard to yield component traits in soybean (Lehman & Lambert, 1960) canola (Shahin & Valiollah, 2009) and wheat (Joseph et al., 1985). Variability in plant height, branching patterns and seed yield are reported for pennycress, specifically in response to differences in environment, seeding rates and stand densities (Best & McIntyre, 1975; Matthies, 1990; Johnson et al., 2015). Phippen, Gallant & Phippen (2010) and Matthies (1990) both reported similar results – that plants were able to compensate under low densities to produce more branches and pods. It is possible that plants within the single row environment responded to the reduced competition and yielded differently. These results stress the importance of evaluating and selecting within the target planting environment (plot vs. row vs. broadcast) and utilizing the appropriate method of harvest.

The yield Indices #1 (weight of seeds pod^{-1}) and #2 (weight of seeds $plant^{-1}$) were calculated to identify a yield component trait that was highly indicative of overall yield. However, neither correlated significantly to either combine plot yield or single row hand

harvested yields. It appears the strongest relationships with overall yield were high one hundred seed weight, high yield stem^{-1} and a lower number of stems plant^{-1}, which may be target traits for selection to identify high yielding ideotypes in a breeding program. A previous observation by Matthies (1990) identified a negative correlation between the mean weight of the seeds and the number of seeds per capsule. A similar trend was observed in the present collection, although the relationship was not significant (r = -0.21, P = 0.18). Weight ranges from 0.4 – 1.3 and 0.6 - 1.3 mg per seed were observed in the WIU and USDA accessions accordingly (Sedbrook et al., 2014). Another report comparing two European accessions grown in a greenhouse reported one hundred seed weight values of 0.123 and 0.101 g (or 1.23 mg and 1.01 mg seed^{-1}) (Claver et al., 2017). One hundred seed weight values in the present study are very similar and range from 0.085 – 0.114 g (or 0.85 – 1.14 mg seed^{-1}). As observed with other traits, the values for seed weight that were estimated in this collection are very similar to the NPGS accessions and less variable than the WIU collection as reported in Sedbrook et al. (2014).

Hierarchical Clustering

This is the first report of clustering of pennycress accessions based on their morphological and yield component traits. Hierarchical clustering is useful for identifying trends among the accessions and relationships between traits. For example, plants that tend to exhibit large basal widths, also tend to be taller, have later flowering and maturity and higher one hundred seed weight (Table 2.12). This approach is superior to simply looking at correlations because it clusters individual accessions and based on their similarity and one can visually observe groupings. This demonstrates that if the breeding program desires shorter plants to reduce lodging, they may be selecting for lower yielding, smaller plants overall. From a breeding perspective, it may also be beneficial to focus on crossing accessions from different clusters to maximize diversity in the progeny.

Conclusions

Here we report the characterization of 10 morphological and 9 yield component traits in a collection of 42 wild, winter type pennycress accessions from the University of Minnesota and the NPGS collections. Significant variation and pairwise differences were detected for the majority of traits assessed and high heritability estimates were observed, indicating potential for selection within the present collection, which serves as the basis for the University of Minnesota breeding program. Methodologies presented for assessing morphological and yield component traits in pennycress are reported, in particular with regards to phenotyping days to flowering and maturity using an adapted BBCH scale, that may be utilized by the community of pennycress researchers. This is the first reported maturity and development scale reported for pennycress, with the exception of a "Seed Stage Development Scale" proposed in Claver et al. (2017), which outlines four stages of silicle development based on color (green, green-yellow, yellow-green and dry). The trait values for the accessions calculated here will inform the breeding efforts and provide a baseline for gains from selection. Morphological and yield component traits that remain as challenges for researchers working to develop pennycress as a crop include early maturity and shattering. Despite our efforts to evaluate collections from a wide host range, we were unable to detect both significant variation and pairwise differences for these traits, indicating that additional sources of genetic variation are needed to make sizable gains in improvement, both in the form of targeted/untargeted mutagenesis and additional collections.

Tables and Figures

Table 2.1. Accession name, origin and source of the 27 wild spring type pennycress accessions that were culled from the original collection on the basis of growth type. Sources include the United States Department of Agriculture's Germplasm Resource Information Network (USDA NPGS) and the University of Minnesota Collection (UMN Collection).

No.	Accession	Origin	Source
1	TAMN115	St. Louis, MO	UMN Collection[¶]
2	TAMN121[†‡]	Roseau, MN	UMN Collection
3	TAMN123[†‡]	Roseau, MN	UMN Collection
4	TAMN124[†‡]	Roseau, MN	UMN Collection
5	TAMN125[†‡]	Roseau, MN	UMN Collection
6	TAMN128	Montana	UMN Collection
7	TAMN129[§]	Montana	UMN Collection
8	TAMN131[†‡]	Howard Springs, Montana	UMN Collection
9	TAMN133[§]	Montana	UMN Collection
10	TAMN134	Montana	UMN Collection
11	TAMN135	Montana	UMN Collection
12	Ames29512[†‡]	Canada	USDA NPGS
13	Ames31493[‖]	D'Arcy, Saskatchewan, Canada	USDA NPGS
14	Ames31489[†‡]	Saskatoon, Canada	USDA NPGS
15	Ames31490[†‡]	North Battleford, Saskatchewan, Canada	USDA NPGS
16	Ames31491[†‡]	Lashburn, Saskatchewan, Canada	USDA NPGS
17	Ames31492[†‡]	Makwa, Saskatchewan, Canada	USDA NPGS
18	Ames31500[†‡]	Beaverlodge, Alberta, Canada	USDA NPGS
19	PI633415[†‡§]	Belgern, Saxony, Germany	USDA NPGS
20	PI650286[†]	Groitzsch, Saxony, Germany	USDA NPGS
21	PI650285[†]	Leipzig-Mockau, Saxony, Germany	USDA NPGS
22	PI650284[†‡]	Kefferhausen, Thuringia, Germany	USDA NPGS
23	PI633414[†‡]	Wachstedt, Thuringia, Germany	USDA NPGS
24	Ames22461[‡]	Slawinek, Lubin, Poland	USDA NPGS
25	PI650287[†‡]	Molsheim, Bas-Rhin, France	USDA NPGS
26	Ames31021	Shawnee, Colorado	USDA NPGS
27	Ames31024[†‡]	Nathrop, Colorado	USDA NPGS

[†] Indicates that spring type growth habit was also reported by Kevin Dorn (2015)
[‡] Indicates that the causative FLC mutation was identified (Kevin Dorn, 2015; Kevin Dorn, personal communication).
[§] Indicates an accession that displayed winter annual habit in the growth chamber (Katherine Frels, personal communication), indicating divergent populations may also exist for these accessions.
[¶] UMN Collection accessions were made by Dr. Donald L. Wyse.

Table 2.2. Accession name, origin and source of the 42 wild winter annual pennycress accessions surveyed. Sources include the United States Department of Agriculture's National Plant Germplasm System at the North Central Regional PI Station in Ames, Iowa (USDA NPGS). and the University of Minnesota Collection (UMN Collection).

No.	Accession	Origin	Source
1	TAMN101	Coates, MN	UMN Collection[¶]
2	TAMN102	Sherburne, MN	UMN Collection
3	TAMN103	Rosemount, MN	UMN Collection
4	TAMN104	Rosemount, MN	UMN Collection
5	TAMN105	Coates, MN	UMN Collection
6	TAMN106[‡]	Coates, MN	UMN Collection
7	TAMN107	Coates, MN	UMN Collection
8	TAMN108[‡]	Sherburne, MN	UMN Collection
9	TAMN109	Rosemount, MN	UMN Collection
10	TAMN110	Rosemount, MN	UMN Collection
11	TAMN111[†]	New York, NY	UMN Collection
12	TAMN112	Illinois	UMN Collection
13	TAMN114	St. Louis, Missouri	UMN Collection
14	TAMN116	Springfield, IL	UMN Collection
15	TAMN117	Springfield, IL	UMN Collection
16	TAMN118	Springfield, IL	UMN Collection
17	TAMN120	Roseau, MN	UMN Collection
18	TAMN126	Montana	UMN Collection
19	TAMN127	Montana	UMN Collection
20	TAMN130[§]	Montana	UMN Collection
21	TAMN132	Montana	UMN Collection
22	Ames29513	Ames, Iowa	USDA NPGS
23	Ames30982	Saylorville, Iowa	USDA NPGS
24	Ames29531	Fargo, ND	USDA NPGS
25	Ames30985	Yankton, SD	USDA NPGS
26	Ames30984	Fort Pierre, SD	USDA NPGS
27	Ames29118	Illinois	USDA NPGS
28	Ames30983	Illinois	USDA NPGS
29	Ames29509	Columbus, Ohio	USDA NPGS
30	Ames31488	Ontario, Canada	USDA NPGS
31	Ames31487	Ontario, Canada	USDA NPGS
32	Ames23761	Ontario, Canada	USDA NPGS
33	Ames31501	Manitoba, Canada	USDA NPGS
34	Ames24499	Serbia/Montenegro	USDA NPGS
35	Ames30999	Bellvue, CO	USDA NPGS
36	Ames31012	Briggsdale, CO	USDA NPGS
37	Ames31017	Red Feather Lakes, CO	USDA NPGS
38	Ames31018	Pine, CO	USDA NPGS
39	TAMN76	Red Cloud, NE	UMN Collection
40	TAMN77	Sand Hills, NE	UMN Collection

41	TAMN78	Greeley, NE	UMN Collection
42	TAMN79	Creighton, NE	UMN Collection

† Indicates that winter type growth habit was also reported by Kevin Dorn (2015)
‡ Indicates that a divergent spring annual line was identified within the same accession and the causative FLC mutation identified (Kevin Dorn, 2015; Kevin Dorn, personal communication).
§ Indicates an accessions that displayed winter annual habit in the growth chamber (Katherine Frels, personal communication), indicating divergent populations may also exist for these accessions.
¶ Accessions from the UMN Collection were collected by Dr. Don Wyse with the exception of TAMN76, TAMN77, TAMN78 and TAMN79, which were collected by Dr. David Marks.

Table 2.3. Description of the layout, crop history and plot size and management for all locations in 2014 and 2015.

Year	Location	Reps	Plot Size	Row Spacing	Seeds/Plot	Seeding Rate (kg/ha)	Seed Treatment	Preceding Crop	Planting Date	Harvest Date
2014	St. Paul, MN - South	1	1.524 m row	0.61 m	100 seeds	2.67	GA[†]	Soybean	15-Aug	2-Jul
2014	St. Paul, MN - North	1	1.524 m row	0.61 m	100 seeds	2.67	GA	Soybean	30-Aug	2-Jul
2015	St. Paul, MN	2	3.048 m row	0.61 m	0.5 grams	6.46	GA	Buckwheat	27-Aug	19-Jun
2015	Rosemount, MN	2	1.524 x 1.524 m plot	25.4 cm	2.6 grams	11.19	None	Soybean	6-Sep	16-Jun
2015	Waseca, MN	2	1.524 x 1.524 m plot	25.4 cm	2.6 grams	11.19	None	Soybean	18-Sep	30-Jun

[†] Seed was soaked overnight in a 2% solution of Gibberellic Acid (ProGibb) to promote germination.

Table 2.4. Description of site locations, geographic coordinates, elevation, temperature and precipitation at trial sites in the 2014 and 2015 pennycress trials. National Weather Service (NOAA Online Weather Data, 2016) estimates show total snowfall (TSNW), maximum snow depth (MXSD), and accumulated precipitation (TPCP), all in cm, from 1 August to 1 July.

Growing Season	Coordinates	Soil Type	Elevation (m)	Temperature (C)			Precipitation (cm)		
2014				Min	Mean	Max	TSNW:	MXSD:	TPCP:
St. Paul	44° 59' 06" N, -93° 10' 57" W	Waukegan Silt Loam	298	-31.1	3.6	34.4	174.5	63.5	81.6
2015									
St. Paul	44° 59' 06" N, -93° 10' 57" W	Waukegan Silt Loam	298	-24.4	5.3	32.8	87.4	12.7	51.3
Rosemount	44° 41' 16" N, -95° 4' 30" W	Waukegan Silt Loam	295	-26.6	5.5	32.2	66.8	17.8	52.7
Waseca	44° 3' 52" N, -93° 31' 30" W	Nicollet Clay Loam	353	-28.3	5.4	35.0	113.3	17.8	67.0

Table 2.5. Trait names, acronyms, units and number of readings per replication. Traits that were recorded in a particular year's environments are indicated with an "X".

No.	Category	Trait	Acronym	Units	No. Readings	2014	2015
1	Morphological	Emergence	FPPM	Count	1		X
2	Morphological	Basal Width	BSW	cm	5	X	X
3	Morphological	Stem Width	STMW	mm	6		X
4	Morphological	Pre-Maturity Height	PMHT	cm	5		X
5	Morphological	Vigor	VIG	1-9 Scale	5	X	X
6	Morphological	Stems Meter $^{-1}$	STPM	Count	2		X
7	Morphological	Lodging	LODG	% Standing	1	X	X
8	Morphological	Plant Habit	PHBT	1-4 Scale	1		X
9	Morphological	Height	HT	cm	5	X	X
10	Morphological	Days to Flowering	DTF	Julian Days Count	1	X	X
11	Morphological	Days to Maturity	DTM	Julian Days Count	1	X	X
12	Yield Component	Combine Plot Yield	CPY	kg ha $^{-1}$	1	X	X
13	Yield Component	Hand Harvest Single Row	HHSR	g	1	X	X
14	Yield Component	One Hundred Seed Weight	HSW	g	3	X	X
15	Yield Component	Yield Stem^{-1}	YPS	g	2		X
16	Yield Component	Stems Plant^{-1}	STPPL	Count	2		X
17	Yield Component	Pods Plant^{-1}	PPPL	Count	2	X	X
18	Yield Component	Seeds Pod^{-1}	SPPD	Count	5	X	X
19	Yield Component	Raceme Length	RACEL	cm	5	X	X
20	Yield Component	Shattering	SHAT	1-5 Scale	1		X
21	Yield Component	Weight of Seeds Pod^{-1}	WSPPD	g	1	X	X
22	Yield Component	Weight of Seeds Plant^{-1}	WSPPL	g	1	X	X

Table 2.6. BBCH type maturity scale used to measure maturity throughout the growing season of pennycress. Scale abridged and adapted from a camelina scale by Martinelli & Galasso (2011) (see Figure 2.3).

BBCH Code		Description
Principle Growth Stage 4: Floral Initiation		
	48	Basal rosette stage, no signs of floral initiation.
	49	Visible leaf clustering in center of rosette
Principal Growth Stage 5: Inflorescence Emergence		
	50	Inflorescence present but still enclosed by leaves
	51	Inflorescence visible from above
	55	Individual flower buds visible but still closed
	59	First petals visible outside the sepals but all flowers still closed
Principal Growth Stage 6: Flowering		
	60	First flowers open
	61	10% of flowers open
	62	20% of flowers open
	63	30% of flowers open
	64	40% of flowers open
	65	50% of flowers open (full flowering)
	67	70% of flowers open, first pods visible …
	69	90% of flowers open, first pods reaching final size
Principal Growth Stage 7: Development of Fruit		
	71	10% of pods have reached final size
	72	20% of pods have reached final size
	73	30% of pods have reached final size
	74	40% of pods have reached final size …
	79	Nearly all pods have reached final size
Principal Growth Stage 8: Ripening		
	81	10% of pods are ripe (seeds are black and visible through yellowed, dried pod)
	82	20% of pods are ripe
	83	30% of pods are ripe
	84	40% of pods are ripe …
	89	Nearly all pods ripe and crop ready for harvest, basal leaves dried
Principal Growth Stage 9: Senescence		
	97	Plant dead or dry
	99	Harvested product

Table 2.7. Analysis of Variance (ANOVA) of accession as a fixed effect in the linear mixed model analyses for morphological and yield component traits using Satterthwaite degrees of freedom approximation.

Morphological Traits

Trait	Sum Sq	Mean Sq	NumDF[¶]	DenDF[#]	F Value	Pr(>F)	Significance	Pairwise Differences	Heritability
Emergence	26546	647.46	41	117.03	2.56	0.000	***	Y	0.59
Basal Width	127.17	3.10	41	136.11	2.19	0.000	***	Y	0.54
Stem Width	48.86	1.19	41	80.04	2.79	0.000	***	Y	0.64
Prematurity Height	298.06	7.27	41	41.00	1.43	0.129	NS	N	0.30
Vigor	175.66	4.28	41	123.84	1.82	0.006	**	N	0.45
Stems Meter^{-1}	16205	395.24	41	198.07	1.75	0.006	**	N	0.43
Lodging	1.07	0.03	41	87.23	1.18	0.257	NS	N	0.21
Plant Habit	95.04	2.32	41	203.02	6.26	0.000	***	Y	0.84
Height	4323.20	105.44	41	99.12	2.01	0.003	**	Y	0.50
Maturity[‡]									
Julian Days	421421	421421	1	1470.10	27791.4	0.000	***		
Accession	778	19	41	1470.0	1.30	0.1343	NS	N	0.33

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Yield Component Traits

Trait	Sum Sq	Mean Sq	NumDF[¶]	DenDF[#]	F Value	Pr(>F)	Sig.	Pairwise Differences	Heritability
Single Row Hand Harvest Yield	147.61	3.60	41	107	1.60	0.030	*	Y	0.33
One Hundred Seed Weight	4.58E-03	1.12E-04	41	141.25	3.09	0.000	***	Y	0.57
Yield Stem^{-1}	13.23	0.32	41	196.03	1.70	0.009	**	N	0.40
Stems Plant^{-1}	34.31	0.84	41	364.20	1.59	0.015	*	N	0.41
Pods Plant^{-1}	1940.20	47.32	41	425.01	1.17	0.222	NS	N	0.20
Seeds Pod^{-1}	260.39	6.35	41	103.75	2.06	0.002	**	Y	0.52
Raceme Length	1321.90	32.24	41	88.57	1.16	0.272	NS	N	0.19
Weight of Seeds Pod^{-1}	2.31E-04	5.64E-06	41	234.96	2.80	0.000	***	N/A	0.63
Weight of Seeds Plant^{-1}	509.23	12.42	41	229.20	1.42	0.060	.	N/A	0.23
Combine Yield[‡]	**Df**	**Sum Sq.**	**Mean Sq.**	**F Value**	**Pr(>F)**	**Significance**			
Accession	41	3365325	82081	2.39	0.003	**		Y	0.57
Residuals	41	1409854	34387						
Shattering‡		**Sum Sq.**	**Mean Sq.**	**F Value**	**Pr(>F)**	**Significance**			
Accession	41	6.41	0.16	0.53	0.99	NS		N	N/A
Residuals	123	36.58	0.29						

*** P < 0.001, ** P < 0.01, * P < 0.05, . P < 0.1.

[†] Model for maturity had Julian days, accession and year as fixed effects.

