Heterosis Assessment for Agronomic and Seed Quality Traits in Hybrid Canola (*Brassica napus* L.)

BY

Kevin Gerald Falk

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

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Abstract

Six *ogu* INRA CMS canola hybrids, their parents and three open pollinated check cultivars were grown in replicated yield trials at 6 locations in 2006 and 4 locations in 2008 and assessed for seedling vigour, plot uniformity, days to 50% flower, days to maturity, plant height, oil content, protein content, sum of oil and protein content, glucosinolate content and saturated fatty acid content. The hybrids displayed mid-parent heterosis for seedling vigour, uniformity, days to 50% flower, days to maturity, plant height, seed oil content, sum of oil and protein content and glucosinolate content. The hybrids also displayed high-parent heterosis for seedling vigour, uniformity, plant height, seed oil content and glucosinolate content. Heterosis for seed oil content was frequently observed and suggests that it may be possible to develop high seed yield, high oil content *B. napus* hybrids to grow in western Canada.

Introduction 1.0

Canola/rapeseed production in Canada has grown steadily since the 1940s. In Canada, three Brassica species are currently produced as oilseed crops, *Brassica napus*, *Brassica rapa* and *Brassica juncea*. The transition from rapeseed to canola was made by efforts of Dr. Baldur Stefansson and Dr. Keith Downey, in which they reduced the content of erucic acid in the seed oil and glucosinolates in the seed meal creating a product suitable for human food and livestock consumption. Tower (*B. napus*), released by B. Stefansson in 1974 was the world's first canola variety. As canola/rapeseed acreage in Canada grew, breeders focused on increasing seed yield, other agronomic traits and improving seed quality. *B. napus* is a naturally partially outcrossing plant, however; in Canada it has a high tendency to self pollinate (Cuthbert and McVetty, 2001). Therefore, the development of open pollinated cultivars was the breeding method of choice for Canadian canola/rapeseed breeders for much of the last sixty years. More recently, Canadian canola/rapeseed breeders have begun to develop hybrid cultivars.

There are several reports in the literature indicating that hybrid vigour or heterosis for seed yield occurs in *B. napus* hybrids. Allard (1960) defined the term heterosis as "hybrid vigour such that an F_1 hybrid falls outside the range of the parents with respect to some character or characters." Hybrids utilize this heterosis in yield and agronomic performance, thus having an advantage over current elite open pollinated populations Poehlman and Sleper, 1995). Sernyk and Stefansson (1983) and Grant and Beversdorf (1985) reported that 30 to 60% mid-parent heterosis for seed yield occurred for inter-

cultivar crosses of canola. Brandle and McVetty (1989) reported that 20 to 120% high parent heterosis for seed yield occurred for inter-inbred line crosses of canola.

The levels of heterosis observed for seed yield in canola hybrids were sufficient to encourage hybrid cultivar development. Unfortunately, the development of hybrids canola cultivars was delayed until a stable male sterility system could be found and then used to produce commercial quantities of hybrid canola seed. A number of different male sterility systems have been explored including cytoplasmic male sterility (CMS), genic male sterility (GMS), chemical induction of male sterility and male sterility through recombinant DNA technology (McVetty, 1998). The pollination control system of choice for most hybrid crop breeders has been cytoplasmic male sterility. Considerable research was done to develop CMS systems for use in hybrid canola. During the 1970s and through to the 1990s many cytoplasms were identified and tested including *pol*, *mur*, *nap* and *ogu*; however, each one had one or more undesirable attributes. INRA (l'institut national de la recherché agronomique) in France was able to genetically engineer the *ogu* cytoplasm to remove its negative effects thus creating a workable CMS system in canola referred to as *ogu* INRA. This system has permitted the development of hybrid canola cultivars and is now the predominant pollination control system used to produce hybrid canola cultivars around the world.

Commercial hybrid canola cultivars were first grown in Canada 20 years ago. Since their introduction, hybrid canola cultivars have increased in acreage as the breeding has focused on exploiting heterosis to increase vigour and seed yield. Currently, hybrid canola cultivars claim over 70% of the canola acreage in Canada, and this trend continues

to increase annually (Statistics Canada, 1995). Nearly all major canola breeding programs within North America now focus exclusively on the development of hybrid cultivars, abandoning research on open pollinated varieties. The market shift has been driven by canola growers and the canola industry wishing to capitalize on the high yield potential and superior agronomic performance offered by hybrids.

Reports of heterosis for other agronomic or seed quality traits in canola hybrids are contradictory. Grant and Beversdorf (1985) reported that there was little to no heterosis for oil or protein content in their study. These results have been supported by various other studies (Brandle and McVetty, 1990; Banks and Beversdorf 1994; Ali et al. 1995; Esch and Wricke, 1995). In contrast, Sernyk and Stefansson (1983) found significant heterosis for oil content in about half of the 8 hand-crossed canola hybrids studied. Similarly, Cuthbert (2006) reported that high parent and commercial heterosis for oil content was common in hand-crossed hybrids derived from inter-cultivar crosses of high erucic acid rapeseed HEAR (*B. napus*). Finally, Jovan Djordjevic (personal communication) of Monsanto Inc. has noticed that both seed yield and oil content appears to increase simultaneously in some newly developed *ogu* INRA CMS hybrids. Heterosis for oil content in *B. napus* remains to be confirmed. In addition, the conditions under which heterosis for oil content occurs need to be elucidated. Could genetically divergent parents produce high heterosis for oil content as well as for seed yield? Could the cytoplasmic – nuclear reactions resulting from the bioengineered cytoplasm play a role in the genesis of oil content heterosis?

This study's null hypothesis predicted that we would not detect or quantify heterosis for agronomic traits in selected Monsanto-developed *ogu* INRA CMS F_1 canola hybrids. We also hypothesize not to detect or quantify heterosis for seed quality traits, including oil and protein content in selected Monsanto-developed *ogu* INRA CMS F_1 hybrids. From the trial data, we estimate no positive or negative mid- and high-parent and commercial heterosis levels for selected agronomic and seed quality traits in selected Monsanto developed *ogu* INRA CMS F_1 canola hybrids. The alternative hypothesis states that we detect and quantify heterosis for agronomic and seed quality traits in selected Monsanto-developed *ogu* INRA CMS F_1 canola hybrids. In addition, we use this data to estimate positive or negative mid- and high-parent and commercial heterosis levels for selected agronomic and seed quality traits in selected Monsanto developed *ogu* INRA CMS F_1 canola hybrids.

Literature Review 2.0

2.1 Origin and History of Rapeseed

The term rapeseed or rape is derived from the latin word for turnip 'rapum'. Both *Brassica napus* and *Brassica rapa* are classified as rapeseed and are both members of the crucifer family, also known as *Brassicaceae.* This family also includes common vegetables such as turnip, Brussels sprouts, cabbage, mustard and rutabaga. *B. napus* and *B. rapa* are commonly known as Argentine and Polish rape (or canola) due to the sources of their original introductions. The origins of these species were first identified by a Korean botanist by the name of Woo Jang-Choon. Woo made three interspecific crosses, using three diploid species in the Brassica genus to produce three synthetic hybrids. The hybrids can be identified, and differentiated from their diploid parents, by chromosome numbers, which spontaneously doubled to form tetraploids. *B. rapa* is a naturally occurring diploid species with 10 pairs of chromosomes (denoted AA), which if crossed with another naturally occurring diploid species, *B. oleracea*, which has 9 pairs of chromosomes (denoted CC) forms *B. napus*. *B. napus* includes the combination of both genomes resulting in 19 pairs of chromosomes in the tetraploid arrangement (denoted AACC) (Fu and Yang, 1998). Since there are no wild relatives of *B. napus* known, it is believed that its initial cross was somewhat recent with *B. napus* originating in the Mediterranean region (Snowdon et al., 2007). Other crosses in the Brassicaceae family have produced other interspecific tetraploid species which can be found in the Triangle of U (Figure 2.1).

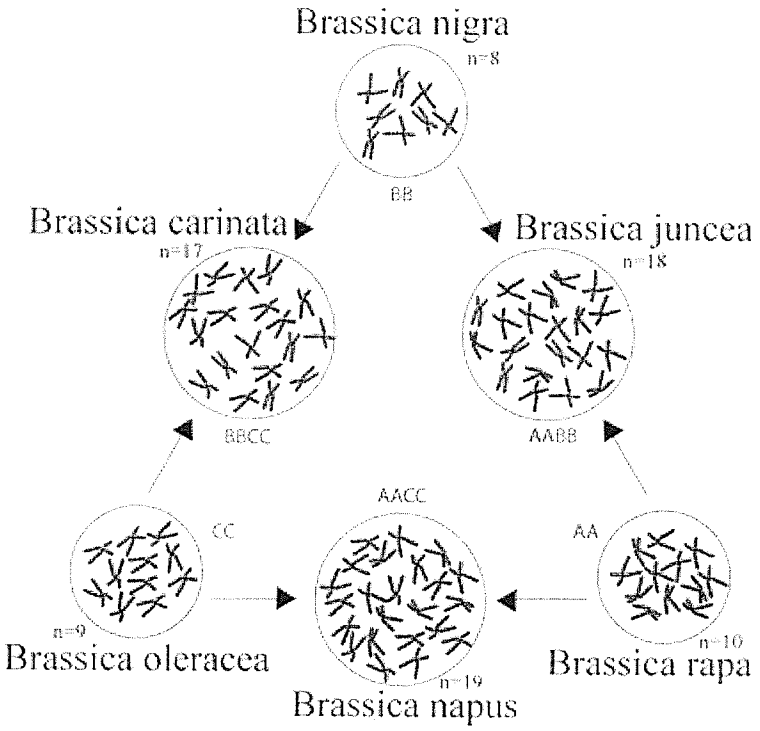

Figure 2.1: Triangle of U – Genomic and chromosome relationship of Brassica species (http://en.wikipedia.org/wiki/Triangle_of_U)

2.1.1 History of Rapeseed Cultivation

The cultivation and use of rapeseed dates back to 3000 BC when early civilizations used its oil to fuel lamps in which it was favourable because it burned smokelessly (Prakash, 1980). In addition, the oil has been used in cooking; however, its use was never widely accepted. Rapeseed use expanded into Europe and was the principle source of lamp oil from the 13th century until the market shifted to petroleum during the 19th century. Rapeseed production again increased in the age of steamships since it was an excellent oil used to lubricate steamship engines. Rapeseed oil was used specifically since it adhered to metal surfaces even when submerged under water or

bathed in steam. Rapeseed production in Canada started during the Second World War; the demand for rapeseed oil was at a premium as rapeseed oil reserves were low during the war. A small number of Canadian farmers had been producing rapeseed prior to the war and quickly increased their seed production as the demand for oil grew. The rapeseed species of choice at this time was *B. rapa* or Polish rapeseed. However, H.J. Newfeld started rapeseed breeding by making selections from breeding stocks originating from Argentina (*B. napus*), the seed of which had been purchased from the United States during the war (Stefansson and Downey, 1995).

Rapeseed production declined significantly after the war ended and rapeseed was not widely grown in Canada for several years. Rapeseed was reestablished through the first licensed Canadian variety of *B. napus* named 'Golden', in 1954. Golden was bred for uniformity, lodging resistance, high oil content and low iodine value in the oil. Rapeseed breeding continued; however, the fatty acid content, namely erucic acid and eicosenoic acid in the oil and glucosinolate content in the seed, kept rapeseed from becoming a major commodity in Canada. Rapeseed meal is high in protein and an ideal supplement to livestock feed; however, the glucosinolate content reduced palatability and proved to be responsible for thyroid enlargement in non-ruminants (Greer, 1950). Dr. Baldur Stefansson of the University of Manitoba bred out both the unhealthy erucic acid from the oil and the glucosinolates from the meal to produce the world's first 'double low' (referring to low erucic acid and low glucosinolate) rapeseed cultivar 'Tower' in 1974. Rapeseed now had the potential to enter mainstream edible oil markets. The elimination of the negative side effects of high erucic acid in the oil and high glucosinolates in the meal opened up a wide spectrum of opportunities for the new

commodity. To differentiate it from conventional rapeseed, this new product was given the name canola by the Rapeseed Crushers of Western Canada in 1979 which stood for **Can**adian **o**il **l**ow **a**cid (Stefansson and Downey, 1995). Previously, rapeseed that was bred to have zero erucic acid in the 1960's was given the name Canbra, a contraction between Canada and Brassica (Downey et al., 1967).

Canola was produced on 13 million acres (5.26 million hectares) in Canada in 2006. The Canola Council of Canada has a goal to push canola production even higher to meet the ever growing market demand for canola oil and meal (Figure 2.2). Canola production increases are influences by a number of factors, namely commodity prices and consumer demand. Canola acreage growth is being fueled by new products being produced from canola oil and/or meal. Along with canola being a healthy alternative to many cooking oils, canola oil is also used in the production of newly popular biodiesel fuel.

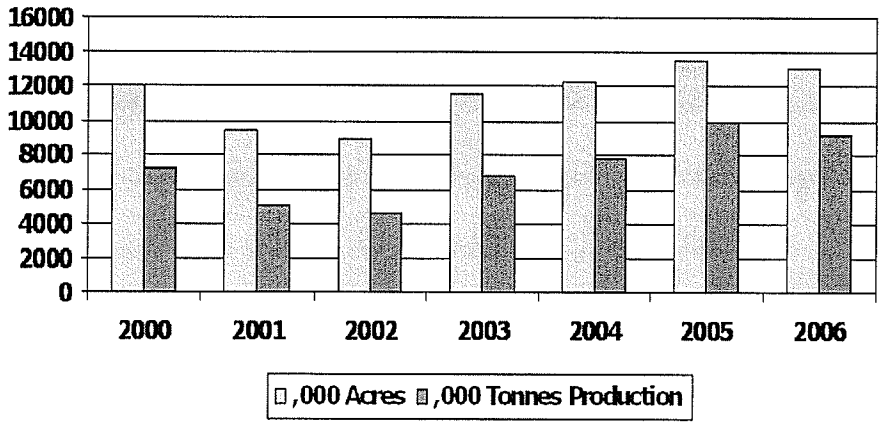

Figure 2.2: Canola acres and production in western Canada, 2000 – 2006 (Canola Council of Canada, 2008)

2.2 Agronomic Traits

2.2.1 Seedling Vigour

Seedling vigour describes the ability of the plant to emerge through the soil surface and grow vigoursly in the early growing season. Heterosis is often referred to as hybrid vigour, which is the earliest and most notable trait affected by the phenomenon. Although hybrid vigour is often remarkable when comparing the hybrid to its parents, few studies refer directly to the trait. This neglect may be due to the lack of direct economic importance conferred by hybrid vigour. Nonetheless, many vegetable, forage crop and horticultural plants utilize the enhanced vegetative growth produced by heterosis of vigour. Sernyk and Stefansson (1983) referred to the vegetative vigour in hybrids as being easily distinguishable from their parents. They also noted that the enhanced vigour appeared to give greater tolerance to flea beetle injury.

2.2.2 Days to First Flower

Days to flowering has a low narrow sense heritability of 14%. This is most likely being the result of flowering being the consequence of many complex processes including physiological, biochemical and gene action (Murfet, 1977). Thurkal and Singh (1987) observed decreased number of days to first flower due to partial dominant gene action. They also identified additive gene action as being important to days to first flower. Sernyk and Stefansson (1983) reported that hybrids on average flowered earlier than their parents. Grant and Beversdorf (1985) confirmed these results noting the hybrids flowered prior to or intermediate to both parents. Starmer et al. (1998); however, did not see any significant difference in flowering date between hybrids and parents.

2.2.3 Plant Height

Plant height in *B. napus* is quite variable. Winter growth habit cultivars grow substantially longer resulting in larger and taller plants than spring growth habit types. In Canada, plants are generally bred to be shorter, as this decreases the risk of lodging resulting in yield loss. In addition, shorter plants produce less straw residue resulting in a higher harvest index (Thompson and Hughes, 1986). Both additive and dominant gene action play a role in the inheritance of plant height (Govil et al., 1984). Positive heterosis for plant height was reported by Sernyk and Stefansson (1983), however, height was not significantly different in a similar study by Grant and Beversdorf (1985).

2.2.4 Days to Maturity

Days to maturity is defined as the time elapsed between planting and approximately 60% seed colour change on the main raceme. When breeding *B. napus* in western Canada days to maturity is crucial to ensure the crop matures before the first fall frost. Campbell and Kondra (1978) reported that high yields were positively correlated with earliness and rapid development. Unlike days to first flower, days to physiological maturity has a high narrow sense heritability of 57% (Ringdahl et al., 1986).

2.3 Seed Quality Traits

2.3.1 Protein Content

High protein content is also desirable, but secondary to oil content. Because protein content is negatively correlated with oil content, increasing protein content most likely means decreasing valuable oil content. Therefore, breeders looking to increase both oil content and

protein content will observe the sum of oil and protein content, with a focus on using more total space in the seed. Protein content is increased when the plant is under flooding or heat stress (Henry and MacDonald, 1978). These circumstances cause the movement of resources from oil production to protein production. As well, protein production is increased under high nitrogen fertility (Henry and MacDonald, 1978).

Protein contributes to 20 to 40% of the total seed weight. Protein is used as a source of amino acids for the germinating seed to reduce its dependence on external nitrogen. The proteins within the Brassica seed consist of albumins and globulins. The albumins are water-soluble, metabolically active proteins which are responsible for the housekeeping functions of the cell including the synthesis and degradation of the other storage proteins, globulins. These globulins serve as nitrogen reserves and are sequestered in protein bodies in the storage cells of the seed (Lonnerdal and Janson, 1972). The globulins make up the majority of protein within the seed. As with oil content, the estimated narrow sense heritability for protein content is low at about 26% (Grami and Stefannson, 1977), which would suggest genetic advancement for this trait could be difficult.

2.3.2 Sum of Oil and Protein Content

Oil and protein content have an inverse relationship with each other. Because of oil's economic importance compared to protein, breeders and growers wish to maximize oil content. However, due to environmental effects, protein can often offset oil within the seed. When considered together, both oil and protein content have a narrow sense heritability of 0.33 (Grami and Stefannson, 1977), higher than either alone. Robbelen (1978) suggests by focusing simultaneously on genetic advancement of oil and protein

content, the intrinsic value of the seed is maximized.

2.3.3 Saturated Fatty Acid Content

Canola is generally referred to as a healthy alternative to other edible vegetable oils due to its relatively low saturated fatty acid (SFA) content. The movement to healthier eating has been accelerated by research linking SFA's to coronary heart disease among other illnesses. Canola, having an average content of SFA's under 7.1% is labeled as low, the lowest of all current vegetable oils, and is half the content of that found in maize, soybean and olive oil. The canola SFA content has tended to increase over time as less *B. rapa* cultivars are being grown in western Canada. Because *B. rapa* characteristically has lower a SFA content, the change to almost entirely *B. napus* production in western Canada has raised the SFA content of canola oil. SFA contents are also affected by growing conditions, where higher temperatures tend to elevate SFA contents (Aksouh et al. 2001).

2.3.4 Glucosinolate Content

Glucosinolates are sulfur containing compounds found in the meal of the Brassica seed. These compounds produce negative effects in two ways. First, they produce a by-product called oxazolidinethione, which inhibits the function of the thyroid gland of non-ruminants (Greer, 1950). Second, glucosinolates produce a bitter taste that reduces palatability and thus feed intake for livestock. Glucosinolates are hydrolyzed by the enzyme myrosinase to produce thiocyantes, isothiocyantes or nitriles which result in bitter-tasting and toxic compounds (Figure 2.3)

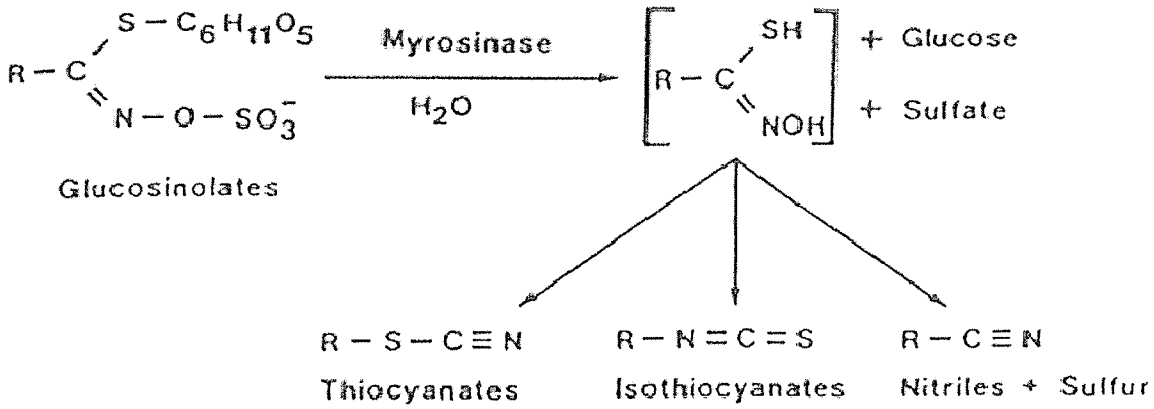

Figure 2.3: Chemical structure and enzymatic hydrolysis products of glucosinolates (Downey, 1983)

Glucosinolates have largely been bred out of current *B. napus* cultivars in Canada. The Canola Council of Canada sponsored definition of canola meal requires that it have less than 20 micromoles total glucosinolates per gram seed at 8.5% moisture. Kondra and Downey (1969) indicated that glucosinolates were controlled by three to five gene loci. In addition, through reciprocal crosses, Sernyk and Stefansson (1983) identified that glucosinolate content is determined by the maternal genotype and not the embryo. Glucosinolates have also been known to increase as temperature stress increases (Aksouh et al. 2001).

2.4 Oil Content

2.4.1 Oil Production

Oil content is the most valuable component of the *B. napus* seed, having approximately two to five times the value of the meal. The seed is crushed to extract the oil, of which 80% is concentrated in the cells of the cotyledons, 7 – 12% in the endosperm layer and the remainder in the seed coat (Downey et al. 1975). After the oil as

been extracted through crushing the seed combined with solvent extraction, only the seed meal remains.

Oil and protein content have an inverse relationship which is often affected by environment. Both moisture and temperature play a large role in determining the content of both oil content and subsequently protein content. In cool, wet conditions, the plant tends to produce higher oil content, reducing its production of protein. In hot, dry conditions, the opposite is true (Goodwin, 2006). Timing within the growing season of temperature and moisture is also a factor. Oil content is one of the last components synthesized in the developing seeds, therefore oil and protein contents are highly dependent on the environment at seed set. The Brassica plant utilizes two main storage components for reserves in its seeds, lipids and protein. The percentage of these storage reserves is subject to genetics and environment, but, it is based loosely on a 2:1 ratio (oil/protein). Deposition of both protein and oil into the embryo begins days after flowering and continues until senescence in a sigmoidal pattern (Figure 2.4) (Norton and Harris, 1975). The products form organelles in the seed known as oil bodies and protein bodies. The formation of these organelles is broken down into four stages. The first stage takes place from 0-3 weeks after flowering when very little deposition of storage products takes place. The second stage involves the rapid deposition of triacylglcerols from week 4-7 after which it decreases but continues until maturity. Stage three begins at six weeks after flowering with the deposition of cruciferin and napin proteins and continues to 11 weeks. The fourth stage involves the production of the synthesis of the oil body protein layer that surrounds the oil bodies. This takes place from 8 to 12 weeks after flowering. The oil bodies are essentially naked until the final stage when they are

packaged into the proteinaceous membrane during the final stage of development (Murphy et al., 1989).