[‡] Indicates model excluded all random effects on the basis of a log likelihood test and had only accession as a fixed effect.

[§] Indicates that when converted to a random effects model, the variance component accession was zero and heritability could not be estimated.

[¶] Denotes numerator degrees of freedom.

[#]Denotes denominator degrees of freedom.

Table 2.8. Morphological and yield component traits, units, mean ± standard error of mean (SEM), minimum values and accessions, maximum values and accessions, standard deviation (SD) and coefficient of variation (C.V.) for all accessions based on best linear unbiased estimates (BLUEs).

Trait	Units	Mean ± SEM	Min.	Max.	SD	C.V.
Emergence	Count	36.4 ±1.98	16.13	66.25	12.85	35%
Basal Width	cm	8.75 ± 0.10	7.51	10.91	0.62	7%
Stem Width	mm	3.87 ± 0.04	3.30	4.63	0.27	7%
Prematurity Height	cm	22.40 ± 0.27	19.32	26.20	1.76	8%
Vigor	1-9 Scale	5.81 ± 1.98	4.54	6.99	0.57	10%
Stems Meter^{-1}	Count	64.17 ± 1.26	47.17	79.33	8.16	13%
Lodging	% Standing	0.55 ± 0.02	0.30	0.80	0.12	21%
Plant Habit	1-4 Scale	2.04 ± 0.10	1.00	3.17	0.63	31%
Height	cm	90.98 ± 0.81	81.47	106.86	5.23	6%
Days to Flowering	Julian Count	132.70± 0.11	131.43	134.46	0.73	0.6%
Days to Maturity	Julian Count	165.04 ± 0.11	163.77	166.81	0.73	0.6%
Plot Combine Yield	kg/ha^{-1}	1210.45 ± 31.28	829.76	1657.32	202.70	17%
Single Row Hand Harvest Yield	g	80.39 ± 2.86	38.54	145.1980185	18.55	23%
One Hundred Seed Weight	g	0.1031 ± 0.001	0.08	0.11	0.01	6%
Yield Stem^{-1}	g	1.30 ± 0.37	0.74	1.75	0.24	19%
Stems Plant^{-1}	Count	4.97 ± 0.23	2.73	8.72	1.48	30%
Pods Plant^{-1}	Count	503.60 ± 14.68	348.08	790.68	95.14	20%
Seeds Pod^{-1}	Count	12.27 ± 0.10	10.87	13.58	0.62	5%
Raceme Length	cm	32.04 ± 0.23	28.42	35.09	1.52	5%
Shattering	1-5 Scale	4.66 ± 0.03	4.25	5	0.20	4%
Index 1: Weight of Seeds Pod^{-1}	g	0.0126 ± 0.0001	0.01	0.01	0.00	7%
Index 2: Weight of Seeds Plant^{-1}	g	6.81 ±0.23	4.58	13.28	1.51	22%

Table 2.9. Random effects variance components (V) and their percent contribution (C) to the final, fit model's variance for each of the models' random terms: accession*environment (A*E), replication nested within environment (R/E), environment (E) and residual (R). Contributions were calculated by dividing the variance of each component by the sum of all variance components. Models were fit using a backwards elimination approach where maximum likelihood was used to compare a model with and without the variance component in question and compared to a Chi-square (χ^2) distribution (df=1).

Morphological Traits

Term	Component	Emergence	Basal Width	Stem Width	Prematurity Height	Height	Lodging	Plant Habit	Stems Meter^{-1}	Vigor	Maturity
A*E	**C**	-	8.2%	7.2%	26.1%	15.2%	26.1%	-	-	8.1%	-
R/E	**C**	6.2%	1.0%	3.2%	4.0%	3.1%	4.0%	-	1.8%	3.7%	-
E	**C**	69.7%	71.2%	22.6%	49.9%	62.9%	49.9%	3.6%	51.8%	37.9%	76.3%
R	**C**	24.1%	19.6%	67.0%	20.0%	18.7%	20.0%	96.4%	46.5%	50.2%	23.7%
A*E	**V**	-	0.59	0.05	0.03	42.78	0.03	-	-	0.38	-
R/E	**V**	64.48	0.07	0.02	0.00	8.64	0.00	-	8.62	0.18	-
E	**V**	730.51	5.13	0.14	0.06	176.58	0.06	0.01	251.74	1.78	48.91
R	**V**	252.44	1.41	0.43	0.02	52.58	0.02	0.37	226.00	2.36	15.19

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Yield Component Traits

Term	Component	Combine Yield[†]	Single Row Hand Harvest Yield[‡]	One Hundred Seed Weight	Seeds Pod^{-1}	Pods Plant^{-1}	Stems Plant^{-1}	Raceme Length	Meter Yield Stem^{-1}	Shattering[†]	Index 1: Weight of Seeds Pod^{-1}	Index 2: Weight of Seeds Plant^{-1}
A*E	**C**	-	-	36.8%	9.9%	-	-	8.9%	-	-	-	-
R/E	**C**	-	-	6.0%	-	-	-	2.5%	7.2%	-	2.3%	-
E	**C**	.	.	26.6%	11.1%	17.4%	2.7%	18.5%	46.0%	.	44.3%	21.8%
R	**C**	-	21.0%	30.6%	79.0%	82.6%	97.3%	70.1%	46.7%	-	53.4%	78.2%
A*E	**V**	-	-	4.3E-05	0.39	-	-	3.50	-	-	-	-
R/E	**V**	-	-	7.1E-06	-	-	-	0.97	0.03	-	8.7E-08	-
E	**V**	-	.	3.1E-05	0.43	8.51	0.01	7.32	0.19	-	1.7E-06	2.44
R	**V**	-	2.26	3.6E-05	3.08	40.36	0.53	27.68	0.19	-	2.0E-06	8.78

[†] Indicates a model with no random effects as a result of model fitting.
[‡] The random effect seeding rate remained in the Single Row Hand Harvest Yield model ($\alpha = 0.10$); C = 79.0%, V = 8.49, SD = 2.91.
[§] Components that were not significant ($\alpha = 0.10$) were removed from the model, indicated by "-".

Table 2.10. Phenotypic correlations among the best linear unbiased estimates (BLUEs) for the 10 morphological traits recorded. The below-diagonal elements are Pearson's correlation coefficients (r) followed by their level of significance.

	Emergence	Basal Width	Stem Width	Prematurity Height	Height	Lodging	Plant Habit	Stems Meter^{-1}	Vigor	Days to Flowering	Days to Maturity
Emergence	1	-	-	-	-	-	-	-	-	-	-
Basal Width	-0.22	1	-	-	-	-	-	-	-	-	-
Stem Width	-0.61 ***	0.18	1	-	-	-	-	-	-	-	-
Prematurity Height	0.57 ***	0.12	-0.20	1	-	-	-	-	-	-	-
Height	0.07	0.68 ***	0.03	0.38 *	1	-	-	-	-	-	-
Lodging	-0.08	-0.02	0.18	-0.14	-0.20	1	-	-	-	-	-
Plant Habit	-0.67 ***	0.11	0.63 ***	-0.62 ***	-0.20	0.13	1	-	-	-	-
Stems Meter^{-1}	0.44 **	-0.28	-0.75 ***	-0.01	-0.19	-0.10	-0.35	1	-	-	-
Vigor	0.40 **	0.47 **	-0.20	0.68 ***	0.70 ***	-0.18	-0.49 **	-0.09	1	-	-
Days to Flowering	-0.33 *	0.17	-0.04	-0.59 ***	-0.05	0.02	0.21	0.17	-0.51 ***	1	-
Days to Maturity	-0.33 *	0.17	-0.04	-0.59 ***	-0.05	0.02	0.21	0.17	-0.51 ***	1.00 ***	1

*** P < 0.001, ** P < 0.01, * P < 0.05

Table 2.11. Phenotypic correlations among the best linear unbiased estimates (BLUEs) for the 10 yield component traits recorded. The below-diagonal elements are Pearson's correlation coefficients (r).

	Combine Plot Yield	Single Row Hand Harvest Yield	One Hundred Seed Weight	Seeds Pod^{-1}	Pods Plant^{-1}	Stems Plant^{-1}	Raceme Length	Yield Stem^{-1}	Shattering	Weight of Seeds Pod^{-1}	Weight of Seeds Plant^{-1}
Combine Plot Yield	1	-	-	-	-	-	-	-	-	-	-
Single Row Hand Harvest Yield	-0.04	1	-	-	-	-	-	-	-	-	-
One Hundred Seed Weight	0.05	0.32 *	1	-	-	-	-	-	-	-	-
Seeds Pod^{-1}	-0.26	-0.16	-0.21	1	-	-	-	-	-	-	-
Pods Plant^{-1}	-0.19	-0.07	-0.54 ***	0.15	1	-	-	-	-	-	-
Stems Plant^{-1}	-0.34 *	-0.38 *	-0.35 *	0.09	0.65 ***	1	-	-	-	-	-
Raceme Length	-0.48 **	0.00	0.11	0.10	0.07	0.08	1	-	-	-	-
Yield Stem^{-1}	0.18	0.44 **	0.41 **	-0.09	-0.14	-0.51 ***	0.26	1	-	-	-
Shattering	0.27 .	- 0.22	-0.16	-0.38 *	0.03	0.09	0.17	0.09	1	-	-
Weight of Seeds Pod^{-1}	-0.12	0.12	0.66 ***	0.52 ***	-0.37 *	-0.18	0.12	0.21	0.37 **	1	-
Weight of Seeds Plant^{-1}	-0.19	-0.01	-0.39 *	0.34 *	0.87 ***	0.49 **	0.00	-0.07	0.14	-0.11	1

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

Table 2.12. Mean trait values for accessions grouped into four clusters as revealed by Ward's D hierarchical clustering.

	Cluster			
Trait	A1	A2	B1	B2
Emergence	48.46	24.75	31.24	31.00
Basal Width	8.57	8.28	9.93	8.72
Stem Width	3.69	3.90	3.92	3.98
Prematurity Height	24.13	19.53	22.99	21.55
Vigor	6.19	4.88	6.48	5.57
Stems Meter^{-1}	67.01	68.95	62.87	61.83
Lodging	0.54	0.56	0.46	0.58
Plant Habit	1.36	2.78	2.00	2.40
Height	92.13	86.50	101.29	88.89
Days to Flowering	132.31	134.05	133.30	132.65
Days to Maturity	164.65	166.39	165.64	164.99
Combine Plot Yield	1375.92	1090.32	1043.19	1149.16
Single Row Hand Harvest Yield	85.43	74.92	113.34	71.53
One Hundred Seed Weight	0.1038	0.0875	0.1101	0.1035
Yield Stem^{-1}	1.42	0.88	1.50	1.25
Stems Plant^{-1}	4.02	6.34	4.51	5.50
Pods Plant^{-1}	465.13	656.93	542.72	499.88
Seeds Pod^{-1}	11.93	12.46	12.05	12.50
Raceme Length	31.65	30.94	33.25	32.23
Shattering	4.80	4.77	4.53	4.62
Weight Seeds Pod^{-1}	0.0124	0.0108	0.0131	0.0129
Weight Seeds Plant^{-1}	6.14	9.08	7.80	6.76

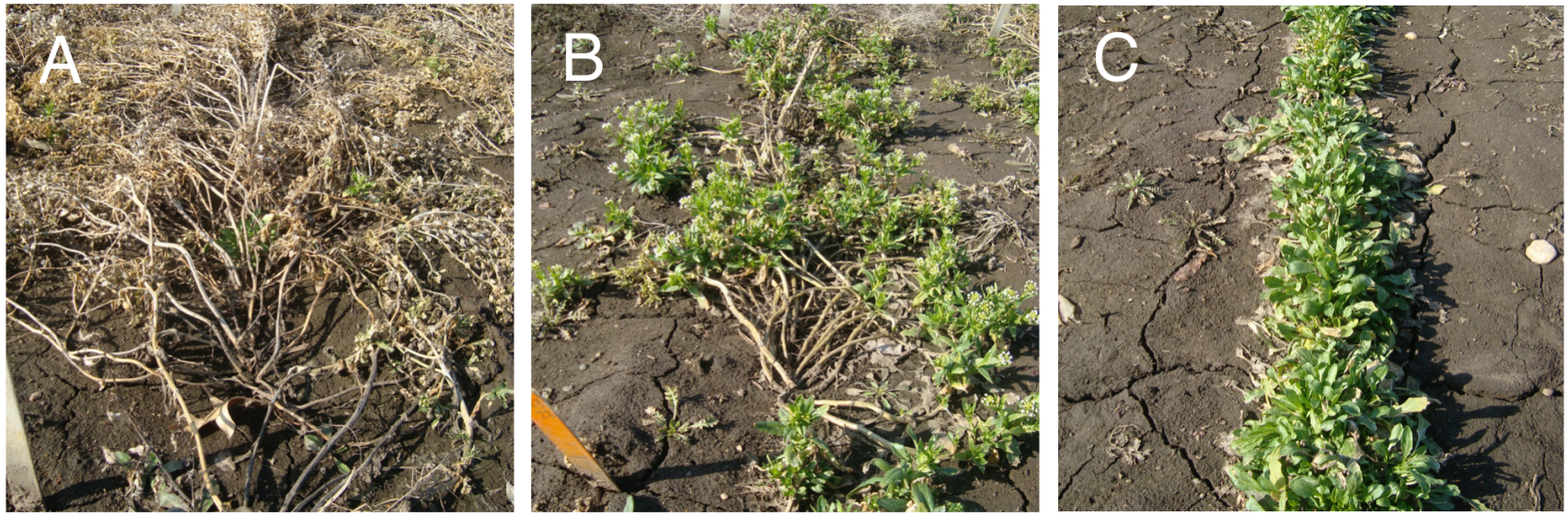

Figure 2.1. Pennycress growth habit as observed in St. Paul, MN on April 15, 2014. (A) Spring type accessions that initiated flowering and seed set in the fall before vernalization and did not survive winter. (B) Spring type accession that initiated stem elongation in the fall, overwintered and continued to flower in the spring. (C) Winter type accession that did not initiate floral growth until after vernalization. Accessions that displayed growth as shown in A and B were culled from the experiment.

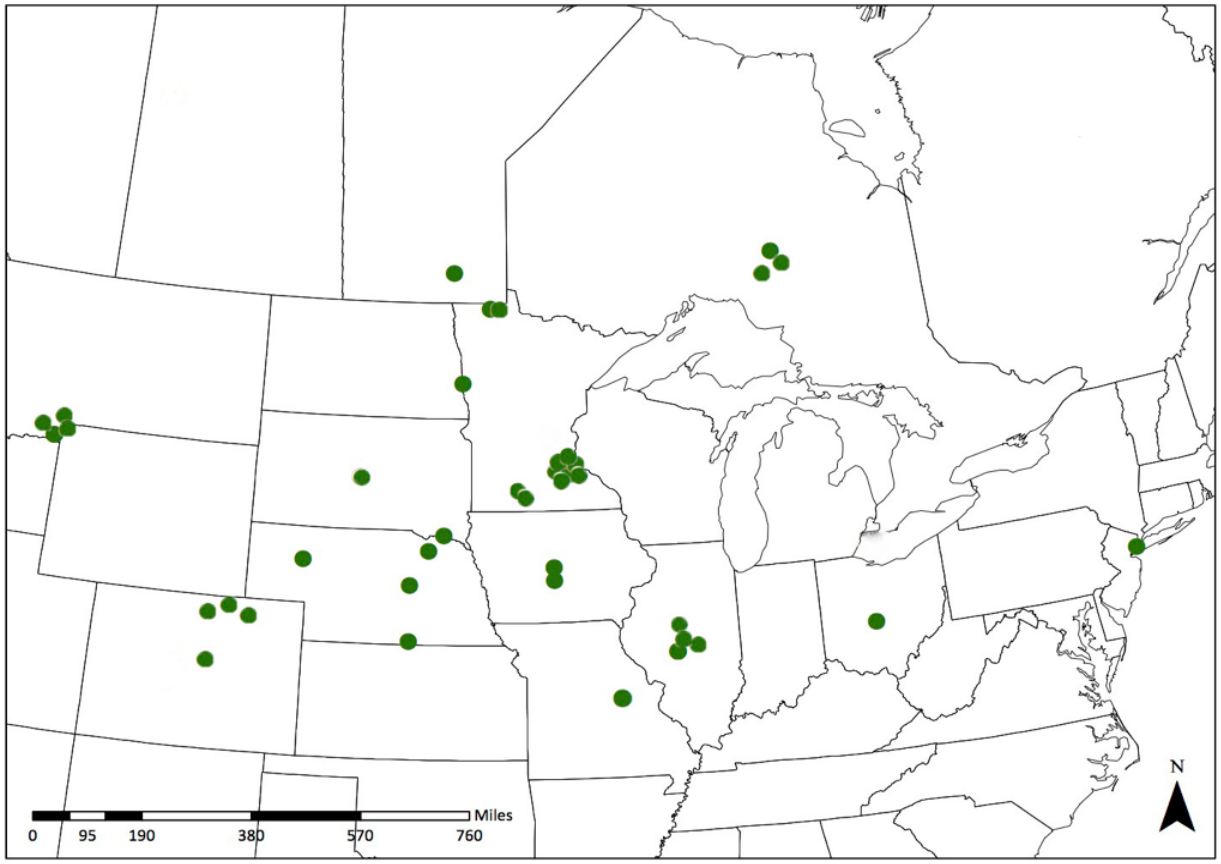

Figure 2.2. Geographic distribution of the origins of the winter type pennycress accessions surveyed. Green dots indicate collection sites for both accessions from NPGS and the UMN collections. Not pictured is NPGS accession Ames24499 from the State Union of Serbia and Montenegro.