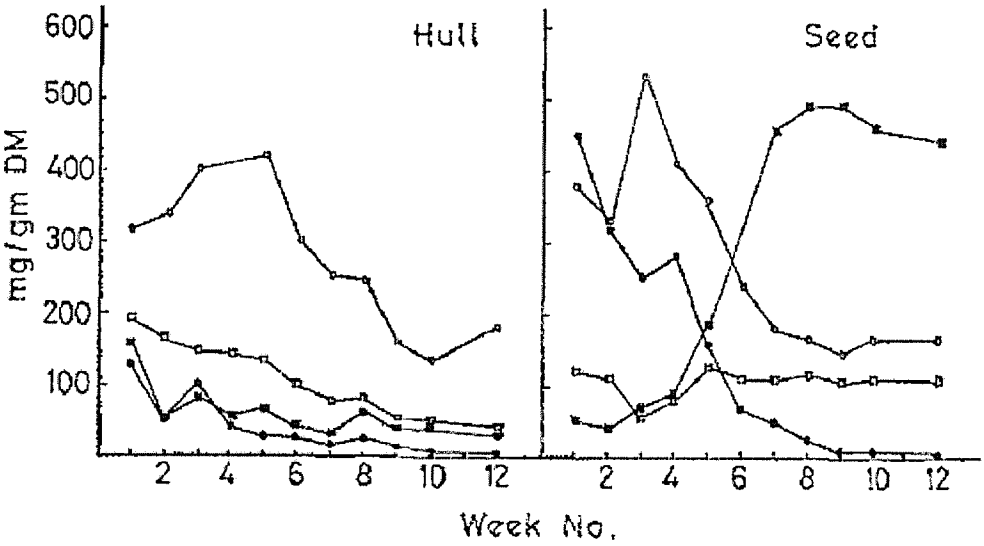

Figure 2.4: The content of storage materials in the hull and seed of Brassica napus during development. Aqueous soluble materials o---o, protein □---□, lipid ■---■, starch ●---●

Throughout the embryogenesis process, oil bodies continue to coalesce from small protein free droplets. These oil bodies are then inserted into the protein membrane after its synthesis by ribosomes (Murphy et al. 1989). The 19 kDa oil-body protein membrane comprises 20-25% of the total seed protein is not found anywhere else in the embryo (Murphy et al. 1989). Unlike the oil bodies, the synthesized protein-bodies accumulate into large bodies and are not packaged into a membrane. Murphy et al. (1989), claim that there are several specific genes and promoters that control deposition of seed storage tissues during embryogenesis. This suggests that the process can be manipulated.

In addition, nitrogen fertilizer has also been identified to affect oil content. As nitrogen fertilizer application continues to be applied, from 30 lbs/ac (34 kg/ha) to 150 lbs/ac (168 kg/ha), oil content steadily decreases (Figure 2.5). Lastly, genetics also play a role in oil content. Oil content is controlled by both additive gene action and overdominance gene action (Govil et al., 1984). Oil content's narrow sense heritability is about 0.26 (Grami and Stefannson, 1977). Jovan Djordjevic (personal communication), identified within a series of new canola hybrids, higher narrow sense heritability for oil content ranging from 0.5 to 0.8. The same study showed that higher yielding environments tend to favour higher oil content almost exclusively, (i.e. phenotypic correlations were 0.8 – 0.9).

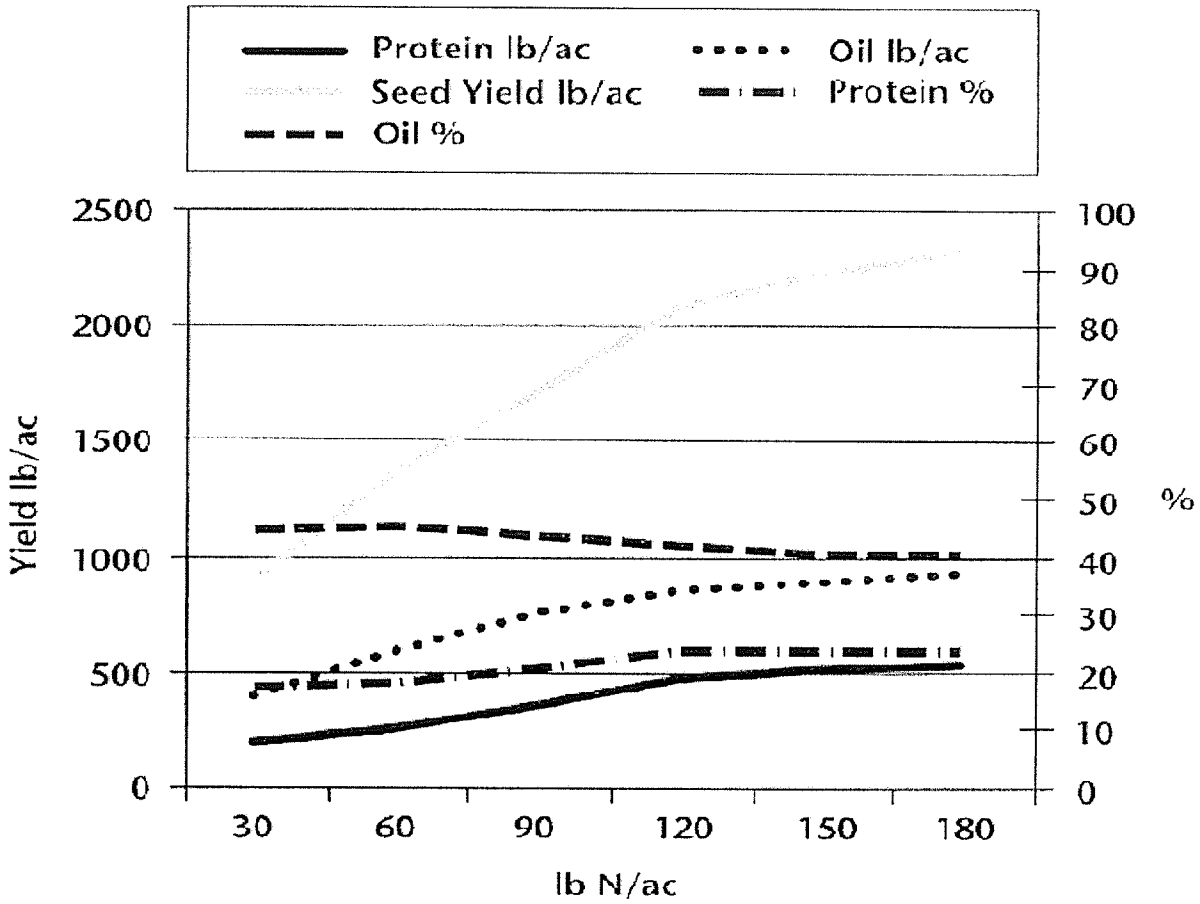

Figure 2.5: Effect of nitrogen fertilizer on yield and oil content of canola (2003 Canola Growers Manual)

Vegetable oils contain naturally occurring fatty acids from C12 to C24 chain length. These fatty acid chains not only vary in chain length but also in degree of saturation and each plant species has distinct oil profiles. The purpose of modification of these oil profiles is to produce natural oils with improved nutrition and functional properties. Thus, plant breeding can essentially take the place of processing prior to use, thus saving time and expense. Two methods can be taken from a plant breeding approach. As a result, both traditional breeding and biotechnology are used to modify fatty acid profiles in oilseed crops (McVetty and Scarth, 2002). Although numerous plants, both domesticated and wild, produce a variety of fatty acids, wild plants are difficult to

domesticate and thus domesticated species are modified (Thelen and Ohlrogge, 2002; Jaworski and Cahoon, 2003). Brassica oil in particular is low cost and renewable making it a popular choice for fatty acid modification (Thelen and Ohlrogge, 2002). Traditionally, rapeseed oil produced from Brassica oilseed cultivars (*B. napus, B. rapa, and B. juncea*) has a fatty acid composition of 5% palmitic (16:0), 1% Steric (18:0), 15% oleic (18:1), 14% linoleic (18:2), 9% linolenic (18:3) and 45% erucic (22:1) (Table 1.2) (Downey, 1990). However, through breeding efforts in the 1960's, specifically by Dr. Baldur Stefansson at the University of Manitoba and Dr. Keith Downey at the Saskatoon Research Station, the oil profile has been changed to a healthier fatty acid composition allowing for human consumption. The term 'canola' was coined by the Canola Council of Canada to identify *Brassica napus* and *Brassica rapa* which produce less than 2% of erucic acid and less than 20 μmol g^{-1} seed glucosinolates at 8.5% moisture. A typical canola profile has a composition of 4.7% palmitic, 1.8% steric, 63.0% oleic, 20.0% linoleic, 8.6% linolenic, 1.9% eicosenoic and 0.0% erucic (Table 2.1).

Table 2.1: Percent fatty acid composition of Canadian oils (Downey, 1990)

Fatty Acid	Formula	Oilseed Rape (Rapeseed)	Oilseed Rape (Canola)
Palmitic	C16:0	4.0%	4.7%
Steric	C18:0	1.5%	1.8%
Oleic	C18:1	17.0%	63.0%
Linoleic	C18:2	13.0%	20.0%
Linolenic	C18:3	9.0%	8.6%
Eicosenoic	C20:1	14.5%	1.9%
Erucic	C22:1	41.0%	0.0%

2.4.2 Conventional Breeding for Fatty Acid Profile

Improving fatty acid profile has been a primary objective in Brassica breeding programs around the world (Scarth and Tang, 2006). Conventional breeding has led the way in developing different types of Brassica cultivars with different fatty acid profiles. The key to breeding is identifying the desired fatty acid profile for a specific end use application and quickly developing and registering a cultivar that supplies that oil profile at a competitive price (McVetty and Scarth, 2002). The breeding techniques essentially increase or reduce particular fatty acid(s), producing many divergent types of Brassica oils (Burton et al., 2004; Przybylski, 2005). The specialty oil types have been bred using conventional, artificially induced mutations within the species and biotechnology approaches (Scarth and Tang, 2006).

2.4.3 Selecting for High Oil Content Canola

Since the first Canadian commercial CMS *B. napus* hybrid, Hyola 40, was registered in 1989, breeders have been tapping into the genetic potential of high yields

through heterosis. The pursuit of higher yields was market driven, as producers continued to demand ever increasing revenue. The demand was filled by increasing seed yield as well as seed quality and oil content. Seed quality and oil content increases were minimal but sufficient to meet progressively growing minimums (Figure 2.6). Breeders are required to meet registration committee mandated standards for components like oil, protein and glucosinolates. It hasn't been until recently that the industry has begun to focus its efforts on healthier oil and higher oil content. However, even with the industry's sights set on healthy or high oil content canola, yield still predominates and hinders the development of high oil varieties, unless they are grown under special contracts. Dave Charne, the Director of Research for Pioneer Hi-Bred states that "High-oil content canola varieties are still not yet on the fast track because no one is willing to sacrifice seed yield for oil content. Seed yield, along with grower profitability, would inevitably decline if breeders began shooting for better oil content, or any other end-use trait exclusively" (Canola Council of Canada, 2008). High oil content canola may be in the future, but for now breeders will not sacrifice potential seed yield increases by focusing on increased oil content. Shen et al. (2005), however, did identify a positive correlation between high seed yield traits and high oil content, making it possible for breeders to select for both.

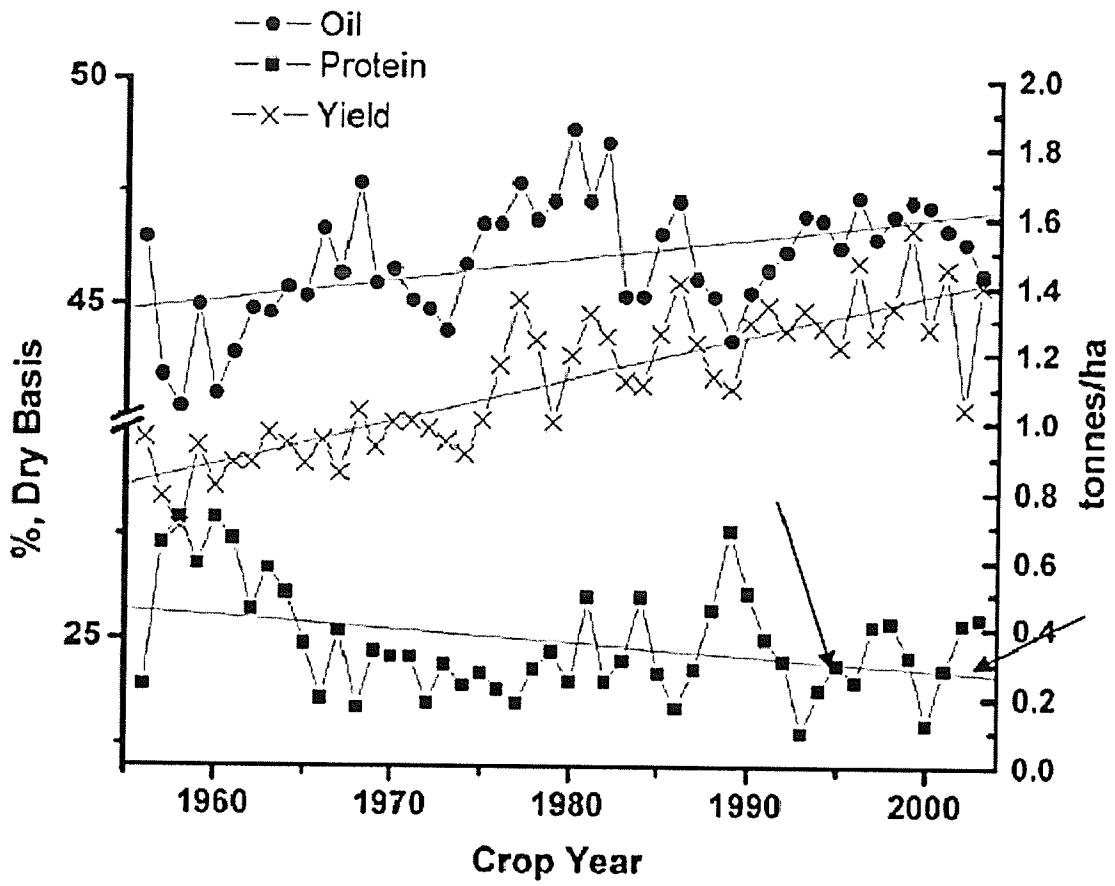

Figure 2.6: A review of the impact of environment and agronomic practices on quality of Canadian canola seed, Canola Council of Canada Report (Goodwin, 2006)

Even though breeders focused strongly on improving seed yield and disease resistance, seed oil content has been trending higher over the past several decades (Goodwin, 2006). Due to canola oil content being highly dependent on environmental effects, it is difficult to pursue high oil content in a breeding program which already focuses on seed yield and disease resistance. However, if indeed heterosis exists for seed oil content it could be utilized along with selection for seed yield. The question that remains is do the parental varieties that produce high yielding hybrid cultivars also produce high oil content hybrids as well? With oil content heterosis incorporated into

breeding programs, improvements in oil content could change from making small increments over years to large leaps.

2.4.4 Low Erucic Acid

As stated earlier, rapeseed breeding started in Canada in the 1950s headed by Dr. Baldur Stefansson at the University of Manitoba and Dr. Keith Downey at the Saskatoon Research Station of AAFC. Due to the nutritional concerns of erucic acid in then current rapeseed cultivars, Drs. Stefansson's and Downey's aim was to reduce erucic acid content through conventional breeding, producing healthier seed oil. The production of low erucic acid is a result of the blocking of the oil biosynthesis pathway in which oleic acid, a precursor, is elongated into erucic (Figure 2.7). Mutants with low erucic acid were found in a German forage variety of *B. napus* named 'Liho' in 1959 (Stefansson et al., 1961). This variety was used in backcrosses to adapted cultivars to exploit its low erucic acid properties. The result was the first low erucic acid cultivar of *B. napus* and *B. rapa*, Oro (1968) and Span (1971) respectively (Stefansson and Downey, 1995). 'Double low' rapeseed cultivars which contain both low glucosinolates in rapeseed meal and low erucic acid in rapeseed oil was established in Canada, taking the name 'canola' with the licensing of the first canola variety 'Tower' in 1974 (Canola Council of Canada, 2008). The conversion to low erucic acid, low glucosinolate rapeseed was fairly rapid in Canada. Not only in Canada, but Europe, China, Australia and other countries have seen a steady conversion from rapeseed to canola.

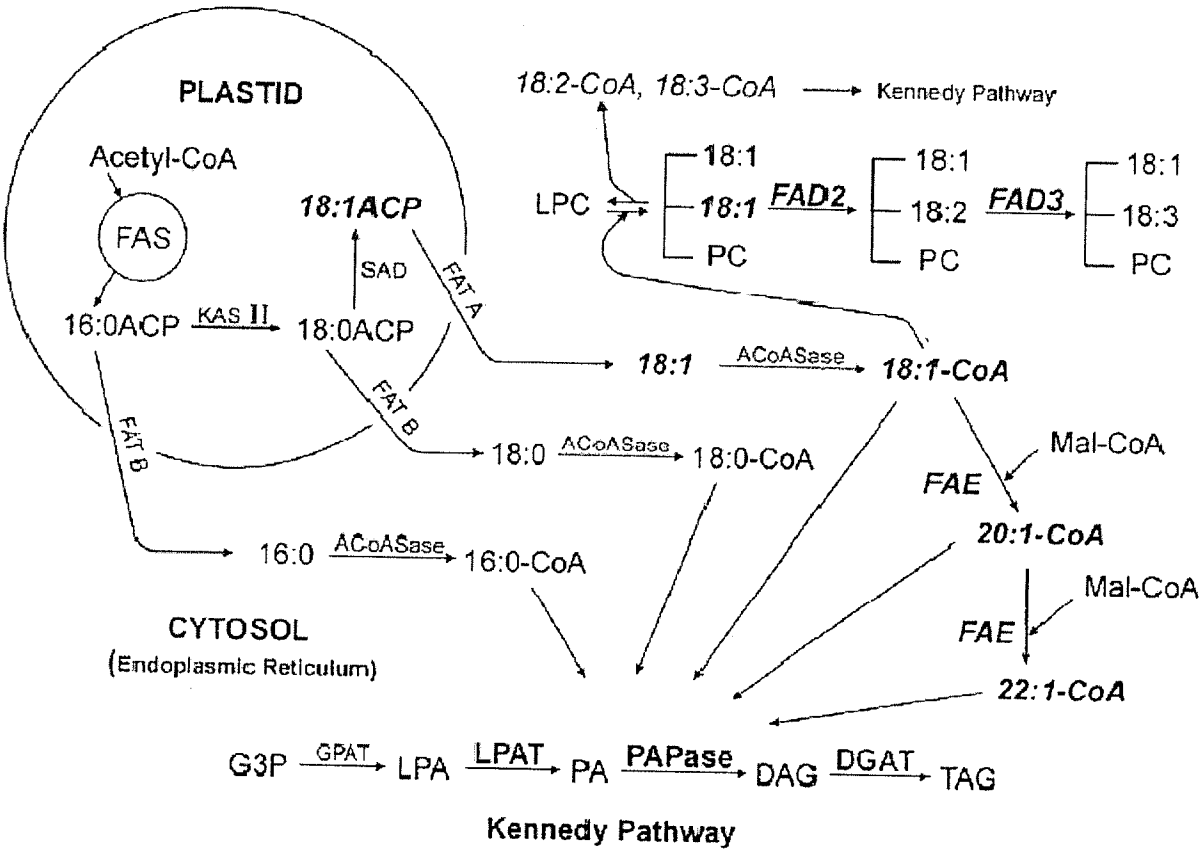

Figure 2.7: Oilseed rape biosynthesis pathway (Taylor, 2003)

2.4.5 High Erucic Acid

The manipulation of fatty acids in Brassica species didn't stop with the reduction of erucic acid; it also included the enhancement of erucic acid. The alleles e, E^a, E^b, E^c, and E^d, at each loci contribute <1%, 10%, 15%, 30%, and 3.5% of erucic acid produced, respectively. When these alleles are recessive, erucic acid content falls below 1%, when they are dominant, erucic acid is 45% (Siebel and Pauls, 1989). Oleic acid is a precursor to erucic acid, when the lipid biosynthesis pathway is blocked, erucic acid production is greatly reduced, allowing for a build up of oleic acid. A study by Bao et al. (1998) in *B. rapa* showed that erucic acid isn't produced immediately from newly synthesized oleic acid; rather it uses an intermediate pool of oleic acid. They found that this process is

inhibited with the addition of 2-[{3-chloro-5-(trifluromethyl)-2-pyridinyl}oxyphenoxy] propanoic acid which resulted in increased accumulation of oleic acid. Even though erucic acid is undesirable nutritionally, its properties can be advantageous for many industrial applications such as an additive to lubricants and solvents, as a softener in textiles, the manufacture of polymers, surfactants, plasticizers, surface coatings and pharmaceuticals (Katavic et al., 2000; McVetty and Scarth, 2002; Taylor et al., 1994; Sonntag, 1991, 1995; Leonard, 1994). Essentially, for these industrial purposes, the higher the erucic acid content, the better. Using breeding techniques, the content of erucic acid in the seed have increased from 45% to a ceiling of 55% (McVetty et al. 1998, 1999). In addition, new approaches involving resynthesizing *B. napus* from its ancestral diploids *B. rapa* and *B. oleracea* have been undertaken to attempt to produce erucic acid contents as high as 60% (Luhs and Friedt, 1995). Interspecific hybridization between *B. napus* and *Crambe abyssinica* (55% erucic acid) showed increase of erucic acid up to 61.5% (Wang et al., 2003; Schroder-Pontoppidan et al., 1999).