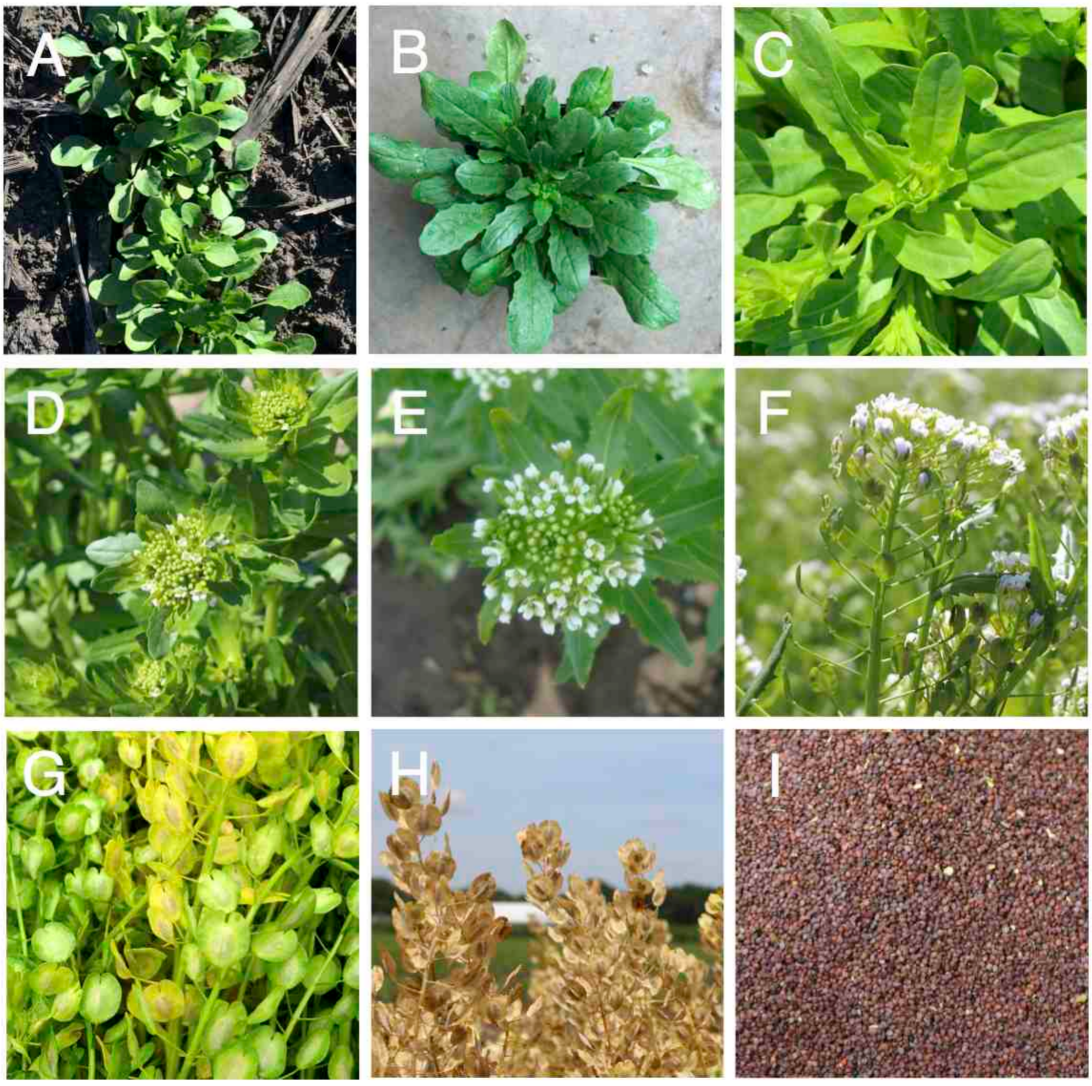

Figure 2.3. The various growth stages (GS) of pennycress. (A) GS 48; basal rosette stage, no signs of floral initiation. (B) GS 49; visible leaf clustering in center of rosette. (C) GS 50; inflorescence present but still enclosed by leaves. (D) GS 60; first flowers open. (E) GS 65; 50% of flowers open (full flowering). (F) GS 69; 90% of flowers open, first pods reaching final size. (G) GS 81; 10% of pods are ripe (seeds are black and visible through yellowed, dried pod). (H) GS 97; plant dead or dry. (I) GS 99; harvested product.

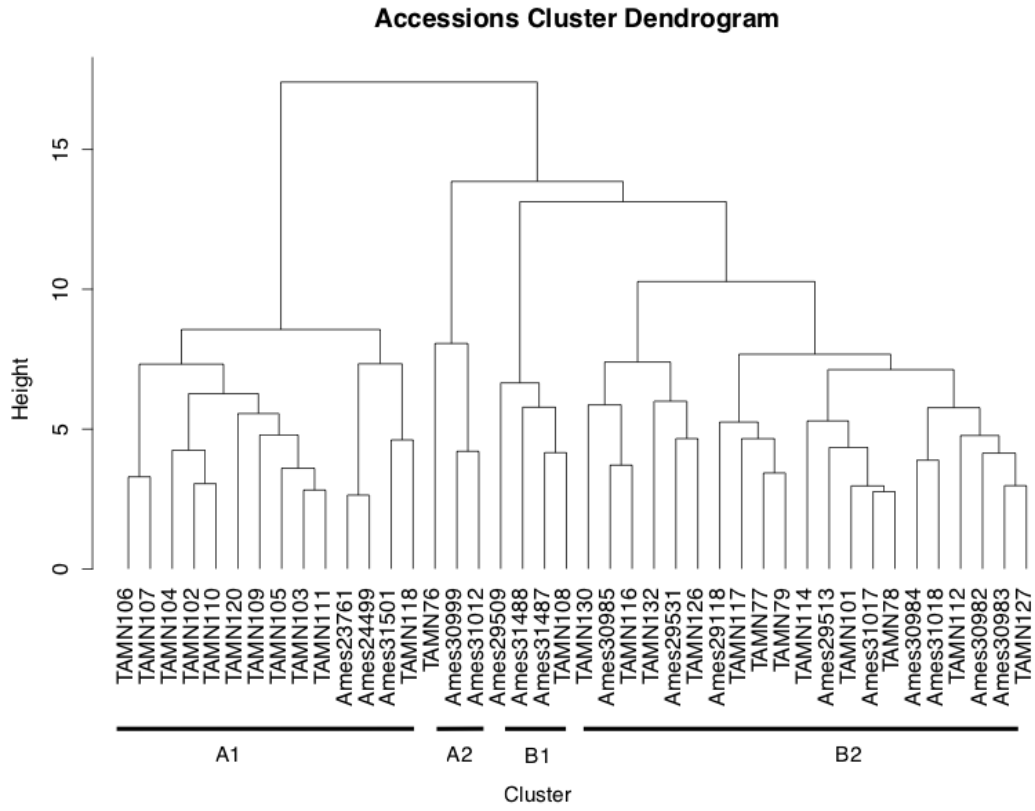

Figure 2.5. Hierarchical clustering of the pennycress accessions using best linear unbiased estimates (BLUEs) for both morphological and yield component traits. A1, A2, B1 and B2 designate clusters that exhibited similar phenotypes.

Chapter 3: Oil Chemistry Traits of Winter Annual Pennycress Germplasm

Introduction

The lack of soil cover throughout the late fall, winter and early spring in the corn and soybean-dominated agricultural landscape of the Midwestern United States is of growing concern. Exposed soil is susceptible to nutrient runoff and erosion, which is problematic for maintaining surface and subsurface water quality. The benefits of planting cover crops are well documented and include reduced NO_3 losses and soil erosion, increased organic matter, weed suppression, carbon sequestration and habitat as well as food resources for beneficial pollinators (Kladivko et al., 2004, 2014; Kaspar et al., 2007, 2008, 2012; Strock et al., 2004; Lal et al., 1991; Bowman et al., 1998; Sindelar et al., 2015; Eberle et al., 2015). Despite these benefits, cover crops were planted on less than 2% of all U.S. cropland in 2010-2011 (Wade et al., 2015). Barriers to planting cover crops include difficulty of establishment/termination, interference with cash crops, perceptions of risk and uncertainty and finally, lack of improved cover crop cultivars (Strock et al., 2004; Snapp et al., 2003; Arbuckle & Roesch-McNally, 2015; Runck et al., 2014; Brummer et al., 2011). To aid in incentivizing cover crops, researchers are looking for ways to develop new cover crops that have economically-viable end uses. One area in particular is the use of alternative winter annual oilseed crops for biofuel production.

There is increasing demand and regulatory push for the production and use of domestic, renewable biofuels due to their environmental benefits over petroleum-derived fuels. Biodiesel and renewable diesel, specifically, have advantages compared to their petroleum counterparts. They can be derived from renewable, domestic feedstocks, have improved lubricity, lower sulfur content, excellent flash point and biodegradability, reduced toxicity and overall lower regulated exhaust emissions (Moser et al., 2009a). They burn cleaner than petroleum-based fuels and can offset their associated carbon emissions by the carbon the feedstock plants assimilate during growth (Fan et al., 2013). One major barrier that limits the production of biodiesel and renewable diesel fuels is the sometimes prohibitive cost of feedstock acquisition, or the price of the feedstock to the processing plant, which can be up to 85% of the total production cost (Paulson & Ginder,

2007). The common biofuel feedstocks in the United States are soybean, corn, canola, palm, and animal fats (EIA, 2016), many of which have competitive food-related uses. One way to reduce feedstock costs is to utilize alternative, non-food sources. Viable alternative feedstocks need an acceptable fatty acid profile, low production costs, few agricultural inputs and production during the off-season or on marginal lands (Moser et al., 2009a).

Field pennycress (*Thlaspi arvense* L.) is a winter annual oilseed currently under investigation for its dual use as a feedstock for biodiesel and renewable diesel production as well as for a cover crop. Pennycress is a self pollinating, diploid (2n=14) plant that is related to canola, camelina and the cruciferous vegetables (Warwick et al., 2002; Franzke et al., 2011). The winter annual habit of pennycress allows it to be seeded in late August or early September, meaning it can be grown in systems that allow sowing during that time. Some examples in the Upper Midwest include: sweet corn, silage corn and early-harvested sugar beets. The most commonly proposed methods include interseeding pennycress into standing corn (*Zea mays* L.) prior to or immediately following corn harvest in the fall and harvesting before soybean [*Glycine max* (L.) Merr.] planting in the spring (sequential double cropping system) or over the top of V3 soybean plants (relay cropping system) (Sindelar et al., 2015) (Figure 1.3). It can be grown in the rotation without requiring additional land or displacing other food crops, which has proven to be a major concern with other biofuels, such as ethanol (Boateng et al., 2010; Pimentel et al., 2009).

Pennycress oil in its current state is an acceptable feedstock for biodiesel production and meets the United States American Society for Testing and Materials (ASTM) D6751 regulations (Moser et al., 2009a). Its oil is composed of 55.6% monounsaturated fatty acids (one double bond) (16:1, 18:1, 20:1, 22:1, 24:1), 38% polyunsaturated fatty acids (18:2, 18:3, 20:2, 20:3, 22:2, 22:3) (two or more double bonds) and 4.6% saturated fatty acids (only single bonds) (14:0, 16:0, 18:0, 20:0, 22:0) (Moser et al., 2009b). Erucic, linoleic and linolenic are the primary fatty acids with 32.8,

22.4 and 11.8% respectively (Moser et al., 2009b). Other fatty acids to note are oleic, gondoic and nervonic at 11.1, 8.6 and 2.9% (Moser et al., 2009b).

For use in general industrial applications, pennycress-derived oil is reported to have excellent low temperature fluidity and lubrication properties (Moser et al., 2009a; Cermak et al. 2013). A standard transesterification process with methanol and a sodium methoxide catalyst at 60° C can yield 82% wt. biodiesel in the form of methyl esters (Moser et al. 2009a). This process is described in more detail in Chapter 1. Pennycress oil methyl esters have a cetane number (CN) of 59.8 due to the presence of erucic and gondoic methyl esters, which is above the minimum of 47 set by ASTM D6751 (Moser et al., 2009a; American Society for Testing and Materials, 2008). Biodiesel typically has poor low temperature operability compared to petro-diesel. Pennycress methyl esters, however, exhibit better lower temperature operability even compared to soybean oil, which can be attributed to the low level of saturated fatty acids (Moser et al., 2009a; Moser, 2008; Moser et al., 2009b). An alternative method for creating biodiesel from pennycress oil has been investigated and that is the use of ethyl esters as opposed to methyl esters. This process uses fermentation of sugars to yield a fuel composed of renewable carbon (Kim & Dale, 2005). In a study that compared pennycress-derived ethyl esters and methyl esters, they were found to behave similarily (Moser et al., 2016). The production of rewewable diesel or green diesel (i.e. jet fuel) from pennycress oil has yet to be specifically outlined in the literatre. The process and the fuel itself is commonly mentioned and a life-cycle assessmenet exists (Fan et al., 2013), but the specifics of the production from pennycress are not yet described.

The capacity to utilize pennycress oil in targeted end use applications depends on the plants' ability to produce profitable yields and seeds with high oil content. Seed yield reports of pennycress range from 1086 – 1387 kg ha^{-1} (Johnson et al., 2015). The oil content of cold-press, dried wild pennycress seeds collected near Peoria, IL was 29.0% on a dry weight basis (dwb), which corresponds to previous reports of 24-39% and 32.9%, and stands in contrast to a particularly high estimate from European accessions, 39.1 – 41.2% (Moser et al. 2009a; Dolya, 1974; Sedbrook et al., 2014; Evangelista et al.,

2012; Claver et al., 2017). It is estimated that if the proposed dual or relay crop pennycress system were implemented on the 16.2 million ha of the Midwest agricultural landscape that are under corn and soybean rotation, a total of 15 billion liters of biofuel could be produced annually (Fan et al., 2013). Domestic biodiesel production was 4.8 billion liters in 2015 (EIA, 2017). Relay cropping may result in reduced yields due to competition between plants, but it has been shown that total system oilseed yield (pennycress and soybean) is higher when grown together (Johnson et al., 2015).

One component of a viable feedstock is a reliable, profitable end use for the seedmeal co-product that remains after oil extraction. In the case of other common biofuel feedstocks, including canola and soybean, the seedmeal is sold as a high value source of protein for livestock. Pennycress plants and seeds, however, contain high concentrations of glucosinolates (β-thioglucoside-*N*-hydroxysulfates), which are a class of secondary plant compound. Pennycress seedmeal contains solely the glucosinolate sinigrin with 36.71 ± 0.41 mg gram^{-1} sample (92.36 ± 1.03 μmol g^{-1} sample) (Hojilla-Evangelista et al. 2015). Cruciferous vegetables with sinigrin typically produce allyl isothiocyanate (AITC) via hydrolysis by the enzyme myrosinase, but in pennycress, a non-isothiocyanate, allyl thiocyanate (ATC), is produced (Gmelin & Virtanen, 1959). ATCs are toxic to animals, causing gastric distress, abortion, and death, among other symptoms when consumed by cattle (Smith & Crowe, 1987), and milk and flesh products from livestock that consume it may exhibit an unpalatable flavor (Warwick et al., 2002). Due to the potential toxicity of the glucosinolate hydrolysis products in pennycress presscakes and the need for a profitable co-product, researchers have investigated other potential uses to ensure the economic integrity of the system. Several uses have been investigated: pyrolysis oil (Boateng et al., 2010) biofumigants (Vaughn et al., 2005) and protein-derived emulsions (Hojilla-Evangelista et al., 2015).

The most concentrated fatty acid in pennycress oil is erucic and methods are proposed for increasing its concentration to expand the utility of the oil (Isbell et al., 2015). Enrichment strategies can increase erucic acid in pennycress oil content from 36% to greater than 70% using distillation or soap crystallization (Isbell et al., 2015). Oil with

high erucic acid has utility as an additive to lubricants, solvents, softeners, and in the manufacturing of polymers, high-fluidity lubricants, surfactants, surface coatings and pharmaceuticals (Leonard, 1994). In order to be competitive in this marketplace and to limit purification costs, oil should have at minimum 45% erucic acid (Sovero, 1993). The demand for these uses has provoked the development of high erucic acid rapeseed (HEAR) as well as the initial investigation into erucic acid enrichment and biosynthesis in pennycress (Scarth & Tang, 2006; Isbell, Evangelista, et al., 2015; Claver et al., 2017).