2.4.6 Low Linolenic Acid

Polyunsaturated fatty acids in *B. napus* include primarily linoleic (18:2) and linolenic (18:3) acid. These fatty acids have increased numbers of double bonds, two and three respectively, which make them susceptible to oxidation (Browse et al., 1998; Lauridsen et al., 1999). Due to these double bonds, these fatty acids have 10 and 25 times higher oxidation rates compared to oleic acid (18:1). These polyunsaturated fatty acids are essential fatty acids for human health and development (James et al., 2000; Ghafoorunissa et al., 2002). However, because of their increased susceptibility to oxidation, the shelf life of oils containing these fatty acids is greatly reduced. These oils

usually undergo partial hydrogenation to increase their stability; however, this process leads to the formation of trans fats which cause health concerns such as coronary heart disease (Kochar, 2000). The reduction of linolenic acid began with the introduction of the cultivar Stellar in 1987 through seed chemical mutagenesis, which contained 3% linolenic acid. Further decrease of linolenic acid was achieved through breeding; however, the total elimination of linolenic acid is unlikely since it plays an essential role in plant growth and reproduction (Tanhuanpaa and Schulman, 2002). Also, significant increase in linolenic acid were produced by mid-parent heterosis (Starmer et al. 1998), suggesting that heterosis does not always work in favor of improvement.

2.4.7 High Oleic Acid

Standard canola varieties have approximately 61% oleic acid which has been increased from the original 15% in rapeseed (Scarth and McVetty, 1999). Oils with high oleic content are desirable because they are monounsaturated, resulting in healthier oil. As well, they are much more stable than linoleic and linolenic fatty acids thus eliminating partial hydrogenation leading to the production of trans fats. An optimal oleic acid oil profile for increased heat stability while cooking is 67 to 75%; however, over 90% oleic acid content would result in near homogeneous starting materials allowing for reduced costs (Katavic et al., 2001). Conventional breeding techniques have allowed for an increase to 85-90% oleic acid (Vilkki and Tanhuanpaa, 1995). Mutagenesis treatments have also been used to bring the oleic content up to 86%. Starmer et al. (1998) noticed significant mid-parent heterosis for oleic content in hybrid cultivars over their parents. These wide ranging techniques could all be used to help raise oleic content in future

canola cultivars.

Market acceptance for these specialty canola types has been slow due to lack of encouragement, through premiums, for breeders to produce these varieties. The current market for these specialty oils requires a contract to maintain segregation from the field to the end use market. Unfortunately, drawbacks include maintaining the segregation and insufficient price premiums. One of the most interesting observations during the development of canola from rapeseed is the lack of significant change in total oil content despite the displacement of 40% erucic acid and 10% eicosenoic acid (Downey and Craig, 1964). The reason for this is that oleic acid is the precursor in the pathway to erucic (22:1) and eicosenoic (20:1) acids. The development of erucic acid from oleic acid is controlled by two genes in the embryo, which have no dominance but additive effects. Reciprocal crosses between plants with high erucic acid and zero erucic acid demonstrate that it is in fact embryonic genes controlling this process and not maternal genes (Downey and Craig, 1964). This opens up the idea that heterosis could play a part in oil biosynthesis since the process is controlled by the heterotic genes. The genes control a pathway which takes the 18:1 carbon chain of oleic acid, which is produced in the plastid, and adds four carbon molecules (or 2 acetates) onto the carboxylic end of the chain to produce erucic acid (22:1) in the cytosol. It is this model that explains why the percentage of oil in different rapeseed varieties remains constant while the fatty acid composition sees wide variation (Downey and Craig, 1964). However, Starmer et al.(1998) found that both oleic and linolenic fatty acids showed significant increase in hybrids over their parents in *B. napus*.

2.5 Heterosis

2.5.1 Definition

Hybrid vigour in crossbreeds has been observed for centuries (Shull, 1914). It was first studied by Kolreuter in 1763 (East and Hayes, 1912) and noted by Darwin in 1877. The term 'heterosis' was coined early in the 20th century by G.H. Shull to describe the genetic effect that results in hybrid vigour. Shull (1952) defined heterosis as, "the interpretation of increased vigor, size, fruitfulness, speed of development, resistance to disease and to insect pests, or to climatic rigors of any kind, manifested by crossbred organisms as compared with corresponding inbreds, as the specific results of unlikeness in the constitutions of the uniting parental gametes." East and Hayes (1912) provided an operational definition of heterosis as, "the decrease in vigour due to inbreeding naturally cross-fertilized species and the increase in vigour due to crossing naturally self-fertilized species are manifestations of one phenomenon. This phenomenon is heterozygosis. Crossing produces heterozygosis in all characters by which the parent plants differ. Inbreeding tends to produce homozygosis automatically." The greater the genetic distance between parents, the greater the resulting heterosis. Heterosis and hybrid vigour, however, are not synonymous. Whaley (1944) states, "heterosis refers to the developmental stimulation resulting, by whatever mechanism, from which different gametes unite." 'Hybrid vigour' denotes the manifest effects of heterosis.' The calculation of heterosis can be achieved in three contexts: mid-parent, high-parent and commercial heterosis.

Mid-parent heterosis refers to the contrast of the trait; yield for example, between the phenotype of the F_1 hybrid and the average phenotype of its two parents. Mid-parent

heterosis, although a widely used term, does not contribute any novelty or synergistic effect as the hybrid may not exceed both parents. The term high-parent heterosis refers to just that, the hybrids phenotype performance surpassing the phenotypic performance of the phenotypic performance of the better parent. The third term, commercial heterosis compares the phenotypic performance of the hybrid to the current phenotypic performance of the standard cultivar in the industry. Though commercial heterosis is an unscientific term, hybrids must perform substantially better than commercial non-hybrid cultivars to be commercially successful (Pandey and Zehr, 1999).

The genetic phenomenon of inbreeding depression has been well known for many years. It is caused by self-fertilizing naturally outcrossing plants (or animals) to close relatives. It produces a general loss in vigour and decrease in size and fertility of the progeny which subsequently decreases with further generations of inbreeding. However, as the parents relation spreads further apart, from siblings to cousins, the inbreeding effect or depression, lessens. In contrast, if lines are used to cross with other distantly related lines the opposite phenomenon, known as heterosis occurs. (Figure 2.8). Also, heterosis has the opposite effect of inbreeding, the greater the genetic diversity of the parents, the greater effect of heterosis on the progeny. Shull proposed a practical method to harness the heterotic advantage in maize in 1909 (Shull, 1909) changing the breeding landscape of maize and other naturally outcrossing crops forever.

Hybrids have 4 main advantages over open pollinated cultivars

1. More productive. Higher yields. (Lamkey and Edwards, 1999)
2. Production is more reliable. Hybrid crops seem to be more resilient to environmental stress than open pollinated cultivars.
3. Greater uniformity among F_1 plants
4. Superior agronomic and possibly seed quality characteristics

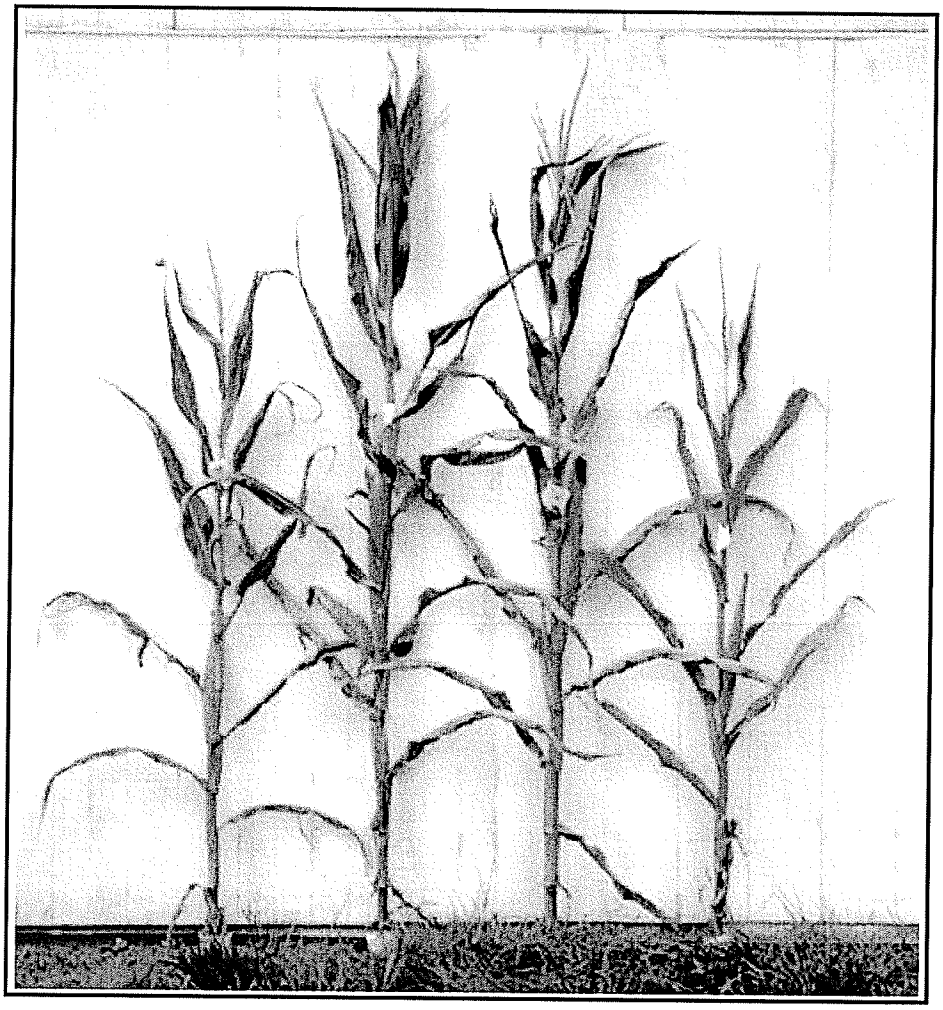

Figure 2.8: Representative individuals from two inbred maize lines (far left and far right) and the progeny of reciprocal hybrid crosses (left center and right center) are shown. Both parents are two high-quality inbred lines. Nonetheless, the progeny of a hybrid cross between these two lines are taller and more productive than either parent, illustrating the concept of heterosis. (Birchler, 2003)

Heterosis is not due to any single genetic cause, but a multitude of causes. It is basically achieved by a greater proliferation of cells in some, but not all tissues (East,

1936). Since the introduction of maize hybrids, researchers have been attempting to identify the exact mechanisms which control heterosis. Although the answer is still not known, scientists have formed two opposing theories. Shull's definition, one of many to define the term, focuses solely on the phenotype produced and not the genetic background. This is partially due to the genetic understanding of the phenomenon still remaining a mystery. The scientific community has produced three theories to describe the mechanism of heterosis. These theories attempt to explain heterosis through non-additive gene action and include: dominance (Davenport 1908, Bruce 1910, Keeble and Pellew 1910), overdominance (Shull 1908, East 1936) and epistasis (Stuber et al. 1973, Wright 1977).

2.5.2 Dominance Theory

The dominance theory states that genetically, heterosis is the masking of unfavourable or deleterious recessive alleles in the heterozygote (Bernardo 2002). The dominance theory was developed by Davenport (1908) and Bruce (1910) refers to the concealing of recessive alleles by the dominant allele found in the opposite parent (Figure 2.9). The result of crossing two pure lines produces a mean vigor slightly greater than that of the mean of the parents (Bruce, 1910). Also, the medium genetic distance between parents maximizes the difference in dominance for a trait at each locus. However, in this scenario, the value of the heterozygote does not exceed the value of its superior parent. However, it is possible for the F_1 to exceed the value of its superior parent when there is more than one gene controlling a trait if neither parent contains the advantageous alleles at all loci controlling that trait. Theoretically, it should be possible to achieve an inbred

which contains the same level of advantageous alleles as the hybrid (Shull, 1911; East and Hayes, 1912); however, this has yet to be attained. As well, the F_2 generation phenotype distribution should be asymmetrical due to the 3:1 segregation. This is not the case since F_2 phenotypes distribute symmetrically. These two flaws put the dominance theory into question.