On the contrary, there is interest in reducing the concentration of erucic acid in pennycress oil so much as to make the oil edible for human consumption, modeling successful development development of canola from rapeseed. In animal studies of diets high in erucic acid, myocardial damage can result due to fatty deposits around the heart and kidneys (Eskin et al., 1996). To address this concern, breeding for low erucic acid rapeseed was initiated in the 1950s. Initial low erucic acid mutants were identified in the German spring type forage cultivar "LIHO" (Stefansson et al., 1961), which was then backcrossed into adapted cultivars (Stefansson & Downey, 1995). Approximately 20 years after the identification of "LIHO," 95% of all rapeseed in Canada was low erucic acid (Eskin et al., 1996). Today, by regulation, canola must contain < 2% (down from 50% or more) erucic acid and must also have < 30 μmol g^{-1} glucosinolates sample (Schmidt & Bancroft, 2011; Fahey et al., 2000). Glucosinolates in canola are expressed as μmol g^{-1} because there are six different types of glucosinolates and their molar masses and concentrations vary (Slominski et al., 2015). If pennycress oil is to be used for human consumption, erucic acid would need to be reduced, probably near levels required for canola. Furthermore, for the seed meal presscakes (the co-product of oil extraction) to be utilized for animal feed, glucosinolate content would need to be lowered similarly. Edible oil would not necessarily be incompatible with biofuel production, but reducing erucic acid content may decrease the cold temperature operability and high CN inherent to pennycress-derived biodiesel in its current state. Furthermore, developing edible pennycress oil could drive up the price of the feedstock and may not address the current concern of prohibitive feedstock prices. Breeders may choose, however, to develop

different varieties (low or high erucic acid, both with low glucosinolate content) of pennycress to meet market demands, as is done with rapeseed, with the development of high erucic acid rapeseed (HEAR) and low erucic acid rapeseed (LEAR) (Schmidt & Bancroft, 2011).

Pennycress fits the requirements for an alternatively sourced biofuel feedstock. It does not compete with food uses or displace food crops, has an acceptable fatty acid profile, can be grown on marginal lands and may provide environmental services in the form of a cover crop (Moser et al., 2009a; Boateng et al., 2010). Considering these benefits, several breeding programs have been established to improve pennycress from its undomesticated state. The first was initiated at Western Illinois State University (WIU) in 2009 with the goal of selecting for good stand establishment and early flowering (Sedbrook et al., 2014). In 2013, the University of Minnesota established a breeding program focused on the agronomic, seed chemistry and genotypic evaluation of wild collections from the northern United States as well accessions available through USDA-ARS NPGS. At the same time, a mutation (Ethyl methanesulfonate - EMS, gamma and fast neutron) breeding program and inbred development efforts were also initiated. A start-up company in Missouri, Arvegenix, was established in 2013 and is also working to evaluate and breed pennycress (Avegenix, 2013). These breeding programs have focused primarily on characterizing germplasm and domestication traits thus far.

Presently available pennycress germplasm is considered wild and undomesticated, having undergone very limited formal selection with the exception of the PI registration of the fast-germinating variety ‘Katelyn’ by the USDA in 2015 (Isbell et al., 2015). In order to fully realize the potential widespread ecosystem services and economic benefit of integrating pennycress into the corn and soybean cropping system, breeding and selection must be carried out. Improvements in the end use traits of pennycress are required to ensure demand and utility for both the oil and seedmeal. The improvement of a species via traditional breeding methods depends on genetic variation for particular traits of interest (Fehr, 1991). Available pennycress germplasm must be characterized to quantify genetic variation and to identify superior individuals for breeding. A

comprehensive assessment of the available winter type pennycress germplasm for variation in total oil and fatty acid profile is warranted.

The objectives of this study were to:
1) Assess the phenotypic variation for total oil and fatty acid profiles in a collection of 42 winter type pennycress accessions.

2) Least-squares means (lsmeans) as trait values for each accession and trait combination, assess summary statistics of trait distributions, and estimate broad sense heritability.

Materials and Methods

Experimental Design

Winter type accessions were surveyed only as this growth type fits most appropriately in the corn and soybean rotation in the Upper Midwest and provides a longer period of soil cover. The development of the present collection of winter type accessions is described in more detail in Chapter 2. Seed of pennycress from 42 winter type accessions (Table 2.1) originating from both wild collections and the USDA Germplasm Resource Information Network (USDA - NPGS) was harvested from plots over two years at five unique location–year combinations in Minnesota as described in Chapter 2.

Oil Percentage

Oil percentage was calculated on a dry weight basis using pulsed nuclear resonance (pNMR) of seeds as described in Isbell et al. (2015). Equipment details, instrument settings and standard curve development using pennycress oil can be found in Isbell et al. (2015). Briefly, approximately 2.5 g of pennycress seed was weighed into a Pyrex vial and heated to 40° C for 2 hrs. Response factors were measured to the tenth milligram of oil within each sample. A total of 10% of the samples were dried using the Moisture and Volatile Matter Air Oven Method AOCS Ca 2c-25 (AOCS 1995) to measure their average moisture content at the time of analysis. The percent dry matter

was calculated for each sample and multiplied by the sample mass (g). This value was then divided into the total grams of oil as reported by the pNMR to obtain % oil (dwb).

Fatty Acid Profile

Gas chromatography (GC) was performed on pennycress fatty acid methyl esters (FAMEs) using a method also described by Isbell et al. (2015). GC was conducted using an Agilent Technologies 7890N equipped with a flame-ionization detector and an autosampler. A capillary column by Supelco (SP-2389 30-m by 0.25-mm i.d. polysiloxane) was used to separate FAMEs. Full detail regarding temperature ramps and helium carrier gas flow rate can be found in Isbell et al. (2015). A total of 50 pennycress seeds were ground using a 100v homogenizer equipped with a T10 dispersing tool and 2/7 rotor/stator from Finemech (Portola Valley, CA) for 1 minute in a 20 mL scintillation vial with 5 mL of a 0.25 M sodium methoxide solution. Vials were covered with a foil line cap, transferred to a heating block maintained at 60° C for 30 minutes. Vials were removed and 5 mL each of hexane and saturated sodium chloride were added. Contents were allowed to rest until separated. A 0.5 mL aliquot from the top hexane layer was pipetted out and placed in a 2-mL GC vial, diluted to 2 mL with hexane, sealed and placed into the GC autosampler. Samples were run in biological triplicate.

Mixed Modeling

All mixed effect model analyses were performed in Program R version 3.2.3 (R Core Team, 2015) using the "lme4", "lmerTest" and "lsmeans" packages (Bates et al., 2014; Kuznetsova et al., 2016; Lenth, 2016). Data from biological triplicate fatty acid samples were averaged prior to analysis. A linear mixed effects modeling approach was used to account for the unbalanced nature of the dataset and to allow for random effects in the model (Vargas et al., 2013). Accession was treated as a fixed effect in the model because: 1) comparisons among accessions are of interest and; 2) accessions were selected and did not represent a random sample of all wild pennycress (Piepho & Bu, 2003). Environment was treated as a random effect because locations were not intentionally chosen specifically for inference, but served primarily as replication (Piepho

& Bu, 2003; Bernardo, 2002). Replication nested within environment and accession-by-environment interaction were also both treated as random because both terms contain random factors (Piepho & Bu, 2003). A random term for seeding rate was included to account for differences across environments. Seeding rate was treated as random because the values used represent a random sample from a set of possible values and were chosen arbitrarily (and not for inference) based on seed availability. Models were fit using a backwards elimination approach, which included sequentially removing and testing the significance of each random term to the model. A log likelihood ratio test was used and compared to a Chi-squared (χ^2) distribution with one degree of freedom (Tiede et al., 2015). Terms with a p-value exceeding $\alpha = 0.10$ were eliminated. The variance contribution, standard deviation of each remaining random term was estimated using the "summary()" function in R. The significance of the fixed effect's contribution to the model was estimated using the lmerTest "anova()" function and a Satterthwaite approximation for degrees of freedom (Kuznetsova et al., 2016; Satterthwaite, 1946).

Fixed effects were estimated as Best Linear Unbiased Estimates (BLUEs) to account for differences among means of different environments (Bernardo, 2002) and predicted using as "lsmeans" the "lsmeans" package in R (Lenth, 2016). BLUEs were used as the phenotypes in all subsequent analyses. Trait summary statistics including minimum, mean, maximum, standard deviation, standard error, coefficient of variation (standard deviation/mean; C.V.) and model intercepts were recorded. Pairwise differences were calculated and adjusted for multiple comparisons using Tukey's Honest Significant Difference (HSD) using the compact letter display (a) function in "lsmeans" using $P < 0.05$ (Lenth, 2016).

Heritability

Most heritability equations assume balanced data but it is common for plant breeding trials to contain some imbalance (Piepho & Möhring, 2007). To account for the unbalanced dataset, an *ad-hoc* method utilizing lsmeans described in (Holland et al., 2003) was used:

$$\overline{H}^2 = \frac{\sigma_g^2}{\sigma_g^2 + \overline{v}/2} \qquad \text{Equation 1}$$

Where $\overline{H}^2$ is the broad sense heritability, σ_g^2 is the variance component due to genotype (i.e. accession) and $\overline{v}$ is the average variance of a difference of lsmeans pairwise comparisons. The average variance of a difference between lsmeans was calculated using the R function "difflsmeans" in the package "lmerTest" (Kuznetsova et al., 2016). The fixed effect accession was changed to a random effect in all the final, fitted models and random effect summaries were generated to estimate the genetic variance component (σ_g^2).

Results and Discussion:

This study is the first reported multi-environment, common garden trial of a winter pennycress collection grown over multiple years and assessed for variability in fatty acid profile and total oil percentage. One other study (Sedbrook et al., 2014) reports the fatty acid profiles and total oil percentage of 80 wild pennycress accessions (referred to hereafter as the WIU Collection) and 34 accessions from USDA Agriculture Research Service National Plant Germplasm System (referred to hereafter as the WIU - NPGS Collection), some of which are contained in the present study. These analyses were performed on seeds acquired from wild collections and/or directly from NPGS and were not grown in a common garden experiment prior to analysis (Sedbrook et al., 2014). These results serve as a basis for comparison and are therefore referenced throughout.

Oil Percentage

Second only to reducing the glucosinolate content of pennycress seedmeal, total oil percentage is arguably the most important end use trait to improve. Considering the acceptability of the oil's fatty acid profile in its current state for biodiesel and renewable diesel production, focus should be primarily on increasing oil as opposed to significantly altering its specific fatty acid constituents. Attention on particular alterations to the fatty acid profile may come secondarily, as multiple directions may be taken to meet specific

end use goals. For example, one breeding objective may be to decrease erucic acid content to improve edibility, whereas another may be to enrich it to improve the utility of the oil in oleochemical industry.

Within the germplasm assessed, significant variation ($P < 0.001$) and pairwise differences were detected among accessions for total oil percentage. The range of oil percentages observed was 28.5 – 32.7% (dwb). This range is similar to previous reports of 24.7 – 38.7% (dwb) in the WIU - NPGS collection and a more variable range of 13.5 - 38.7% (dwb) was observed in the WIU collection (Sedbrook et al., 2014). In a study that compared eight pennycress accessions (six from the NPGS collection and two from a collection at North Dakota State University), a range of 27.9 – 35.1% (dwb) and significant differences among accessions were detected (Diaz, 2014). A recent assessment from two European accessions (one spring and one winter type) grown in a growth chamber, a range of 39.1 – 41.2% (dwb) was observed, but no significant difference was detected between the two (Claver et al., 2017). A line known as "W-12" from WIU consistently is reported to contain 36% oil, and two lines from the NPGS, Patton and 'Beecher,' contain 34% and 36% (Isbell, Evangelista, et al., 2015). The cultivar 'Beecher' is suggested as presently being the most suitable for commercial production (Isbell et al., 2015) likely due to its consistently high oil content and familiarity within the pennycress research community. It should be noted that the present as well as all the aforementioned oil percentage assessments, with the exception of Claver et al. (2017), were conducted in the same laboratory of Dr. Terry Isbell at the USDA Bio-Oils Laboratory in Peoria, IL.

The values observed in the present germplasm for oil percentages are similar to previous reports; however, others show slightly more variability and sometimes overall higher values. For example, the variability in the WIU collection was greater. It is important to note that these samples were derived directly from collection sites and were not grown together in a common garden experiment prior to analysis. It is possible that some of the variation observed may be due to environmental effects, as well as collection time and storage. In the present study the effect of environment was moderately

significant ($P < 0.10$) in the model and explained a large proportion (69.2%) of the total variance (Table 3.2). The literature on canola indicates environmental factors play a large role in determining both fatty acid constituents and total oil % (Pritchard et al., 2000).

The high broad sense heritability for oil percentage ($H^2 = 0.82$) and the significant variation ($P < 0.001$) among accessions indicates that there is potential to observe gain from selection within the present collection. The coefficient of variation for oil percentage was 3% with a standard error of 0.16 between BLUEs. The practical variability, however, is somewhat limited. Additional genetic variation may be required to make sizable changes in the oil content, and this may come in the form of additional collections of pennycress, and untargeted or targeted mutagenesis.

Fatty Acid Profiles

Fatty acids are referred to in their lipid number nomenclature, C:D, where C is the number of carbon atoms in the fatty acid and D is the number of double bonds present. Fatty acids are expressed as a percentage of the total fatty acid methyl esters (FAMEs). All fatty acids detected in this study are listed in Table 3.1 and shown in Figure 3.1. C22:1 occupied on average the highest proportion with an average of 35.9% (Table 3.3). C18:2 occupied the second highest average proportion with an average of 21.2%. The third highest on average was C18:3 with 13.1% followed by C18:1 (average 12.1%) and C20:1 (average 9.7%). Fatty acids C24:1, C16:0, C18:0, C20:2 and C22:2 on an individual basis occupied on average less than 3% of the total FAME profile. Fatty acid C24:1 was not detected in year 2013/2014 and therefore only 2014/2015 data was used. In terms of specific fatty acid, heritability estimates in the present study were all very high and ranged from 0.44 – 0.96, with an average of 0.86. These estimates, in combination with the fact that accession was significant $P < 0.001$ for all the models except C22:2, demonstrates there is great potential for altering the fatty acid components using selection.

Following a similar trend as the oil percentage data, the range of fatty acid composition values observed in this study are slightly more narrow compared to those

described by Sedbrook et al. (2014) for the WIU and WIU – NPGS collections. For example, C22:1 ranged from 27.5 – 38.4% (difference of 10.9 percentage points) in the WIU Collection and 29.8 – 39.0% (difference of 9.2 percentage points) in the NPGS Collection and 35.92 ± 0.28% (difference of 7.39 percentage points) in the present collection. The differing levels of variability may also be due to the variable growing conditions as described previously. Environment was a moderately ($P < 0.10$) significant component for each of the models in the present study. The percent variance contributions to the models for specific fatty acids varied, which implies that the environment may have a differing effect on the composition of fatty acids. A higher percent variance contribution of environment for C18:1 (72.3%), C20:2 (73.9%) and C22:2 (73.3%) suggests they are more heavily influenced by the environment than fatty acid C18:0 (28.7%). Fatty acids C16:0, C18:2, C18:3, C20:1 and C22:1 all had moderate variance contributions near 50% (Table 3.2). A similar trend is also demonstrated in Diaz (2014), where a difference in fatty acid composition was observed between fall and spring planting dates. This suggests one of two things: 1) there may be additional variation for fatty acid profile in the spring types that were not tested as part of the present collection, or 2) that there is an environmental effect. Diaz (2014) suggests that lower mean temperatures experienced by fall-planted pennycress during seed filling and oil-synthesis stages in the spring modify the proportions of C18:1 and C22:1 fatty acids. In their study, C18:1 was lower in the fall plantings (10% and 11.4%) compared to spring planting (13.1%) while C22:1 was higher for fall planted crops (35.5%) and lower in spring (32.6%) (Diaz, 2014).

No direct selection for specific fatty acids has been reported in pennycress. In the development of the fast germinating pennycress accession ‘Katelyn,’ the authors monitored fatty acid profile throughout selection and observed decreases in fatty acid percentages of C16:0, C18:3, C22:1, and C22:2. Changes in percentages were minor and did not exceed 1.4 percentage points, but were significant nonetheless (Isbell et al., 2015). Selection for increasing or decreasing specific fatty acid components should be informed by the proposed end-uses for pennycress oil. As previously mentioned,

pennycress oil in its current state is acceptable as a biodiesel and renewable diesel feedstock (Moser et al., 2009a). In fact, it has several outstanding characteristics. Pennycress-derived biodiesel may be used to enhance the cold temperature performance of biodiesel blends due to its excellent low temperature operability (Isbell et al., 2015). This can characteristic can be attributed to its relatively low level of saturated fatty acids (Moser et al., 2009a). The high levels of erucic and gondoic acids in pennycress oil are also thought to contribute to the high derived cetane numbers (DCN) of the oil methyl esters (Moser et al., 2009a). Pennycress methyl esters DCN is 59.8, which exceeds the minimums set by the American and European fuel standards, ASTM D6751 and EN 14214, which require at minimum 47 and 51, respectively (American Society for Testing Materials, 2008; European Committee for Standardization, 2003). Pennycress oil also shows excellent lubricity (fuels with poor lubricity can cause engine parts to fail), which is an advantage of biodiesel fuels (Moser et al., 2009a). Due to the high content of erucic, gondoic and other 20+ carbons, pennycress-derived biodiesel actually exceeds the EN14214 limit for kinematic viscosity (but is below the ASTM D6751), which could be addressed by blending with a less viscous feedstock (Moser et al., 2009a).