2.5.3 Overdominance Theory

A competing theory, the overdominance theory presented by Shull (1908) and East (1908) suggests that heterosis is due to the interaction of diverse alleles that create a superior function in the hybrid (Figure 2.9) (Bernardo, 2002). This means that the inbred plants cannot achieve the same values as the hybrid. Therefore, an interaction between two alleles may produce a molecular mechanism that outperforms the inbreds. Unfortunately, no molecular mechanisms present themselves as obvious explanations for overdominance currently (Birchler, 2006). Unlike the dominance or epistatic theory, overdominance relies on the interaction between loci (Hedgecock et al. 1996). Supporters of the dominance theory countered this with the pseudo-overdominance theory. This theory, presented by Jones (1917), suggests that pseudo-overdominance is the cause of heterosis. It is a similar theory to the two locus dominance hypothesis but requires there is repulsion linkage between the two alleles producing complementation and appears as overdominance (Figure 2.9). This theory lacks the gene interaction generating a novel product.

2.5.4 Epistasis Theory

Epistatic effect is defined as a form of interaction between nonallelic genes in which one combination of such genes has a dominant effect over other combinations. With regards to heterosis, the interaction in question could be one locus having an influence on another locus for heterozygote adaptive superiority (Gowen, 1952). According to Stuber (1994), the homozygous alleles of an inbred can have an epistatic effect across the genome which is dramatically unmasked in an F_1 cross resulting a heterotic effect. When being compared to additive and dominance variances however, the epistatic effect is small (Banga, 1998).

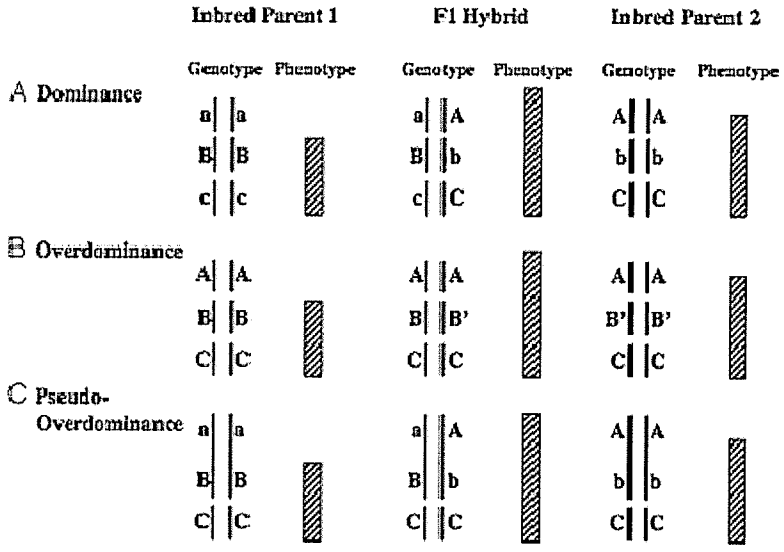

Fig. 2.9 Genetic models for heterosis. (*A*) The dominance model. Inbred parents 1 and 2 carry slightly deleterious homozygous alleles (*B*) The overdominance model. The homozygous alleles at the b locus are different between the inbred parent 1 (BB) and 2 (B'B'). (*C*) The pseudo-overdominance model. The superior phenotype in the F_1 hybrid can be attributed to a small chromosomal region, which contains two or more different loci (e.g., a and b) that are linked in repulsion phase. (Birchler et al. 2006)

New developments in heterosis theory shine light on the subject. A recent study done by Semel et al. (2006) helps to address the genetic basis of heterosis and the three theories surrounding it with some new conclusions. Experiments were done with domesticated tomato (*Solanum lycopersicum* L.) and wild tomato (*Solanum pennellii* L.); in which small segments of the wild tomato's genome was introgressed into many locations within an exhaustive number of domesticated tomato lines. By doing this, the effects of epistasis were reduced to almost nil. The results were illuminating; characteristics within the plant that correlated to reproductive success, such as the number of seeds, showed overdominant action where quantitative traits showed dominant/recessive behavior. Reproductive traits in this situation are defined as any that promotes greater seed number. Since overdominance is utilized only by these reproductive traits, pseudo-overdominance can be rejected. Semel et al. (2006) conclude by saying that heterosis is the result of single genes or gene complexes and that selection promotes interactions between alleles for increased reproductive success.

2.5.5 Heterosis Utilized in Crops

Hybridization has been exploited in crop breeding for over one hundred years. The phenomenon of hybrid vigour was first utilized in maize in the late 19th century (Shull, 1909). Breeding at the time was focused on the production of pure inbred lines. As maize is a natural outcrossing plant, inbreds display what is now known as inbreeding depression. Specific crosses were made using maize inbred lines to create the first hybrids which showed increased vigour over both inbreds and open pollinated lines of the

time, leading to commercial production of hybrid seeds. Maize was the key plant for the development of hybrid technology for number of reasons. The first reason was that maize was an economically important crop in much of the world, including the United States, which made it a target of scientific studies in plant improvement and crop breeding. Another reason was that maize breeding efforts of that time had hit a ceiling and were not improving yield as in previous years, prompting a change in thinking and approach. The third reason was that the plant architecture of maize allows for easy emasculation. The staminate (tassel) and pistillate (ear) inflorescences are separated spatially on the plant creating a unique advantage for manual pollen control in maize. This vastly reduced the time for crossing a male and a female which with perfect flowers must be painstakingly emasculated by hand. Being a naturally outcrossing crop coupled with the ease of emasculation, made it possible for commercial scale maize hybrid production. Maize hybrids quickly took over the market in the United States moving from their major establishment in the 1930s to being 50% of the market in1940 to almost 99% in 1968 (Gardner, 1968). The introduction of hybrid maize in the U.S. substantially increased yields throughout the 20th century (Figure 2.9).

The manual system used to emasculate maize hybrids involves a substantial investment as labourers are required to detassel each plant by hand and due to its morphology is only available to maize. Finding an alternate method of hybrid seed production, such as cytoplasmic male sterility was a needed prior to commercial hybrid production in other crops.

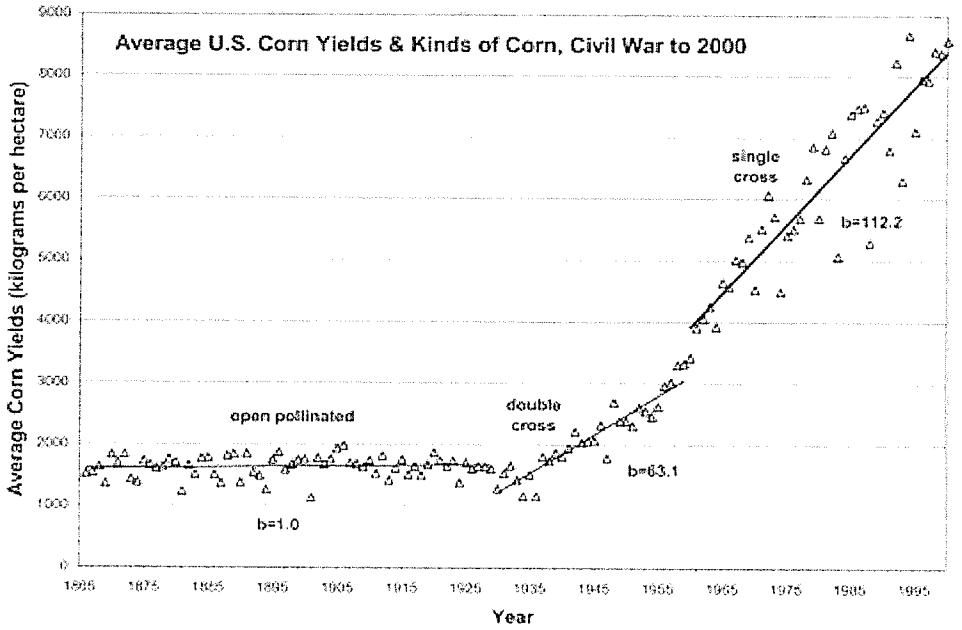

Figure 2.10: Average U.S. maize yields and kinds of maize, Civil War to 2000; periods dominated by open pollinated, by four-parent crosses, and by two-parent crosses are shown; "b" values (regressions) indicate average gain per year (Troyer, 1990)

2.7 Canola Hybrids

2.7.1 Hybrid Breeding

Canola hybrids have been developed within the past twenty years even though the exploitation of plants using heterosis found in hybrids was outlined quite some time ago (Shull, 1909). Prior to canola making the shift to hybrid cultivars, possible heterosis for seed yield had to be determined to ascertain if it would offset the cost of production. Hybrids are produced by crossing two homozygous plants producing F_1 heterozygous progeny which displays heterosis. Hybrid seed is then sold to producers. The hybrid seed production process is dependent on a method of cross pollination (whether by hand or with a male sterility system), strong inbred lines that create reproducible F_1 progeny and genetically diverse germplasm that can maximize the heterotic effect (Brandle and McVetty, 1989). A typical hybrid breeding strategy consists of three steps:

1. Inbred Line Development

2. Testing for Combining Ability

3. Production of Hybrid Seed

2.7.2 Inbred line Development

A breeding program must begin with producing inbred lines. Inbred lines are essential for developing reproducible hybrids. If F_1 hybrids were produced using segregating populations, the F_1 population would also be segregating and thus extremely non-uniform. By crossing two inbred lines, each F_1 hybrid plant will be genetically identical. Inbred lines are developed from germplasm of single, three-way, four-way crosses or open pollinated populations. The key in initial crosses is to obtain desirable traits which will be passed down to the F_1 hybrid. Once these initial crosses are made, the plant is self-pollinated for a number of generations until near homozygousity is reached. Double haploid (DH) line production technology has recently enabled the production of complete homozygous lines through microspore culture and colchicine chromosome doubling. The DH line approach eliminates the extensive inbreeding processes and allows many pure lines to be screened in a timely manner.

2.7.3 Combining Ability

An important step in creating high performance hybrids is determining which combination of inbred parents produces an F_1 with desired traits. Inbred lines can be artificially selected for maximum combining ability, unlike a normal population (Lippman and Zamir, 2007). To assess these potential combinations, two calculations are

made by observing specific crosses. General combining ability (GCA) is the average contribution that a particular inbred makes to the F_1 hybrid in a series of crosses as compared to the other inbred lines contribution in the same series of crosses. The GCA calculation is useful in measuring the additive genetic effects of the crosses in question (Poehlman and Sleper, 2006). However, calculating GCA may be impractical for large breeding programs due to the number of crosses needed.

Once general combining ability for inbred lines has been determined, specific combining ability (SCA) can be used to further distinguish potential hybrid parents. SCA evaluates the non-additive genetic effects and calculates the potential of a specific inbred line in a test with an array of other inbreds in question in diallel crosses (Poehlman and Sleper, 2006). With all potential crosses complete, the two parents producing the best performing hybrid have the superior SCA. Using full diallel mating, breeders can also determine which inbred line performs best as female or male. It is suggested that the two inbred parents which produce a high yielding hybrid have genes that complement each other in yield.

2.7.4 Production of Hybrid Seed

Canola hybrid seed production is dependent on a number of factors. Male sterility is a necessity as *B. napus* grown in western Canada is predominately (97%) self pollinated (Cuthbert and McVetty, 2001). Therefore a male sterility system is required. The movement of pollen from the male to the male-sterile female plant is also required. The hybrid seed production fields also require isolation since contamination increases with other compatible Brassica species growing in the vicinity. In western Canada, hybrid

canola seed production fields are located in the Lethbridge – Taber, Alberta area which designate farming regions prohibiting commercial production. Flowering synchrony between the male and female plants is essential for cross pollination efficiency. Often seeding will be staggered to allow the later parent to flower in synchrony with the earlier parent. Leaf cutter and honey bee hives are also placed in the field as to act as pollen vectors.