When considering the potential of pennycress oil to be used as food-grade oil for human consumption, it is important to recognize the erucic acid content due to its association with negative health effects. The conversion of rapeseed to canola, for example, required a decrease in erucic acid from around 50% or more to less than 2% (Schmidt & Bancroft, 2011). Progress towards this end was made possible by the identification of a low erucic acid (LEA) mutant (Harvey & Downey, 1963). Lowering both the contents of erucic acid and glucosinolates allowed the oil to be used as highly nutritious oil for human consumption and increased its utility as cooking oil. Oils low or high in erucic acid are both suitable for renewable fuel end uses (Schmidt & Bancroft, 2011) so any decrease in erucic acid in pennycress oil would be solely in attempt to improve its edibility. The variation for erucic acid content was significant ($P < 0.05$) with pairwise differences between accessions in the present study. The observed range was 31.8 – 39.2%, and heritability was estimated to be very high ($H^2 = 0.96$). This indicates

there is potential to move the population mean in either direction via selection. It also shows that thus far, there were no specific accessions discovered that could play a role similar to that of the canola mutant. To make progress towards the end of a reduction to levels near canola (< 2%) would require additional genetic variation and likely mutagenesis. In canola, the genetic control of erucic acid stems from two homeologous loci (E^A and E^C) that explain 90% of the variation (Harvey and Downey 1964; Jourdren et al., 1996a). Varieties low in erucic acid are recessive at both loci, which are shown to correspond to two copies of the *Fatty acid elongase* 1 gene (FAE1) originally identified in Arabidopsis (Fourmann et al., 1998; Lemieux et al., 1990). A mutation in FAE1 (*fae1*) is either loss of function, resulting from a 4 bp deletion (Wu et al., 2008), or a point mutation and a single amino acid substitution (Han et al., 2001). The identification and elucidation of this naturally occurring mutant is what made LEA rapeseed a possibility.

Research on the accumulation of erucic fatty acid in pennycress specifically is relatively limited with a single attempt at elucidation in Claver et al. (2017). The authors found that general lipid/mg accumulation in pennycress seed has been shown to increase in a near-linear fashion during seed development (Claver et al., 2017). The percentage of erucic acid increased from 27-28 to 34-47% between pod stage colors of green-yellow to yellow-green, and appears to be controlled at the transcriptional level, especially in the early stages of development (Claver et al., 2017). This increase in erucic acid was observed in conjunction with a decrease in C18:2 and 18:3, suggesting that there is a change in the acyl flux towards elongation that results in less C18:1 CoA substrate (Claver et al., 2017). *Fatty Acid Elongase* 1 (FAE1) was detected in the pennycress genome (TaFAE1), and is shown to have high phylogenetic correlation with other high erucic-acid oilseeds (Claver et al., 2017). Specifically, the protein encoded by this gene showed an 86% similarity (93.67% homology) to Arabidopsis and 91.3% (95.45 homology) to *B. napus* (Claver et al., 2017). There are several cysteine and histidine residues that are part of the active site of the FAE enzyme and were found to be present in the pennycress lines studied (Claver et al., 2017). It is suggested that the high erucic acid phenotype may be a result of the presence of certain amino acid residues or protein

domains and not necessarily only FAE1 (Claver et al., 2017). These authors are investigating the use of *fae1* mutants to determine the influence of residues on the high erucic acid trait. Others are targeting for manipulation using both untargeted mutagenesis and via Targeting Induced Local Lesions IN Genomes (TILLING) (Sedbrook et al., 2014) to potentially uncover a loss of function mutant that would play a role in pennycress breeding similar to "LIHO" in canola.

With the present germplasm, minor but significant variations were observed for particular fatty acid constituent traits, which correspond to a previous report of germplasm characterization (Sedbrook et al., 2014). Significant variation ($P < 0.001$), high estimates for heritability and pairwise differences among accessions indicate that there is potential to make selections to move trait means in particular directions. It is important to direct the focus of breeding to improving pennycress for its intended end use traits. There are multiple directions that one may take to improve the fatty acid profile of pennycress oil. There are three potential markets: oil for human consumption, oil for oleochemical use and oil for biodiesel and renewable diesel. As mentioned previously, oil for human consumption would require significant changes in the fatty acid profile, and are likely unattainable using the germplasm characterized in this study. Mutagenesis or targeted gene editing could accomplish this goal. Oil for oleochemical use is possible, but erucic acid would need to either be enriched via processing or increased through breeding. The oil in its current state is acceptable as a feedstock for biodiesel and renewable diesel production, and specific issues can be addressed via additional processing steps or blending, which is routine practice in fuel formulations.

Current breeding efforts should focus on improving the agronomic feasibility of pennycress via improving shattering resistance, earliness and yield. In terms of end-use traits, selections should be made for improving oil content in general and should focus secondarily on altering fatty acid profile, as the content is suitable for its most well defined end use, namely biodiesel. Other breeding targets that would improve the end use and economic feasibility of pennycress, specifically the presscakes after oil extraction, is reducing the glucosinolate content to make the co-product edible as animal feed. Efforts

are currently underway to assess this collection for sinigrin content in the seedmeal. To make any sizable changes beyond the values presented here, the mutagenesis populations developed at the University of Minnesota will play a crucial role in achieving specific large-scale changes in fatty acids.

Breeders may choose to move forward with directional selection towards varieties of pennycress with low and high erucic acid and lower glucosinolate content. Using the data obtained in the present study, in combination with glucosinolate screening data that is currently underway, researchers at the University of Minnesota are training a near infrared (NIR) prediction equation for rapidly screening pennycress. The equation has assisted researchers in identifying low and high erucic acid as well as low glucosinolate mutants from the large-scale mutagenesis population developed at the University of Minnesota in 2013. These mutants are being subjected to additional field trials and study to validate findings. Once validated these mutants could serve important roles in aiding breeders to significantly alter pennycress' fatty acid profile to meet market demands and improve the profitability of the system.

Tables and Figures

Table 3.1. Analysis of Variance (ANOVA) of accession as a fixed effect in the linear mixed model analyses using Satterthwaite degrees of freedom approximation for individual fatty acid models (with lipid number designation).

Trait	Fatty Acid	Sum Sq	Mean Sq	DenDF[†]	F Value	Pr(>F)	Significance Level	Pairwise Differences
C24:1	Nervonic	31.25	0.76	196.07	3.52	0.000	***	N
C16.0	Palmitic	10.96	0.27	265.99	14.73	0.000	***	Y
C18:0	Steric	0.19	0.00	262.06	5.97	0.000	***	Y
C18.1	Oleic	71.07	1.73	135.51	9.03	0.000	***	Y
C18:2	Linoleic	205.52	5.01	266.06	16.42	0.000	***	Y
C18:3	Linolenic	152.23	3.71	263.02	16.25	0.000	***	Y
C20:1	Eicosenoic	75.67	1.85	263.02	24.57	0.000	***	Y
C20:2	Eicosadienoic	2.82	0.07	263.05	10.72	0.000	***	Y
C22:1	Erucic	990.56	24.16	263.04	25.30	0.000	***	Y
C22:2	Docosadienoic	0.49	0.01	153.06	1.03	0.434	NS	N
Oil %[‡]		301.13	7.34	261.03	5.63	0.000	***	Y

*** P < 0.001, ** P < 0.01, * P < 0.05, . P < 0.1.

[†] DenDF indicates denominator degrees of freedom. Numerator degrees of freedom are not listed and equaled 41 for every model.

[‡] Calculated on a dry weight basis (dwb).

Table 3.2. Random effects components accession*environment (A*E), replication nested within environment (R/E), environment (E) and residual (R) variance (V) and their percent contribution (C) to the final, fit model's variance. Contributions were calculated by dividing the variance of each component by the sum of all variance components. Models were fit using a backwards elimination approach where maximum likelihood was used to compare a model with and without the variance component in question and compared to a Chi-square (χ^2) distribution (df=1).

Term	Component	C24:1	C16:0	C18:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:1	C22:2	Oil %
		Nervonic	Palmitic	Steric	Oleic	Linoleic	Linolenic	Eicosenoic	Eicosadienoic	Erucic	Docosadienoic	
A*E	C	34.6%	-[†]	-	13.2%	-	-	-	-	-	18.7%	-
R/E	C	1.4%	-	4.6%	5.1%	-	12.1%	14.6%	1.4%	3.6%	-	3.3%
E	C	48.2%	51.3%	28.7%	72.3%	47.3%	48.3%	53.0%	73.9%	53.5%	73.3%	69.2%
R	C	15.8%	48.7%	66.8%	9.5%	52.7%	39.6%	32.4%	24.6%	42.9%	8.0%	27.5%
A*E	V	0.17	-	-	0.27	-	-	-	-	-	0.03	-
R/E	V	0.01	-	5.22E-05	0.10	-	0.07	0.03	3.76E-04	0.08	-	0.16
E	V	0.24	0.02	3.29E-04	1.47	0.27	0.28	0.12	0.02	1.19	0.11	3.29
R	V	0.08	0.02	7.65E-04	0.19	0.31	0.23	0.08	0.01	0.95	0.01	1.30

[†] Components that were not significant were removed from the model, indicated by "-".

Table 3.3. End use traits evaluated, units, mean ± standard error of mean (SEM), minimum values and accessions, maximum values and accessions, standard deviation (SD) and coefficient of variation (C.V.) for all accessions best linear unbiased estimates (BLUEs).

Trait	Fatty Acid	Units	Mean ± SEM	Min.	Max.	SD	C.V.	Heritability
C24:1	Nervonic	%	1.36 ± 0.06	1.43	2.85	0.36	17%	0.44
C16:0	Palmitic	%	3.14 ± 0.03	2.78	3.98	0.20	6%	0.94
C18:0	Steric	%	0.40 ± 0.003	0.35	0.49	0.03	6%	0.83
C18:1	Oleic	%	12.10 ± 0.14	10.59	14.19	0.91	8%	0.89
C18:2	Linoleic	%	21.19 ± 0.13	19.54	22.85	0.83	4%	0.94
C18:3	Linolenic	%	13.14 ± 0.11	10.77	14.33	0.73	6%	0.94
C20:1	Eicosenoic	%	9.72 ± 0.08	8.75	10.85	0.50	5%	0.96
C20:2	Eicosadienoic	%	1.59 ± 0.02	1.43	1.80	0.10	6%	0.91
C22:1	Erucic	%	35.92 ± 0.28	31.79	39.18	1.84	5%	0.96
C22:2	Docosadienoic	%	0.516 ± 0.02	0.19	0.86	0.12	24%	0.78
Oil[†]		%	30.87 ± 0.16	28.45	32.74	1.01	3%	0.82

[†] Calculated on a dry weight basis (dwb).

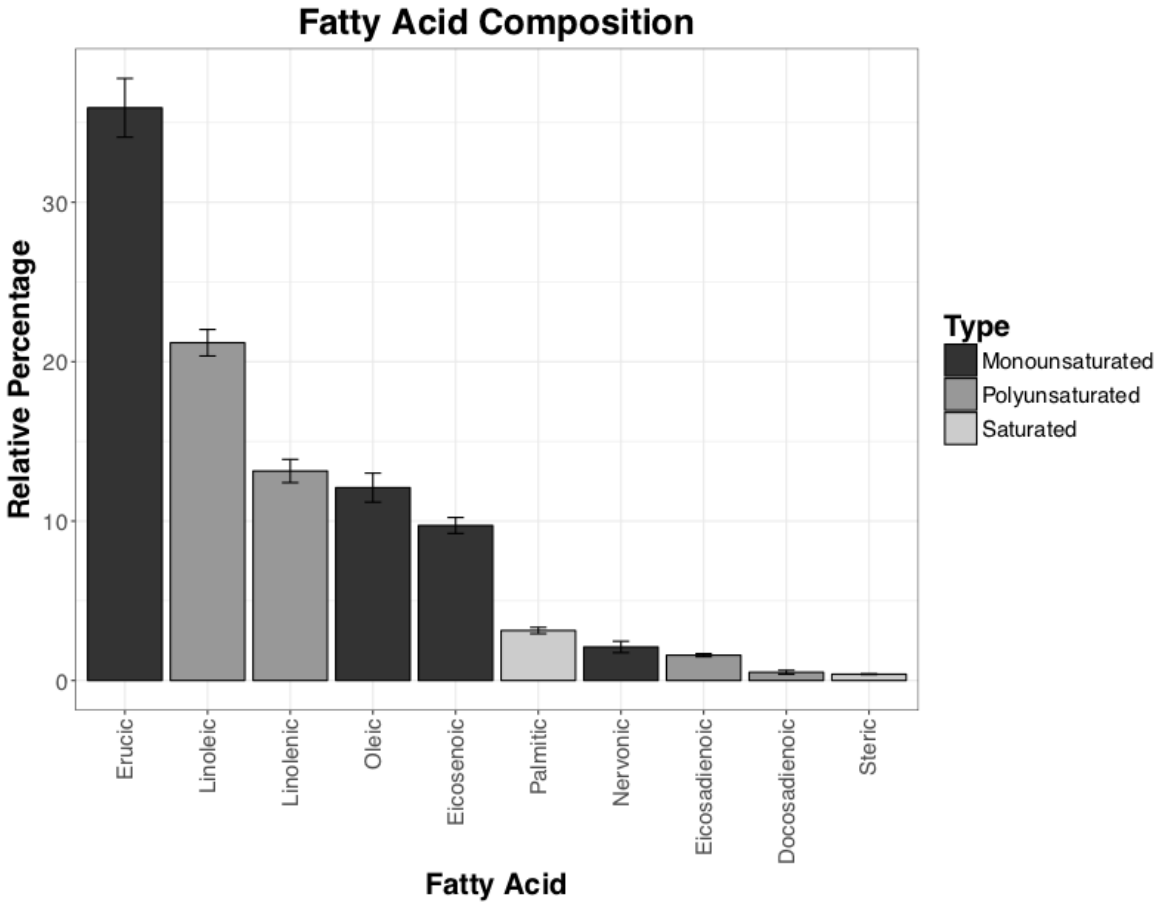

Figure 3.1. Mean relative composition of fatty acid methyl ester in field pennycress oil for all accessions estimated as best linear unbiased estimates (BLUEs). Bars indicate standard deviations.

Bibliography

Air Transport Action Group. (2010). Beginner's guide to aviation efficiency. *Aviation*, (November Issue).

Alexander, R. B., Smith, R. A., Schwarz, G. E., Boyer, E. W., Nolan, J. V., & Brakebill, J. W. (2008). Differences in phosphorus and nitrogen delivery to the Gulf of Mexico from the Mississippi River Basin. *Environ. Sci. Technol.*, *42*(3), 822–830. http://doi.org/10.1021/es0716103

Arbuckle, J. G., & Roesch-McNally, G. (2015). Cover crop adoption in Iowa: The role of perceived practice characteristics. *J. Soil Water Conserv.*, *70*(6), 418–429. http://doi.org/10.2489/jswc.70.6.418

ASTM. (n.d.). American Society for Testing and Materials (ASTM) standard D6751: Standard specification for biodiesel fuel blend stock (B100) for middle distillate fuels. West Conshohocken, PA: ASTM.

Avegenix. (2013). Arvegenix. Retrieved January 1, 2016, from http://www.arvegenix.com/

Bart, J., Palmeri, J., & Natale Cavallaro, S. (2010). Biodiesel science and technology: From soil to oil. CRC Press.

Bastow, R., Mylne, J. S., Lister, C., Lippman, Z., Martienssen, R., & Dean, C. (2004). Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature*, *427*(6970), 164–167. http://doi.org/10.1038/nature02269

Bates, D. M. (2010). lme4: Mixed-effects modeling with R. *Springer*. http://doi.org/10.1177/009286150103500418

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2014). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.*, *67*(1), 1–48. http://doi.org/10.18637/jss.v067.i01.

Beckie, H. (2016). Group B/2 Resistant Thlaspi arvense from Canada, Alberta. Retrieved February 15, 2016, from http://weedscience.org/details/case.aspx?ResistID=5097

Bell, J. M. (1982). From Rapeseed to Canola: A Brief History of Research for Superior Meal and Edible Oil. *Poultry Sci.*, *61*(4), 613–622. http://doi.org/10.3382/ps.0610613

Bernardo, R. (2002). Breeding for Quantitative Traits in Plants (2nd ed.). Woodbury, MN: Stemma Press.

Best, K. F., & McIntyre, G. I. (1972). Studies on the Flowering of Thlaspi arvense L. I. The Influence of Some Environmental and Genetic Factors. *Bot. Gaz.*, *133*(4), 454–459.

Best, K. F., & McIntyre, G. I. (1975). The Biology of Canadian Weeds. *Can. J. Plant Sci.*, *55*, 279–292.

Blackshaw, R. E. (1990). Control of Stinkweed (*Thlaspi arvense*) and Flixweed (*Descurainia sophia*) in Winter Wheat (*Triticum aestivum*). *Can. J. Plant Sci.*, *824*(3878964), 817–824.

Boateng, A. A., Mullen, C. A., & Goldberg, N. M. (2010). Producing stable pyrolysis liquids from the oil-seed presscakes of mustard family plants: Pennycress (*Thlaspi arvense* L.) and Camelina (*Camelina sativa*). *Energy Fuels*. Retrieved from http://pubs.acs.org.ezp3.lib.umn.edu/doi/abs/10.1021/ef101223a

Bowman, G., Shirley, C., & Cramer, C. (1998). *Managing cover crops profitably*. (G. Bowman, Ed.) *Sustainbale Agriculture Network* (2nd ed.). Beltsville, MD.