2.7.5 Hybrid Canola History

Open pollinated populations of canola were prevalent in Canada from canola's development in the 1970's until fairly recently when hybrid cultivars became available. In 1989, the first *B. napus* hybrid, Hyola 40, was registered. Since this milestone, the market demand has progressively shifted from open pollinated cultivars to hybrids. Hybrid cultivar adoption has increased from just over 15% of planted acres in 2002 to 55% of planted acres in 2006 (Figure 2.11).

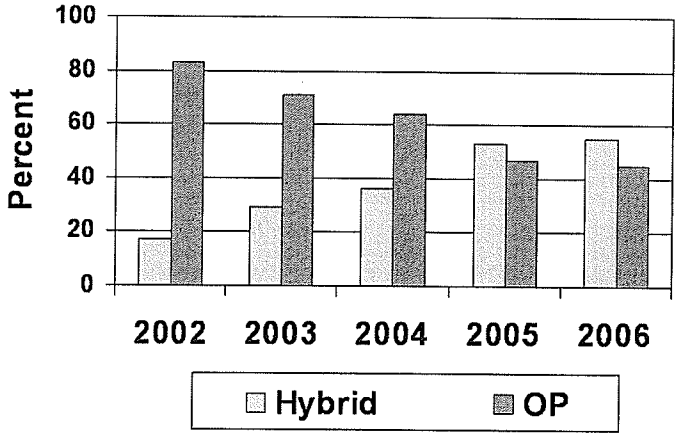

Figure 2.11: Acreage of hybrid and open pollinated canola cultivars in western Canada. (Canola Council of Canada, 2008)

2.5 Male Sterility

2.8.1 Introduction

Over the last 20 years, breeders have been attempting exploit heterosis in partially cross pollinated plants such as *B. napus* on a commercial scale. The difficulty lies in three components: understanding how to exploit heterosis, pollination control (restricting self-pollination) and sufficient pollen transfer (Grant and Beversdorf, 1985). This process is compounded by the plants high tendency to self pollinate. Unlike maize, hand emasculation on a commercial scale is impossible; pollination must be controlled using a non-labour intensive technique. The key in unlocking this secret has been found in male sterility and self incompatibility.

Although self incompatibility in *B. napus* is now being effectively used in China (Shen et al. 2005), male sterility has been chosen as the popular route to take. Cytoplasmic male sterility has been the method of choice to be used in hybrid breeding as it has been observed in over 150 plant species (McVetty, 1998). Male sterility describes plants that are unable to produce pollen and thus are dependent on pollen produced by another pollinator. A number of different mechanisms producing male sterility have been

explored including cytoplasmic male sterility (CMS), genic male sterility (GMS), chemical induction of male sterility and male sterility through recombinant DNA technology (McVetty, 1998).

2.8.2 Hybrid Production via CMS

Originally, the CMS systems were differentiated by the restorer gene used; now, however, it is the different cytoplasmic organelles that distinguish between sterile and normal plants. CMS systems consist of a male-sterile female 'A-line,' a male-fertile maintainer 'B-line', and a male-fertility restorer 'R-line'. The female (A-line) is constructed to have the sterile CMS cytoplasm and homozygous recessive restorer gene(s) (rr). These two factors together produce male sterility thus producing a female plant. The male line (R-line), unlike the female line, is homozygous for the restorer gene (RR) and can have either fertile (F) or sterile (S) cytoplasm, in both cases being fertile. The maintainer (B-line) has fertile cytoplasm (F) and is homozygous for the restorer gene (rr) and is used to cross with the female A-line to produce the next generation of the A-line. While both the A- and B-lines are genetically identical with the exception of the cytoplasms, the R-line is genetically dissimilar to produce heterosis through genetic diversity (Figure 2.12). The commercial hybrid seed is produced by growing A-lines and R-lines together in an open field allowing the pollen from the fertile R-lines to pollinate the male sterile A-lines. The seed from the A-line is then harvested and sold as F_1 seed. Hybrid seed production fields are set up in rows or bays in which the A- and R-lines grow contiguously. Due to the bulk of pollen the male R-lines produce, the ratio of A- to R-lines is about four to one.

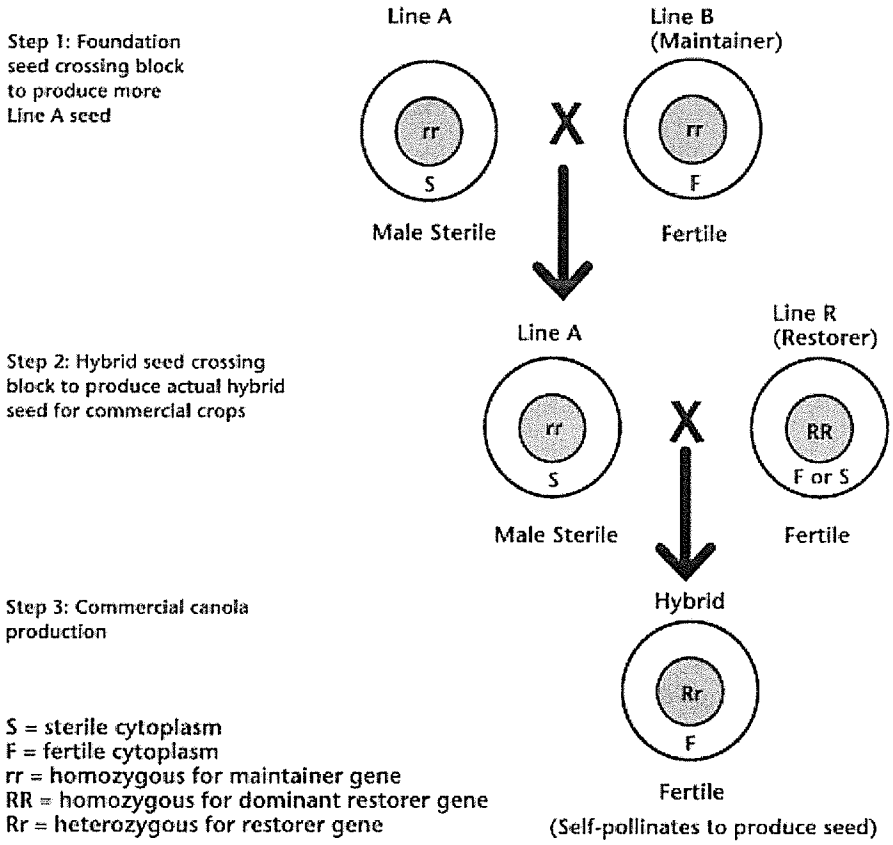

Figure 2.12: Hybrid seed production using a cytoplasmic male sterility system (Bernardo, 2002).

2.8.3 CMS Origins

Cytoplasmic male sterility systems originate from a number of different sources. Intergenic, interspecific and intraspecific crosses commonly produce male sterility. In addition, mutagens can be utilized to induce male sterility as well as male sterility occurring naturally through spontaneous means.

In intergeneric crosses, the key is to cross two genera of divergent origin to attempt to capture incompatible nuclear-cytoplasm interactions. As the crosses become

more divergent, the potential of incompatibility between nucleus and cytoplasm rises, thus producing CMS. Initial crosses and then numerous backcrosses are made to remove undesirable traits from the new CMS lines. However, the negative pleiotrophic and agronomic effects resulting from intergenic crosses are often difficult to fully remove. Interspecific crosses, genetic diversity is minimized thus reducing the likelihood of CMS arising. These crosses, however, are easier to make and are not as prone to negative effects (Pearson, 1981). Intraspecific crosses involve crosses of lines in different cytoplasms within the same species which are genetically diverse to maximize likelihood of CMS. CMS is commonly found in species which naturally have more than one type of cytoplasm (one possibly being male-sterile) in which the CMS system can be adapted for commercial use. This has been successfully done in onion, maize, rice and sorghum (Pearson, 1981). Cytoplasmic male sterility systems can also arise spontaneously (Edwardson 1956, 1970) in which male sterile plants have been discovered in male fertile populations. Mutagens have been used to induce male sterility; however, none of these sources of CMS have been adequate for use in hybrid breeding (Edwardson 1956, 1970).

2.8.4 CMS Deficiencies

Currently, cytoplasmic male sterility is a commonly used form of pollination control in a wide range of crops; however, getting from initial discovery to commercial use of the CMS was a difficult process. CMS systems commonly had insufficient or unstable male sterility. Many of the difficulties also lay within the restoration systems and with seed production. And once the CMS system is stable, breeders still have to deal with the undesirable pleiotropic and agronomic effects from the original CMS system.

How the CMS system works in particular is not well known, what is known is that the CMS system utilizes the interaction between foreign mitochondria and nuclei causing reduced efficacy and subsequently male sterility (Edwardson, 1970). Female fertility however, is not compromised even when male fertility is completely eliminated. These plants will not set seed unless an alternate source of pollen is available. Pollen production in the plant happens in the male reproductive organs known collectively as the stamen (anther and filament). The process of pollen production is the highest metabolic demand in the life cycle of the plant and thus requires maximum energy resources and optimal conditions. CMS is responsible for inhibiting the production of pollen due to insufficient or mistiming of resources (McVetty, 1998).

All hybridization techniques have had their challenges; CMS has often been the pollination control technology of choice. CMS requires that the sterility to be maternally inherited through the cytoplasm. Different Brassica breeders have attempted to harness CMS by developing and/or assessing different cytoplasms that could be adapted for use in pollen control. These cytoplasms include *nap* (Thompson, 1972), *ogu* (Bannerot et al. 1977), *pol* (Fu 1981) and *mur* (Riungu et al. 2000). The *ogu* CMS cytoplasm used in Brassica is derived from radish (*Raphanus sativus* L.).

2.8.5 CMS Cytoplasms

During early CMS development, a number of cytoplasms were discovered or developed for Brassica. Each cytoplasm was given a three-lettered name, four common systems were as follow: *nap, pol, mur* and *ogu. Nap* cytoplasm was derived from intraspecific crosses within *B. napus* while *pol* cytoplasm was derived from a

spontaneous mutation in a polish rape variety, Polima (Thompson, 1972). *Ogu* cytoplasm originates from a Japanese radish (*Raphanus sativus*), a crucifer and is named after the scientist who discovered it Dr. Ogura (Ogura, 1968). *Mur* cytoplasm was derived also from a crucifer cross, which is considerably wider, with a crucifer weed known as sand rocket (*Diplotaxis muralis*). One of the main hurdles with working with male sterility is restoring male fertility. Finding a functional male fertility restorer gene for each system was essential to success. In addition, with male sterility, other negative side effects were also present in these cytoplasms. Hybrids with *nap* cytoplasm are unstable at mid to high temperatures (over 21°C) in which the plant may revert to male fertility (Fan and Stefansson, 1986). *Pol* cytoplasm hybrids have a similar effect at high temperature (over 26°C+) and the *pol* hybrids perform poorly with regard to seed yield, total dry matter, harvest index and oil content in comparison to hand-crossed counterparts in the *nap* cytoplasm (McVetty et al. 1990). *Mur* hybrids display a morphological effect called faciation in which the racemes on the plant grow together reducing its productivity. *Ogu* hybrids display low temperature chlorosis in temperatures below 12°C and low nectar production.