Boyd, N. S., & Van Acker, R. C. (2003). The effects of depth and fluctuating soil moisture on the emergence of eight annual and six perennial plant species. *Weed Science*, *51*(5), 725–730. http://doi.org/10.1614/P2002-111

Carr, P. M. (1993). *Potential of Fanweed and Other Weeds as Novel Industrial Oilseed Crops*. (J. Janick & J. E. Smon, Eds.) *New Crops* (Vol. 1.2). Wiley, New York. Retrieved from https://hort.purdue.edu/newcrop/proceedings1993/V2-384.html

Charles Brummer, E., Barber, W. T., Collier, S. M., Cox, T. S., Johnson, R., Murray, S. C., Thro, A. M. (2011). Plant breeding for harmony between agriculture and the environment. *Frontiers in Ecology and the Environment*, *9*(10), 561–568. http://doi.org/10.1890/100225

Chen, S., Kurle, J., Malvick, D., Potter, B., & Orf, J. (2011). Soybean Cyst Nematode Management Guide. *University of Minnesota Extension*. Retrieved from http://www.extension.umn.edu/agriculture/soybean/soybean-cyst-nematode/EFANS-Soybean-SoybeanCystNematode-WebQuality.pdf

Claver, A., Rey, R., López, M. V., Picorel, R., & Alfonso, M. (2017). Identification of target genes and processes involved in erucic acid accumulation during seed development in the biodiesel feedstock Pennycress (Thlaspi arvense L.). *Journal of Plant Physiology*, *208*, 7–16. http://doi.org/10.1016/j.jplph.2016.10.011

Clopton, J. R., & Triebold, H. O. (1944). Fanweed Seed Oil Potential Substitute for Rapeseed Oil. *Industrial and Engineering Chemistry*, *36*(3), 218–219.

Coers, L. M., & Phippen, W. B. (2010). Determining the Role of Day Length and Temperature on Vernalization of Field Pennycress (*Thlaspi arvense* L.). Retrieved from: http://www.wiu.edu/pennycress/current-experiments/Flower%20Induction%202011.pdf

Cruz, V. M. V, Luhman, R., Marek, L. F., Rife, C. L., Shoemaker, R. C., Brummer, E. C., & Gardner, C. A. C. (2007). Characterization of flowering time and SSR marker

analysis of spring and winter type *Brassica napus* L. germplasm. *Euphytica*, *153*(1–2), 43–57. http://doi.org/10.1007/s10681-006-9233-1

Daxenbichler, M. E., Spencer, G. F., Carlson, D. G., Rose, G. B., Brinker, A. M., & Powell, R. G. (1991). Glucosinolate Composition of Seeds from 297 Species of Wild Plants. *Phytochemistry*, *30*(8), 2623–2638.

Delourme, R., Falentin, C., Huteau, V., Clouet, V., Horvais, R., Gandon, B., Renard, M. (2006). Genetic control of oil content in oilseed rape (Brassica napus L.). *Theoretical and Applied Genetics*, *113*(7), 1331–1345. http://doi.org/10.1007/s00122-006-0386-z

Demirbas, A. (2007). Importance of biodiesel as transportation fuel. *Energy Policy*, *35*(9), 4661–4670. http://doi.org/10.1016/j.enpol.2007.04.003

Diaz, I. M. A. (2014). *An integrative approach for germplasm utilization , genetic diversity and QTL mapping in Camelina spp. and crop-production issues in Thlaspi arvense, new promising oilseed crops for bioenergy and industrial uses*. Iowa State University.

Dolya, V. S., Koreshchuk, K. E., Shkurupii, E. N., & Kaminskii, N. A. (1974). Oils of three representatives of the family Cruciferae. II. *Ukranian Scientific-Research Institute of the Oils and Fats Industry*, *4*(4), 440–442.

Dorn, K. M. (2015). *Genomics and domestication of field pennycress (Thlaspi arvense)*. University of Minnesota.

Dorn, K. M., Fankhauser, J. D., Wyse, D. L., & Marks, M. D. (2013). De novo assembly of the pennycress (*Thlaspi arvense*) transcriptome provides tools for the development of a winter cover crop and biodiesel feedstock. *The Plant Journal*, *75*(6), 1028–1038. http://doi.org/10.1111/tpj.12267

Dorn, K. M., Fankhauser, J. D., Wyse, D. L., & Marks, M. D. (2015). A draft genome of field pennycress (*Thlaspi arvense*) provides tools for the domestication of a new winter biofuel crop. *DNA Research: An International Journal for Rapid Publication of Reports on Genes and Genomes*, *22*(2), 121–31. http://doi.org/10.1093/dnares/dsu045

Downey, R. K., & Craig, B. M. (1964). Genetic Control of Fatty Acid Biosynthesis in Rapeseed (*Brassica napus* L.). *Journal of the American Oil Chemists' Society*, *41*, 475–478. Retrieved from http://www.sid.ir/fa/VEWSSID/J_pdf/5002713920103.pdf

Drenth, A. C., Olsen, D. ., Cabot, P. E., & Johnson, J. (2014). Compression ignition engine performance and emission evaluation of industrial oilseed biofuel feedstocks camelina, carinata, and pennycress across three fuel pathways. *Fuel*, *136*, 143–155.

Eberle, C. a., Thom, M. D., Nemec, K. T., Forcella, F., Lundgren, J. G., Gesch, R. W., Eklund, J. J. (2015). Using pennycress, camelina, and canola cash cover crops to

provision pollinators. *Industrial Crops and Products*, *75*, 20–25. http://doi.org/10.1016/j.indcrop.2015.06.026

EIA. (2016). U.S. Energy Information Administration. Retrieved January 24, 2017, from http://www.eia.gov/biofuels/biodiesel/production/ and http://www.eia.gov/renewable/

Environmental Protection Agency. (2016). Program overview for renewable fuel standard program. Retrieved January 1, 2016, from https://www.epa.gov/renewable-fuel-standard-program/program-overview-renewable-fuel-standard-program

Eskin, N. A. M., McDonald, B. E., Przybylski, R., Malcolmson, L. J., Scarth, R., Mag, T., Adolph, D. (1996). Edible oil and fat products: Oil and oil seeds. In Y. H. Hui (Ed.), *Canola oil* (pp. 1–96). New York: John Wiley & Sons Inc.

European Committee for Standardization (CEN) (2003). *Automotive Fuels - Fatty acid methyl esters (FAME) for diesel engines - Requirement Methods, EN 14214:2003*. Brussels, Belgium.

Evangelista, R. L., Isbell, T. A., & Cermak, S. C. (2012). Extraction of pennycress (*Thlaspi arvense* L.) seed oil by full pressing. *Industrial Crops and Products*, *37*(1), 76–81. http://doi.org/10.1016/j.indcrop.2011.12.003

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2000). The chemical diversity and distribution of glucosinolates and isothiocyanates amoung plants. *Phytochemistry*, *56*, 5–51. http://doi.org/10.1016/S0031-9422(00)00316-2

Fan, J., Shonnard, D. R., Kalnes, T. N., Johnsen, P. B., & Rao, S. (2013a). A life cycle assessment of pennycress (*Thlaspi arvense* L.)-derived jet fuel and diesel. *Biomass and Bioenergy*, *55*, 87–100. http://doi.org/10.1016/j.biombioe.2012.12.040

Fatihi, A., Zbierzak, A. M., & Dörmann, P. (2013). Alterations in seed development gene expression affect size and oil content of Arabidopsis seeds. *Plant Physiology*, *163*(2), 973–85. http://doi.org/10.1104/pp.113.226761

Federal Aviation Administration. (2014). Sustainable Alternative Jet Fuels. Retrieved June 6, 2016, from https://www.faa.gov/about/office_org/headquarters_offices/apl/research/alternative_fuels/

Feeny, P. (1977). Defensive Ecology of the Cruciferae. *Annals of the Missouri Botanical Garden*, *64*(2), 221–234.

Fehr, W. R. (1991). *Principles of cultivar development*. Library of Congress.

Fenwick, R., Heaney, R., Mullin, J., & VanEtten, C. (1983). Glucosinolates and their breakdown products in food and food plants. *CRC Critical Reviews in Food Science and Nutrition*, *18*(2), 123–201.

Ferrándiz, C., Liljegren, S. J., & Yanofsky, M. F. (2000). Negative regulation of the SHATTERPROOF genes by FRUITFULL during Arabidopsis fruit development. *Science (New York, N.Y.)*, *289*(5478), 436–438. http://doi.org/10.1126/science.289.5478.436

Focks, N., & Benning, C. (1998). wrinkled1: A novel, low-seed-oil mutant of Arabidopsis with a deficiency in the seed-specific regulation of carbohydrate metabolism. *Plant Physiology*, *118*(1), 91–101. http://doi.org/10.1104/pp.118.1.91

Fourmann, M., Barret, P., Renard, M., Pelletier, G., Delourme, R., & Brunel, D. (1998). The two genes homologous to Arabidopsis FAE1 co-segregate with the two loci governing erucic acid content in Brassica napus. *Theoretical and Applied Genetics*, *96*(6–7), 852–858. http://doi.org/10.1007/s001220050812

Franzke, A., Lysak, M. a., Al-Shehbaz, I. a., Koch, M. a., & Mummenhoff, K. (2011). Cabbage family affairs: The evolutionary history of Brassicaceae. *Trends in Plant Science*, *16*(2), 108–116. http://doi.org/10.1016/j.tplants.2010.11.005

Gesch, R. W., & Archer, D. W. (2013). Double-cropping with winter camelina in the northern Corn Belt to produce fuel and food. *Industrial Crops and Products*, *44*, 718–725. http://doi.org/10.1016/j.indcrop.2012.05.023

Gmelin, R., & Virtanen, A. I. (1959). A New Type of Enzymatic Cleavage of Mustard Oil Glucosides. Formation of Allylthiocyanate in Thlaspi arvense L. and Benzylthiocyanate in Lepdium ruderale L. and Lepidium sativum L. *Acta Chemica Scandinavica*, *13*(7), 1959–1960.

Grabowski, M. (2015). Aster Yellows of Garden Flowers and Vegetables. Retrieved July 6, 2016, from http://www.extension.umn.edu/garden/yard-garden/flowers/aster-yellows/

Groeneveld, J. H., & Klein, A.-M. (2014). Pollination of two oil-producing plant species: Camelina (*Camelina sativa* L. Crantz) and pennycress (*Thlaspi arvense* L.) double-cropping in Germany. *GCB Bioenergy*, *6*(3), 242–251. http://doi.org/10.1111/gcbb.12122

Groeneveld, J. H., & Klein, A. M. (2014). Pollination of two oil-producing plant species: Camelina (Camelina sativa L. Crantz) and pennycress (Thlaspi arvense L.) double-cropping in Germany. *GCB Bioenergy*, *6*(3), 242–251. http://doi.org/10.1111/gcbb.12122\r10.1111/gcbb.12080; Dur√°n, X.A., Ulloa, R.B.,

Grundy, S. M. (1986). The New England Journal of Medicine Downloaded from nejm.org at University of Minnesota Libraries on July 23, 2014. For personal use only. No other uses without permission. From the NEJM Archive. Copyright © 2010 Massachusetts Medical Society. All rights. *The New England Journal of Medicine*, *314*, 745–748.

Han, J., Lühs, W., Sonntag, K., Zähringer, U., Borchardt, D. S., Wolter, F. P., Frentzen, M. (2001). Functional characterization of beta-ketoacyl-CoA synthase genes from Brassica napus L. *Plant Molecular Biology*, *46*(2), 229–39. http://doi.org/10.1023/A:1010665121980

Harrel, F. E., & Dupont, C. (2015). Hmisc: Harrell Miscellaneous. R package version 3.17-0.

Harvey, B., & Downey, R. (1963). The inheritance of erucic acid content in rapeseed (Brassica naupus L.). *Canadian Journal of Plant Science*, *44*, 104–111.

Haughn, G. W., Davin, L., Giblin, M., & Underhill, E. W. (1991). Biochemical Genetics of Plant Secondary Metabolites in Arabidopsis thaliana: The Glucosinolates. *Plant Physiology*, *97*(1), 217–226. http://doi.org/10.1104/pp.97.1.217

Helliwell, C. A., Wood, C. C., Robertson, M., James Peacock, W., & Dennis, E. S. (2006). The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. *Plant Journal*, *46*(2), 183–192. http://doi.org/10.1111/j.1365-313X.2006.02686.x

Hogge, L. R., Reed, D. W., Underhill, E. W., & Haughn, G. W. (1988). HPLC separation of glucosinilates from leaves and seeds of Arabidopsis thaliana and their identification using thermospray liquid chromatography-mass spectrometry. *J. Chromatogr. Sci.*, *26*(2), 551–556.

Hojilla-Evangelista, M. P., Evangelista, R. L., Isbell, T. A., & Selling, G. W. (2013). Effects of cold-pressing and seed cooking on functional properties of protein in pennycress (Thlaspi arvense L.) seed and press cakes. *Industrial Crops and Products*, *45*, 223–229. http://doi.org/10.1016/j.indcrop.2012.12.026

Hojilla-Evangelista, M. P., Selling, G. W., Berhow, M. A., & Evangelista, R. L. (2015). Extraction, composition and functional properties of pennycress (*Thlaspi arvense* L.) press cake protein. *JAOCS, Journal of the American Oil Chemists' Society*, *92*(6), 905–914. http://doi.org/10.1007/s11746-015-2653-0

Holland, J. B., Nyquist, W. E., & Cervantes-Martinez, C. T. (2003). Estimating and interpreting heritability for plant breeding: an update. *Plant Breeding Reviews*, *22*, 9–112.

Holm, L. (1997). *World Weeds: Natural Histories and Distribution*. John Wiley & Sons. Retrieved from https://books.google.com/books?hl=en&lr=&id=i7JjRXH6uq4C&pgis=1

Hume, L. (1984). The effect of seed maturity, storage on the soil surface, and burial on seeds of Thlaspi arvense L. *Canadian Journal of Plant Science*, *64*, 961–969.

Isbell, T. A., Cermak, S. C., Dierig, D. A., Eller, F. J., & Marek, L. F. (2015). Registration of Katelyn L. (Pennycress) with Improved Nondormant Traits. *Journal*

of Plant Registrations, *9*(2), 212. http://doi.org/10.3198/jpr2014.08.0053crg

Isbell, T. A., Evangelista, R., Glenn, S. E., Devore, D. A., Moser, B. R., Cermak, S. C., & Rao, S. (2015). Enrichment of erucic acid from pennycress (*Thlaspi arvense* L.) seed oil. *Industrial Crops and Products*, *66*, 188–193. http://doi.org/10.1016/j.indcrop.2014.12.050

Johnson, G. a., Kantar, M. B., Betts, K. J., & Wyse, D. L. (2015). Field Pennycress Production and Weed Control in a Double Crop System with Soybean in Minnesota. *Agronomy Journal*, *0*(0), 0. http://doi.org/10.2134/agronj14.0292

Johnston, J. S., Pepper, Alan., H. (2005). Evolution of Genome Size in Brassicaceae. *Annals of Botany*, *95*(1), 229–235. http://doi.org/10.1093/aob/mci016

Joseph, K. D. S. M., Alley, M. M., Brann, D. E., & Gravelle, W. D. (1985). Row Spacing and Seeding Rate Effects on Yield and Yield Components of Soft Red Winter Wheat. *Agronomy Journal*, *77*, 211–214. http://doi.org/10.2134/agronj1985.00021962007700020009x

Kaspar, T. C., Jaynes, D. B., Parkin, T. B., & Moorman, T. B. (2007). Rye Cover Crop and Gamagrass Strip Effects on NO Concentration and Load in Tile Drainage. *Journal of Environment Quality*, *36*(5), 1503. http://doi.org/10.2134/jeq2006.0468

Kaspar, T. C., Jaynes, D. B., Parkin, T. B., Moorman, T. B., & Singer, J. W. (2012). Effectiveness of oat and rye cover crops in reducing nitrate losses in drainage water. *Agricultural Water Management*, *110*(3), 25–33. http://doi.org/10.1016/j.agwat.2012.03.010

Kaspar, T. C., Kladivko, E. J., Singer, J. W., Morse S., Mutch, D. R. (2008) Potenail and Limitations of Cover Crops, Living Mulches, and Perennials to Reduce Nutrient Losses to Water Sources from Agricultural Fields in the Upper Mississippi River Basin. Final Report: Gulf Hypoxia and Local Water Quality Concerns Workshop. Pp. 127-148 in Upper Mississippi River Sub-basin Hypoxia Nutrient Committee. St. Joseph, MI.

Keck, A.-S., & Finley, J. W. (2004). Cruciferous vegetables: cancer protective mechanisms of glucosinolate hydrolysis products and selenium. *Integrative Cancer Therapies*, *3*(1), 5–12. http://doi.org/10.1177/1534735403261831

Khachatourians, G. G., Sumner, A. K., & Phillips, P. W. B. (2001). An Introduction to the History of Canola and the Scientific Basis for Innovation. In *The biotechnology revolution in global agriculture: innovation, invention and investment in the canola industry* (Vol. 24, pp. 33–47). Retrieved from http://www.cabi.org/cabebooks/ebook/20073012606

Kim, S., & Dale, B. E. (2005). Life cycle assessment of various cropping systems utilized for producing biofuels: Bioethanol and biodiesel. *Biomass and Bioenergy*, *29*(6),

426–439. http://doi.org/10.1016/j.biombioe.2005.06.004

Kladivko, E.J., Kaspar, T.C., Jaynes, D.B., Malone, R.W., Singer, J., Morin, X.K., and Searchinger, T. (2014). Cover crops in the upper midwestern United States: Potential adoption and reduction of nitrate leaching in the Mississippi River Basin. *Journal of Soil and Water Conservation*, *69*(4), 279–291. http://doi.org/10.2489/jswc.69.4.279

Kladivko, E. J., Frankenberger, J. R., Jaynes, D. B., Meek, D. W., Jenkinson, B. J., & Fausey, N. R. (2004). Nitrate leaching to subsurface drains as affected by drain spacing and changes in crop production system. *Journal of Environmental Quality*, *33*(5), 1803–1813. http://doi.org/10.2134/jeq2004.1803

Knothe, G. (2010). Biodiesel and renewable diesel: A comparison. *Progress in Energy and Combustion Science*, *36*(3), 364–373. http://doi.org/10.1016/j.pecs.2009.11.004

Kondra, Z. P., & Stefansson, B. R. (1970). Inheritance of the Major Glucosinolates of Rapeseed (*Brassica napus*) Meal. *Plant Science Department*, *237*, 643–647.

Koornneef, M., & Meinke, D. (2010). The development of Arabidopsis as a model plant. *The Plant Journal : For Cell and Molecular Biology*, *61*(6), 909–21. http://doi.org/10.1111/j.1365-313X.2009.04086.x

Kuchernig, J. C., Backenkohler, A., Lubbecke, M., Burow, M., & Wittstock, U. (2011). A thiocyanate-forming protein generates multiple products upon allylglucosinolate breakdown in Thlaspi arvense. *Phytochemistry*, *72*(14–15), 1699–1709. http://doi.org/10.1016/j.phytochem.2011.06.013

Kuznetsova, A., Bruun Brockhoff, P., & Haubo Bojesen Christensen, R. (2016). lmerTest: Tests in Linear Mixed Effects Models R package version 2.0-30. Retrieved from https://cran.r-project.org/package=lmerTest

Lal, R., Regnier, E., Eckert, D. J., Edwards, W. M., & Hammond, R. (1991). Expectations of cover crops for sustainable agriculture. *Cover Crops for Clean Water*, (c), 1–11.

Lehman, W. F., & Lambert, J. W. (1960). Effects of Spacing of Soybean Plants Between and Within Rows on Yield and Its Components. *Agronomy Journal*, *52*(2), 84–86.

Lemieux, B., Miquel, M., Somerville, C., & Browse, J. (1990). Mutants of Arabidopsis with alterations in seed lipid fatty acid composition. *Theoretical and Applied Genetics*, *80*(2), 234–240. http://doi.org/10.1007/BF00224392

Lenth, R. V. (2016). Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software*, *69*(1), 1–33.

Leonard, C. (1994). Sources and commercial applications of high erucic vegetable oils. *Lipid Tech*, 79–83.

Liljegren, S. J. S., Ditta, G. S. G., Eshed, Y., Savidge, B., Bowman, J. L., & Yanofsky, M. F. (2000). SHATTERPROOF MADS-box genes control seed dispersal in Arabidopsis. *Nature*, *404*(April), 0–4. http://doi.org/10.1038/35008089

Liu, J., Hua, W., Zhan, G., Wei, F., Wang, X., Liu, G., & Wang, H. (2010). Increasing seed mass and oil content in transgenic Arabidopsis by the overexpression of wri1-like gene from Brassica napus. *Plant Physiology and Biochemistry*, *48*(1), 9–15. http://doi.org/10.1016/j.plaphy.2009.09.007

Majak, W., Mcdiarmid, R. E., Benn, M. H., & Willmst, W. D. (1991). Autolysis of *Thalaspi arvense* in bovine rumen fluid, *30*(1), 127–129.

Manitoba Government. (2016). The Noxious Weeds Act. Retrieved from http://www.zambialii.org/zm/legislation/consolidated-act/231

Martinelli, T., & Galasso, I. (2011). Phenological growth stages of Camelina sativa according to the extended BBCH scale. *Annals of Applied Biology*, *158*(1), 87–94. http://doi.org/10.1111/j.1744-7348.2010.00444.x

ASTM - American Society for Testing and Materials (2008). *Standard specification for biodeisel fuel blend stock (B100) for middle distillate fuels, ASTM D6751-08*. *ASTM Annual Book of Standards; American Society for Testing and Materials*. West Conshohocken, PA.

Matthies, D. (1990). Plasticity of reproductive at different stages components of development in the annual plant *Thlaspi arvense L*. *Oecologia*, *83*(1), 105–116.

Mattie Sovero. (1993). *Rapeseed, a new oilseed crop for the United States*. Wiley, New York.

McIntyre, G. I., & Best, K. F. (1978). Studies on the Flowering of *Thlaspi arvense* L. IV. Genetic and Ecological Differences between Early- and Late-Flowering Strains. *The University of Chicago Press*, *139*(2), 190–195.

MDARD - Prohibited and Restricted Noxious Weed Seeds. (n.d.). Retrieved January 14, 2016, from http://www.michigan.gov/mdard/0,4610,7-125-2390_41275-149078--,00.html

Michaels, S. D., & Amasino, R. M. (1999). FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. *The Plant Cell*, *11*(5), 949–56. http://doi.org/10.1105/tpc.11.5.949

Michaels, S. D., & Amasino, R. M. (2001). Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *The Plant Cell*, *13*(4), 935–41. http://doi.org/10.1105/tpc.13.4.935

State of Minnesota. (2015). 21.81 - 2015 Minnesota Statutes. Retrieved February 15,

2016, from https://www.revisor.mn.gov/statutes/?id=21.81

Moser, B. R. (2008). Influence of Blending Canola, Palm, Soybean, and Sunflower Oil Methyl Esters on Fuel Properties of Biodiesel. *Energy & Fuels*, *22*(10), 4301–4306. http://doi.org/10.1021/ef800588x

Moser, B. R., Evangelista, R. L., & Isbell, T. A. (2016). Preparation and fuel properties of Field Pennycress (*Thlaspi arvense*) seed oil ethyl esters and blends with ultralow-sulfur diesel fuel. *Energy and Fuels*, *30*, 473–479. Retrieved from http://pubs.acs.org/doi/pdfplus/10.1021/acs.energyfuels.5b02591

Moser, B. R., Knothe, G., Vaughn, S. F., & Isbell, T. A. (2009a). Production and evaluation of biodiesel from field pennycress (*Thlaspi arvens*e L.) oil. *Energy and Fuels*, *23*(May 2007), 4149–4155. http://doi.org/10.1021/ef900337g

Moser, B. R., Shah, S. N., Winkler-Moser, J. K., Vaughn, S. F., & Evangelista, R. L. (2009b). Composition and physical properties of cress (*Lepidium sativum* L.) and field pennycress (*Thlaspi arvense* L.) oils. *Industrial Crops and Products*, *30*(2), 199–205. http://doi.org/10.1016/j.indcrop.2009.03.007

Nychka, D., Furrer, R., Paige, J., & Sain, S. (2015). fields: Tools for spatial data. http://doi.org/10.5065/D6W957CT

Patterson, H. D., & Thompson, R. (1971). Recovery of inter-block information when block sizes are unequal. *Biometrika*, *58*(3), 545–554. http://doi.org/10.1093/biomet/58.3.545

Paulson, N., & Ginder, R. (2007). The Growth and Direction of the Biodiesel Industry in the US. *American Agricultural Economics*, (May), 1–29. Retrieved from http://ageconsearch.umn.edu/bitstream/21402/1/sp06gi02.pdf

Pearlson, M., Wollersheim, C., & Hileman, J. (2012). Modeling and Analysis: A techno-economic review of hydroprocessed renewable esters and fatty acids for jet fuel production. *Biofuels, Bioproducts and Biorefining*, *6*(3), 246–256. http://doi.org/10.1002/bbb

Petrie, G. A., & Vanterpool, C. (1965). Diseases of rape and cruciferous weeds in Saskatchewan in 1965. *Canadian Plant Disease Survey*, *45*, 111–112.

Phippen, W. B., & Phippen, M. E. (2012). Soybean Seed Yield and Quality as a Response to Field Pennycress Residue. *Crop Science*, *52*(6), 2767. http://doi.org/10.2135/cropsci2012.03.0192

Phippen, W., Gallant, J., & Phippen, M. (2010). Evaluation of Planting Method and Seeding Rates with Field Pennycress (*Thlaspi arvense* L.). Accessed from http://www.wiu.edu/pennycress/current-experiments/Plant%20Density%202010.pdf on Janauary 24, 2017.

Piepho, H. P., Busche, A., & Emirch, K. (2003). A Hitchhiker's Guide to Mixed Models for Randomized Experiments. *J. Agronomy & Crop Science*, *189*, 310–322. http://doi.org/10.1046/j.1439-037X.2003.00049.x

Piepho, H. P., & Möhring, J. (2007). Computing heritability and selection response from unbalanced plant breeding trials. *Genetics*, *177*(3), 1881–1888. http://doi.org/10.1534/genetics.107.074229

Pimentel, D., Marklein, A., Toth, M. A., Karpoff, M. N., Paul, G. S., McCormack, R., Krueger, T. (2009). Food Versus Biofuels: Environmental and Economic Costs. *Human Ecology*, *37*(1), 1–12. http://doi.org/10.1007/s10745-009-9215-8

Pritchard, F. M., Eagles, H. A., Norton, R. M., Salisbury, P. A., & Nicholas, M. (2000). Environmental effects on seed composition of Victorian Canola. *Australian Journal of Experimental Agriculture*, *40*, 679–685.

R Core Team (2016). R: A language and environment for statistical computing. Vienna, Austria: Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Robertson, D. M., Schwarz, G. E., Saad, D. A., & Alexander, R. B. (2009). Incorporating uncertainty into the ranking of SPARROW model nutrient yields from Mississippi/Atchafalaya River basin watersheds. *Journal of the American Water Resources Association*, *45*(2), 534–549. http://doi.org/10.1111/j.1752-1688.2009.00310.x

Rodman, J. E., & Chew, F. S. (1980). Phytochemical correlates of herbivory in a community of native and naturalized cruciferae. *Biochemical Systematics and Ecology*, *8*(1), 43–50. http://doi.org/10.1016/0305-1978(80)90024-1

Runck, B. C., Kantar, M. B., Jordan, N. R., Anderson, J. A., Wyse, D. L., Eckberg, J. O., … Porter, P. M. (2014). The Reflective Plant Breeding Paradigm: A Robust System of Germplasm Development to Support Strategic Diversification of Agroecosystems. *Crop Science*, *54*(5), 1939. http://doi.org/10.2135/cropsci2014.03.0195

Saini, H. S., Bassi, P. K., Goudey, J. S., & Spencer, M. S. (2016)., and Environmental Factors Linked references are available on JSTOR for this article : Breakage of Seed Dormancy of Field Pennycress (*Thlaspi arvense*) by Growth Regulators, Nitrate, and Environmental Factors, *35*(6), 802–806.

Satterthwaite, F. E. (1946). An Approximate Distribution of Estimates of Variance Components. *International Biometric Society*, *2*(6), 110–114. Retrieved from http://www.jstor.org/stable/3002019

Scarth, R., & Tang, J. (2006). Modification of Brassica oil using conventional and transgenic approaches. *Crop Science*, *46*(3), 1225–1236.

http://doi.org/10.2135/cropsci2005.08-0245

Schmidt, R., & Bancroft, I. (2011). *Genetics and Genomics of the Brassicaceae*. *Journal of Chemical Information and Modeling* (Vol. Plant Gene). New York Dordrecht Heidelberg London: Springer Science+Business Media, LLC. http://doi.org/10.1017/CBO9781107415324.004

Schnable, P. S., Ware, D., Fulton, R. S., Stein, J. C., Wei, F., Pasternak, S., … Wilson, R. K. (2009). The B73 Maize Genome: Complexity, Diversity, and Dynamics. *Science*, *326*(5956), 1112–1115. http://doi.org/10.1126/science.1178534

Sedbrook, J. C., Phippen, W. B., & Marks, M. D. (2014). Review: New approaches to facilitate rapid domestication of a wild plant to an oilseed crop: example pennycress (*Thlaspi arvense* L.). *Plant Science*, *227*, 122–132. http://doi.org/10.1016/j.plantsci.2014.07.008

Selling, G. W., Hojilla-Evangelista, M. P., Evangelista, R. L., Isbell, T., Price, N., & Doll, K. M. (2013). Extraction of proteins from pennycress seeds and press cake. *Industrial Crops and Products*, *41*, 113–119. http://doi.org/10.1016/j.indcrop.2012.04.009

Shahin, Y., & Valiollah, R. (2009). Effects of row spacing and seeding rates on some agronomical traits of spring canola (*Brassica napus* L.) cultivars. *Journal of Central European Agriculture*, *10*(1), 115–122.

Sharma, N., Cram, D., Huebert, T., Zhou, N., & Parkin, I. A. P. (2007). Exploiting the wild crucifer *Thlaspi arvense* to identify conserved and novel genes expressed during a plant's response to cold stress. *Plant Molecular Biology*, *63*(2), 171–84. http://doi.org/10.1007/s11103-006-9080-4

Sheldon, C. C., Burn, J. E., Perez, P. P., Metzger, J., Edwards, J. A., Peacock, W. J., & Dennis, E. S. (1999). The FLF MADS Box Gene : A Repressor of Flowering in Arabidopsis Regulated by Vernalization and Methylation, *11*(March 1999), 445–458.

Shofran, B. G., Purrington, S. T., Breidt, F., & Fleming, H. P. (1998). Antimicrobial Properties of Sinigrin and its Hydrolysis Products. *Journal of Food Science*, *63*(4), 1996–1999. http://doi.org/10.1111/j.1365-2621.1998.tb15798.x

Simpson, G. G., & Dean, C. (2002). Arabidopsis, the Rosetta Stone of Flowering Time? *Science*, *296*(April), 285–289.

Sindelar, A. J., Schmer, M. R., Gesch, R. W., Forcella, F., Eberle, C. A., & -Lincoln Sindelar, N. (2015). Winter oilseed production for biofuel in the U.S. Corn Belt: Opportunities and limitations, 1–17. http://doi.org/10.1111/gcbb.12297

Slominski, B., Evans, E., Peirce, A., Broderick, G., Zijlstra, R., Beltranna, E., & Drew, M. (2015). *Canola Meal Feeding Guide* (5th ed.). Canola Council of Canada.

Retrieved from http://www.canolacouncil.org/media/516716/2015_canola_meal_feed_industry_guide.pdf

Smith, R. A., & Crowe, S. P. (1987). Fanweed toxicosis in cattle: case history, analytical method, suggested treatment, and fanweed detoxification. *Veterinary and Human Toxicology*, *29*(2), 155–156.

Snapp, S. S., Swinton, S. M., Labarta, R., Mutch, D., Black, J. R., Leep, R., … Stn, B. (2003). REVIEW AND INTERPRETATION Evaluating Cover Crops for Benefits, Costs and Performance within Cropping System Niches of Crop and impact of foregoing a cash crop, some farmers express Michigan and New York producers are experimenting, (i), *Agronomy Journal*, American Socilety of Agronomy 97:322–332.

Spence, J., Vercher, Y., Gates, P., & Harris, N. (1996). "Pod shatter" in Arabidopsis thaliana, Brassica napus and B. juncea. *Journal of Microscopy*, *181*(2), 195–203. http://doi.org/10.1046/j.1365-2818.1996.111391.x

Stefansson, B. R., & Downey, R. K. (1995). Harvest of gold: The history of field crop breeding in Canada. In A. E. S. and D. R. Knott (Ed.), *Rapeseed.* (p. 140–152.). Univ. Ext. Press, Univ. of Saskatchewan, Saskatoon.

Stefansson, B. R., Wo Hougen, F., & Downey, R. K. (1961). Note on the isolation of rape plants with sed oil free from erucic acid. *Canadian Journal of Plant Science*, *41*(1), 218–219.

Strock, J. S., Porter, P. M., & Russelle, M. P. (2004). Cover cropping to reduce nitrate loss through subsurface drainage in the northern U.S. corn belt. *Journal of Environmental Quality*, *33*(3), 1010–1016. http://doi.org/10.2134/jeq2004.1010

Szmigielska, A. M., Schoenau, J. J., & Levers, V. (2000). Determination of glucosinolates in canola seeds using anion exchange membrane extraction combined with the high-pressure liquid chromatography detection. *Journal of Agricultural and Food Chemistry*, *48*(10), 4487–4491. http://doi.org/10.1021/jf000477u

Taylor, D. C., Zhang, Y., Kumar, A., Francis, T., Giblin, E. M., Barton, D. L., … Zhu, W. (2009). Molecular modification of triacylglycerol accumulation by over-expression of DGAT1 to produce canola with increased seed oil content under field conditions This paper is one of a selection of papers published in a Special Issue from the National Research . *Botany*, *87*(6), 533–543. http://doi.org/10.1139/B08-101

Thompson, K., & Hodgson, J. G. (1993). Seed size and shape predict persistence in soil. *British Ecological Society*, *7*(2), 236–241.

Tiede, T., Kumar, L., Mohammadi, M., & Smith, K. P. (2015). Predicting genetic

variance in bi-parental breeding populations is more accurate when explicitly modeling the segregation of informative genomewide markers. *Molecular Breeding*, *35*(10), 1–13. http://doi.org/10.1007/s11032-015-0390-6

Toole, E. H., & Brown, E. (1946). Final Results of the Duvel Buried Seed Experiment. *Journal of Agricultural Research*, *72*, 201–10.

Traka, M., & Mithen, R. (2009). Glucosinolates, isothiocyanates and human health. *Phytochemistry Reviews*, *8*(1), 269–282. http://doi.org/10.1007/s11101-008-9103-7

U.S. Congress, H. of R. (2007). *Energy Independence and Security Act of 2007. Title II - Energy Security through Increased Production of Biofuels; Subtitle A - Renewable Fuel Standard*. Government Printing Office.

U.S. Government Accountability Office. (2015). *Defense Energy: Observations on DOD's Investments in Alternative Fuels*.

USDA-ARS. (n.d.). USDA-ARS U.S. National Plant Germplasm System; North Central Regional PI System; Regional Plant Introduction Station Ames, IA. Retrieved January 19, 2016, from https://npgsweb.ars-grin.gov/gringlobal/view2.aspx?dv=web_site_taxon_accessionlist&params=:taxonomyid=105522;:siteid=16

USDA - National Agricultural Statistics Service. (n.d.). Retrieved February 15, 2016, from http://www.nass.usda.gov/Research_and_Science/Cropland/metadata/meta.php

USDA, & APHIS. (2015). Weed Risk Assessment for *Thlaspi arvense* L. (Brassicaceae) – Field Pennycress. Retrieved on January 24, 2017 from https://www.aphis.usda.gov/plant_health/plant_pest_info/weeds/downloads/wra/Thlaspi-arvense.pdf

Vanhercke, T., El Tahchy, A., Shrestha, P., Zhou, X. R., Singh, S. P., & Petrie, J. R. (2013). Synergistic effect of WRI1 and DGAT1 coexpression on triacylglycerol biosynthesis in plants. *FEBS Letters*, *587*(4), 364–369. http://doi.org/10.1016/j.febslet.2012.12.018

Vargas, M., Combs, E., Alvarado, G., Atlin, G., Mathews, K., & Crossa, J. (2013). Meta: A suite of sas programs to analyze multienvironment breeding trials. *Agronomy Journal*, *105*(1), 11–19. http://doi.org/10.2134/agronj2012.0016

Vaughn, S. F., & Berhow, M. a. (1999). Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). *Journal of Chemical Ecology*, *25*(11), 2495–2504. http://doi.org/citeulike-article-id:7180576

Vaughn, S. F., Isbell, T. A., Weisleder, D., & Berhow, M. A. (2005). Biofumigant Compounds Released By Field Pennycress (*Thlaspi arvense*) Seedmeal. *Journal of Chemical Ecology*, *31*(1), 167–177. http://doi.org/10.1007/s10886-005-0982-4

Vaughn, S. F., Palmquist, D. E., Duval, S. M., & Berhow, M. a. (2006). Herbicidal activity of glucosinolate-containing seedmeals. *Weed Science*, *54*(4), 743–748. http://doi.org/10.1614/WS-06-007R.1

Venkatesh, R., Harrison, S. K., & Riedel, R. M. (2000). Weed Hosts of Soybean Cyst Nematode (Heterodera glycines) in Ohio. *Weed Technology*, *14*(1), 156–160.

Wade, T., Claassen, R., & Wallander, S. (2015). Conservation-Practice Adoption Rates Vary Widely by Crop and Region, (147).

Wang, X., Liu, G., Yang, Q., Hua, W., Liu, J., & Wang, H. (2010). Genetic analysis on oil content in rapeseed (*Brassica napus* L.). *Euphytica*, *173*(1), 17–24. http://doi.org/10.1007/s10681-009-0062-x

Warwick, S. I., Francis, A., & Susko, D. J. (2002). The biology of Canadian weeds. 9. *Thlaspi arvense* L. (updated). *Can. J. Plant Sci.*, *82*, 803–823.

Weed Seeds Order. (2009). Weed Seeds Order , 2005. *Minster of Justice*. Retrieved from http://laws-lois.justice.gc.ca/PDF/SOR-2005-220.pdf

Wilczek, A. M., Roe, J. L., Knapp, M. C., Cooper, M. D., Laura J. Martin, C. D. M. C. L.-G., Sim, S., … Schmitt, O. (2009). Effects of Genetic Pertubation on Seasonal Life History Plasticity. *Science*, *323*(February), 930–934.

Wittstock, U., & Burow, M. (2007). Tipping the scales--specifier proteins in glucosinolate hydrolysis. *IUBMB Life*, *59*(12), 744–751. http://doi.org/10.1080/15216540701736277

Wittstock, U., & Burow, M. (2010). Glucosinolate Breakdown in Arabidopsis: Mechanism, Regulation and Biological Significance. *The Arabidopsis Book, The American Soceity of Plant Biologists*, 1–14. http://doi.org/10.1199/tab.0134

Wittstock, U., Kliebenstein, D., Lambrix, V., Reichelt, M., & Gershenzon, J. (2003). Glucosinolate hydrolysis and its impact on gerneralist and specialist herbivores. *Elsevier Recent Advances in Phytochemistry*, *37*, 101–125.

Wright, J., Wilcke, B., Usset, E., Stienstra, W., Schmitt, M., Sands, G., … Michael Bennett. (1999). *The Minnesota Soybean Field Book*. (M. Bennett, D. Hicks, S. Naeve, & N. Bennett, Eds.). St. Paul, MN: University of Minnesota Extension Service. Retrieved from http://www.extension.umn.edu/agriculture/soybean/docs/minnesota-soybean-field-book.pdf

Wu, G., Wu, Y., Xiao, L., Li, X., & Lu, C. (2008). Zero erucic acid trait of rapeseed (Brassica napus L.) results from a deletion of four base pairs in the fatty acid elongase 1 gene. *Theoretical and Applied Genetics*, *116*(4), 491–499. http://doi.org/10.1007/s00122-007-0685-z

Appendix

Supplementary Table 1. Pearson's correlation coefficients (*r*) followed by their significance level for Best Linear Unbiased Estimates (BLUEs) all the traits analyzed in this thesis.

	Emergence	Basal Width	Stem Width	Prematurity Height	Vigor	Stems Meter	Lodging	Plant Habit	Height	Days to Flowering	Days to Maturity
Emergence	1	-	-	-	-	-	-	-	-	-	-
Basal Width	-0.22	1	-	-	-	-	-	-	-	-	-
Stem Width	-0.61 ***	0.18	1	-	-	-	-	-	-	-	-
Prematurity Height	0.57 ***	0.13	-0.20	1	-	-	-	-	-	-	-
Vigor	0.40 **	0.47 **	-0.20	0.68 ***	1	-	-	-	-	-	-
Stems Meter	0.44 **	-0.28	-0.75 ***	-0.01	-0.09	1	-	-	-	-	-
Lodging	-0.07	-0.02	0.18	-0.15	-0.18	-0.10	1	-	-	-	-
Plant Habit	-0.67 ***	0.11	0.63 ***	-0.62 ***	-0.49 **	-0.35 *	0.13	1	-	-	-
Height	0.07	0.68 ***	0.04	0.38 *	0.70 ***	-0.19	-0.20	-0.20	1	-	-
Days to Flowering	-0.33 *	0.17	-0.04	-0.59 ***	-0.51 ***	0.17	0.01	0.21	-0.05	1	-
Days to Maturity	-0.33 *	0.17	-0.04	-0.59 ***	-0.51 ***	0.17	0.01	0.21	-0.05	1.00 ***	1
Plot Combine Yield	0.57 ***	-0.45 **	-0.40 **	0.46 **	0.11	0.36 *	-0.07	-0.53 ***	-0.29	-0.31 *	-0.31 *
Single Row Hand Harvest Yield	0.28	0.48 **	-0.19	0.33 *	0.35 *	0.22	-0.10	-0.27	0.54 ***	0.28	0.28
One Hundred Seed Weight	0.27	0.30	-0.12	0.40 **	0.53 ***	-0.01	-0.01	-0.27	0.38 *	-0.30	-0.30
Yield Stem	0.18	0.25	0.23	0.57 ***	0.43 **	-0.30	-0.10	-0.33 *	0.38 *	-0.29	-0.29
Stems Plant	-0.50 ***	0.03	0.24	-0.52 ***	-0.25	-0.13	0.17	0.57 ***	-0.13	0.12	0.12
Pods Plant	-0.44 **	0.04	0.34 *	-0.33 *	-0.27	-0.15	0.01	0.49 ***	0.02	0.20	0.20
Seeds Pod	-0.15	0.07	0.18	-0.37 *	-0.32 *	-0.15	0.17	0.27	-0.07	0.15	0.15
Raceme Length	-0.42 **	0.42 **	0.46 **	0.05	0.21	-0.44 **	0.09	0.18	0.53 ***	-0.13	-0.13
Shattering	-0.27	0.25	0.13	-0.44 **	-0.16	-0.03	0.02	0.23	0.18	0.34 *	0.34 *
Index 1	0.14	0.26	-0.01	0.04	0.13	-0.07	0.15	0.01	0.19	-0.08	-0.08
Index 2	-0.37 *	0.04	0.36 *	-0.34 *	-0.28	-0.17	0.04	0.51 ***	0.04	0.22	0.22
C24.1	0.24	-0.04	-0.29	0.01	-0.11	0.33 *	0.09	-0.13	-0.12	0.17	0.17
C16.0	-0.39 *	-0.41 **	0.17	-0.53 ***	-0.55 ***	-0.08	0.18	0.47 **	-0.45 **	0.20	0.20
C18.0	-0.03	-0.33 *	0.24	-0.24	-0.27	-0.09	0.17	0.43 **	-0.33 *	-0.08	-0.08
C18.1	0.17	-0.22	-0.01	-0.03	-0.18	0.19	0.15	0.17	-0.21	0.01	0.01
C18.2	-0.45 **	0.07	0.18	-0.53 ***	-0.38 *	-0.07	-0.03	0.41 **	-0.20	0.16	0.16
C18.3	0.17	0.10	-0.12	-0.05	-0.05	0.10	0.45 **	0.05	-0.04	0.19	0.19
C20.1	-0.18	-0.19	0.31 *	-0.36 *	-0.38 *	-0.05	0.25	0.57 ***	-0.31 *	0.00	0.00
C20.2	-0.36 *	0.14	0.19	-0.34 *	-0.09	-0.20	-0.03	0.21	-0.03	-0.01	-0.01
C22.1	0.15	0.14	-0.11	0.46 **	0.47 **	-0.10	-0.33 *	-0.49 ***	0.36 *	-0.19	-0.19
C22.2	-0.08	0.28	-0.07	0.05	0.28	-0.04	-0.16	-0.30	0.35 *	0.00	0.00
Oil	0.16	-0.29	-0.07	0.29	0.23	-0.03	-0.19	-0.41 **	0.04	-0.32 *	-0.32 *

	Plot Combine Yield	Single Row Hand Harvest Yield	One Hundred Seed Weight	Yield Stem	Stems Plant	Pods Plant	Seeds Pod	Raceme Length	Shattering	Index 1	Index 2
Emergence	-	-	-	-	-	-	-	-	-	-	-
Basal Width	-	-	-	-	-	-	-	-	-	-	-
Stem Width	-	-	-	-	-	-	-	-	-	-	-
Prematurity Height	-	-	-	-	-	-	-	-	-	-	-
Vigor	-	-	-	-	-	-	-	-	-	-	-
Stems Meter	-	-	-	-	-	-	-	-	-	-	-
Lodging	-	-	-	-	-	-	-	-	-	-	-
Plant Habit	-	-	-	-	-	-	-	-	-	-	-
Height	-	-	-	-	-	-	-	-	-	-	-
Days to Flowering	-	-	-	-	-	-	-	-	-	-	-
Days to Maturity	-	-	-	-	-	-	-	-	-	-	-
Plot Combine Yield	1	-	-	-	-	-	-	-	-	-	-
Single Row Hand Harvest Yield	-0.04	1	-	-	-	-	-	-	-	-	-
One Hundred Seed Weight	0.05	0.32 *	1	-	-	-	-	-	-	-	-
Yield Stem	0.18	0.44 **	0.41 **	1	-	-	-	-	-	-	-
Stems Plant	-0.34 *	-0.38	-0.35 *	-0.51 ***	1	-	-	-	-	-	-
Pods Plant	-0.19	-0.07	-0.54 ***	-0.13	0.65 ***	1	-	-	-	-	-
Seeds Pod	-0.26	-0.16	-0.21	-0.09	0.09	0.15	1	-	-	-	-
Raceme Length	-0.48 **	0.00	0.11	0.26	0.08	0.07	0.10	1	-	-	-
Shattering	-0.27	0.23	0.16	0.09	0.09	0.03	0.38 *	0.17	1	-	-
Index 1	-0.11	0.12	0.66 ***	0.20	-0.18	-0.37 *	0.51 ***	0.11	0.37 *	1	-
Index 2	-0.19	-0.01	-0.39	-0.07	0.49 **	0.87 ***	0.34	0.00	0.14	-0.11	1
C24.1	0.19	0.07	-0.17	-0.19	0.03	0.00	-0.04	-0.24	0.00	-0.12	-0.02
C16.0	-0.13	-0.48 **	-0.72 ***	-0.56 ***	0.48 **	0.51 ***	0.35 *	-0.23	-0.05	-0.38 *	0.49 **
C18.0	-0.05	-0.34 *	-0.17	-0.24	0.12	0.13	0.43 **	-0.26	-0.11	0.14	0.35 *
C18.1	0.08	0.17	0.05	-0.07	-0.08	-0.04	0.07	-0.17	0.04	0.06	0.01
C18.2	-0.41 **	-0.47 **	-0.50 ***	-0.45 **	0.47 **	0.34 *	0.33 *	0.17	0.07	-0.22	0.16
C18.3	0.02	0.21	0.04	-0.17	0.00	-0.21	0.09	-0.09	0.11	0.18	-0.24
C20.1	-0.25	-0.17	-0.15	-0.23	0.22	0.08	0.25	0.10	0.10	0.03	0.06
C20.2	-0.36 *	-0.45 **	-0.26	-0.22	0.39 *	0.13	0.12	0.30	-0.01	-0.11	-0.03
C22.1	0.21	0.20	0.36 *	0.48 **	-0.35 *	-0.15	-0.32 *	0.05	-0.11	0.07	-0.06
C22.2	-0.22	-0.04	0.05	0.16	0.02	-0.05	-0.20	0.31 *	0.03	-0.09	-0.10
Oil	0.32 *	-0.07	0.38 *	0.34 *	-0.28	-0.23	-0.39 *	0.02	-0.17	0.04	-0.18

	C24.1	C16.0	C18.0	C18.1	C18.2	C18.3	C20.1	C20.2	C22.1	C22.2	Oil
Emergence	-	-	-	-	-	-	-	-	-	-	-
Basal Width	-	-	-	-	-	-	-	-	-	-	-
Stem Width	-	-	-	-	-	-	-	-	-	-	-
Prematurity Height	-	-	-	-	-	-	-	-	-	-	-
Vigor	-	-	-	-	-	-	-	-	-	-	-
Stems Meter	-	-	-	-	-	-	-	-	-	-	-
Lodging	-	-	-	-	-	-	-	-	-	-	-
Plant Habit	-	-	-	-	-	-	-	-	-	-	-
Height	-	-	-	-	-	-	-	-	-	-	-
Days to Flowering	-	-	-	-	-	-	-	-	-	-	-
Days to Maturity	-	-	-	-	-	-	-	-	-	-	-
Plot Combine Yield	-	-	-	-	-	-	-	-	-	-	-
Single Row Hand Harvest Yield	-	-	-	-	-	-	-	-	-	-	-
One Hundred Seed Weight	-	-	-	-	-	-	-	-	-	-	-
Yield Stem	-	-	-	-	-	-	-	-	-	-	-
Stems Plant	-	-	-	-	-	-	-	-	-	-	-
Pods Plant	-	-	-	-	-	-	-	-	-	-	-
Seeds Pod	-	-	-	-	-	-	-	-	-	-	-
Raceme Length	-	-	-	-	-	-	-	-	-	-	-
Shattering	-	-	-	-	-	-	-	-	-	-	-
Index 1	-	-	-	-	-	-	-	-	-	-	-
Index 2	-	-	-	-	-	-	-	-	-	-	-
C24.1	1	-	-	-	-	-	-	-	-	-	-
C16.0	0.00	1	-	-	-	-	-	-	-	-	-
C18.0	-0.10	0.55 ***	1	-	-	-	-	-	-	-	-
C18.1	0.00	0.15	0.49 ***	1	-	-	-	-	-	-	-
C18.2	0.03	0.41 **	0.11	-0.12	1	-	-	-	-	-	-
C18.3	0.41 **	0.05	-0.08	0.13	-0.22	1	-	-	-	-	-
C20.1	-0.07	0.33 *	0.56 ***	0.79 ***	0.29	0.12	1	-	-	-	-
C20.2	0.04	0.08	-0.25	-0.72 ***	0.65 ***	-0.08	-0.22	1	-	-	-
C22.1	-0.33 *	-0.49 ***	-0.44 **	-0.62 ***	-0.49 **	-0.43 **	-0.82 ***	0.03	1	-	-
C22.2	0.09	-0.36 *	-0.57 ***	-0.79 ***	0.13	-0.32 *	-0.64 ***	0.61 ***	0.57 ***	1	-
Oil	-0.20	-0.45 **	-0.18	-0.12	-0.37 *	-0.47 **	-0.32 *	-0.10	0.56 ***	0.27	1

*** P < 0.001, ** P < 0.01, * P < 0.05