Due to the various restrictions of the current CMS systems, *ogu* was selected to overcome its shortcomings. The cytoplasm was originally transferred into *B. napus* by transferring the *B. napus* nucleus into the male sterile *ogu* cytoplasm through intergeneric cross with Japanese radish producing an alloplasmic line (Bannerot et al. 1974, Pelletier, 1983). The resulting progeny were then backcrossed to *B. napus* which displayed male sterility and low temperature chlorosis while maintaining female fertility. Screening widely in the Brassica species to find genes to correct the chlorophyll deficiency failed

(Bannerot et al. 1977). To reduce the chlorosis effect of the alien radish cytoplasm, Pelletier et al. (1983) genetically engineered a hybrid cytoplasm built on the radish cytoplasm. Using protoplast fusion, *B. napus* chloroplasts were combined with the radish mitochondria to create a cybrid or cytoplasmic hybrid which eliminated both low temperature chlorosis and low nectar production. Another obstacle with the *ogu* CMS system included a lack of fertility restorers. Restorer genes were found in Raphanobrassica and introgressed into rapeseed (Heyn, 1976).

2.8.6 CMS and Heterosis

Cytoplasmic male sterility comes from nuclear-cytoplasmic interactions (Edwardson, 1970). Michaelis (1954) noted that these interactions may be positive or negative. Various CMS systems have been tested and shown to display negative effects or biological costs. Presently, no research has been found that correlates heterosis of oil content or other seed quality traits and the particular pollen control technique used, such as *ogu* INRA CMS. There has, however, been some interest in the scientific community that the hybrid cytoplasm (cybrid) could play a role. Shen et al. (2007) believes that the more trace pollen the high temperature *pol* CMS lines (there are high temp, low temp and stable *pol* CMS lines) had at low temperatures the higher the yield of the F_1 hybrids. Apparently, the high temperature *pol* CMS lines produce stronger heterosis as compared to stable *pol* CMS lines. This cytoplasmic heterosis theory conflicts with that proposed by Shull (1908), who concluded that heterosis was due to gene combinations and not cytoplasmic factors.

2.9 Heterosis for Agronomic Traits in Canola

There are several reports in the literature indicating that hybrid vigour or heterosis for seed yield occurs in canola (*Brassica napus* L.) hybrids. Allard (1960, p.468) defined the term heterosis as "hybrid vigour such that an F_1 hybrid falls outside the range of the parents with respect to some character or characters." Hybrids utilize this heterosis in seed yield and agronomic performance thus having an advantage over current elite open pollinated populations (Poehlman and Sleper, 2006). Sernyk and Stefansson (1983) and Grant and Beversdorf (1985) reported that 30 to 60% mid-parent heterosis for seed yield for inter-cultivar crosses of canola. Brandle and McVetty (1989) reported 20 to 120% high parent heterosis for seed yield for inter-inbred line crosses of canola.

A number of studies have been done looking at agronomic traits such as thousand seed weight, lodging resistance, days to flower, days to maturity and plant height in *B. napus* with varying conclusions. Grant and Beversdorf (1985), despite finding heterosis for seed yield, found no significant differences in thousand seed weight, percent oil, plant height or flowering date. They did; however, see a negative heterotic effect in lodging resistance. Lodging was explained by suggesting that increased seed yields put more stress on the stalk. These results; however, were contradicted by Sernyk and Stefansson (1983), who concluded their hybrids were equal to or better than the parents for lodging. Sernyk and Stefansson (1983) as well did not see any significant difference in thousand seed weight. Physiological maturity was reached slightly earlier in the hybrids in comparison to the parents which is probably due to their rapid initial growth rate (Grant and Beversdorf, 1985; Sernyk and Stefansson, 1983). This early vigour can also explain

why hybrids are generally earlier to flower and mature in most crops (Banga, 1998). The correlation between early vigour, days to flower and maturity was found in previous studies (Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985). A study done with *B. napus* performed by Thukral and Singh (1987) noted that both overdominance and non-additive gene effects play a role in days to maturity in plants. Overdominance is a phenomenon of heterozygotes having more variable phenotypes than homozygotes. Whether this result is due to one gene locus or multiple genes is unknown (Thukral and Singh, 1987). Heterosis for days to maturity was observed in previous studies by Sernyk and Stefansson (1983) and Grant and Beversdorf (1985). Heterosis has also been identified in root growth (Sinha and Khanna, 1975).

2.10 Heterosis for Seed Quality Traits

Most seed quality traits are controlled by numerous recessive genes (Fu, 2000). Fu and Yang claim that "it appears impossible to utilize heterosis for seed quality characters, e.g., oil and protein, because of the additive genetic variation and quotient nature of these characters." What factors in particular influence the genetic phenomenon heterosis to enhance a trait in one direction or another? Heterosis is utilized by plants to increase survivability and fitness. This genetic improvement; however, may or may not be desirable for human purposes. Starmer et al. (1998) realized that "heterosis is an occurrence which does not always work in favor of improvement." Many studies agree that heterosis in *B. napus* is clearly evident for agronomic traits such as seed yield, vigour, plant height, etc. However, there are some contradictions with seed quality traits,

noticeably oil content. Protein content displayed negative heterosis in *B. napus* in studies done by Sernyk and Stefansson (1983) and Grant and Beversdorf (1985).

2.10.1 Heterosis for Oil Content in Maize

Previous studies in maize have been completed at the University of Illinois by D.E. Alexander and R.J. Lambert and their team revealing the restrictions of breeding for both oil content and seed yield in hybrids. Literature reviews concluded that high yield and high oil content are mutually exclusive and one could only enhance one so far before reducing the other. There are two arguments made when looking at mutual exclusiveness of oil content and yield in maize. The first is the energy required to synthesize carbohydrates. Oil requires 2.25 times the energy of starch to produce. With a fixed amount of energy to spend, high oil content varieties would have a lower grain production. The second is the genotype. High oil content genotypes could be more efficient at producing oil content and thus offset the energy costs (Alexander and Lambert, 1968). Alexander and Lambert continued to look at hybrid breeding strategies to maximize both oil content and seed yield. They reevaluated mutual exclusiveness by using the topcross method. This method uses a high oil content pollinator as the male to a normal oil content CMS sterile female. The experiment included F_1 hybrids crossed to with a high oil content or normal oil content pollinator and observed the xenia effect. Xenia effect is the immediate effect of foreign pollen on the seed. The results were that normal females pollinated by high oil content males lead to a significant increase in seed oil content without loss of grain yield. The increase in oil content of these hybrids comes from an increase in germ weight, oil content with a reduction of the percentage of

endosperm. In theory, the experiment works for increasing oil content without sacrificing yield.

2.10.2 Heterosis for Oil Content in *B. napus*

Previous studies have reported heterosis for seed yield in hybrid *B. napus* varieties; however, no significant heterosis for seed oil content (Grant and Beversdorf, 1985; Lefort-Buson et al. 1987; Brandle and McVetty, 1990; Banks and Beversdorf 1994; Ali et al. 1995; Esch and Wricke, 1995). Some of these studies go so far to suggest it is nearly impossible to use heterosis in *B. napus* to enhance oil or protein content. However, other studies (Shen et al. 2005; Sernyk and Stefansson, 1983; Starmer et al. 1998) discovered heterosis for oil and/or protein content in hybrids; however, due to the contradiction, these results are not very convincing. Shen et al. (2005) found strong heterosis for seed yield and relatively strong heterosis for oil content. The average mid parent heterosis for oil content was 3.13%. The relationship between hybrids and parents regarding oil content was positive. Sernyk and Stefansson (1983) found significant heterosis for oil about half the time in the 8 hybrids studied. Starmer et al. (1998) observed mid-parent heterosis in *B. napus*; however, to a lesser degree than seed yield, only 3.8% above the parental mean. Downey and Craig (1964) identified that Brassica oil content is controlled by embryonic and not maternal genes. If oil content is in fact influenced by the seed and not the plant, heterotic genes are in control of the process and thus heterosis is a possibility. Djordjevic (personal communication) noticed that both seed yield and oil content seem to increase simultaneously in some newly developed

Monsanto hybrids using the *ogu* INRA CMS hybrid system and the same hybrids made by hand in the *nap* cytoplasm also showed increases in both seed yield and oil content.

The question remains whether heterosis for oil content in *B. napus* is possible and exploitable as well as why it was discovered by some researchers and not by others. In addition, does cytoplasmic male sterility contribute in some way through biological benefit? Is there potential in utilizing specific CMS cytoplasms, such as the *ogu* INRA CMS system to enhance heterosis for oil content in *B. napus*?

2.11 Heterosis and Geographical Diversity

Moll et al. (1962) was the first to identify the connection between geographical diversity and heterosis in maize. In time, this concept was translated over to other outcrossing plants such as *B. napus.* Basically, due to the large diversity in alleles, when unlike crosses are made, heterosis of these alleles is maximized. The theory is the opposite of inbreeding depression. Instead of reducing vigour and other traits due to the unmasking of deleterious alleles, divergent alleles from distant relatives can be masked. Initial crosses in oilseed rape were made and it was discovered that the material fell into groups known as heterotic groups specified by their geographic origins. The groups are as follows: European, Canadian and Asian (Brandle and McVetty, 1990). Due to the development of many Canadian cultivars originating in Europe, much of these two groups overlapped. Lefort-Buson et al. (1987) discovered that crosses between European and Asian grouped cultivars lead to the highest expression of heterosis. This theory was confirmed by Brandle and McVetty (1990) in which one Australian and numerous European and Canadian lines were crossed. The crosses using the Australian line showed

the strongest heterosis for seed yield. This specific line had one Asian parent and fell into the Asian pool. Cuthbert (2006) and Vincent (2008) also identified greater heterosis from hybrids stemming from genetically divergent crosses when compared to genetically similar crosses. In fact, molecular marker selection identified genetic distance between *B. napus* lines and could predict heterosis (Vincent, 2008).

Materials and Methods 3.0

3.1 Plant Material

The hybrid material used in this study consisted of six Monsanto hybrid canola cultivars. Three of these F_1 hybrid cultivars were commercially available and registered in Canada. The remaining three hybrid cultivars were Monsanto experimental cultivars that were going through the public co-op trials in western Canada when selected for this study in 2007. The parental material consisted of all the 10 Monsanto inbred lines that were all the parents used to produce the six hybrids used in the study. In addition, three Monsanto open pollinated canola cultivars were used as checks. Three of the hybrids shared a common male parent, providing only 10 inbred parents and not 12. The F_1 hybrids and inbred lines were coded for easy reference, for example, the first hybrid is labeled H1, while its parents are labeled H1P1 (for its female parent) and H1P2 (its male parent). The F_1 hybrids selected for this study were commercial or pre-commercial hybrids that had been performing well in previous trials or large scale farmer field trials. As well, preliminary data collected in 2006 suggested that heterosis for both seed yield and oil content could be occurring in the F_1 hybrids chosen for this study (Djordjevic, personal communication).

3.2 Yield Trials Sites

Replicated yield trials of hybrids, inbred parents and open pollinated checks were grown at selected Monsanto locations across western Canada in 2007 and 2008. These

yield trials were grown in the three Prairie Provinces in 2007 and in Manitoba and Saskatchewan in 2008 (Figure 2.13).

All of the research sites were Monsanto research facilities operated by Monsanto Canada Inc. In 2007, the trials were located in Swan Lake and Oakville, Manitoba, Yorkton and Saskatoon, Saskatchewan and Leduc and Barrhead, Alberta. In 2008 the trials were located in Swan Lake Manitoba (approximately 5 km from the previous year's location), Yorkton (approximately 3.5 km from previous year's location), Saskatoon, Vanscoy and North Battleford, Saskatchewan. The Vanscoy site was abandoned early in the 2008 season due to heavy weed pressure.

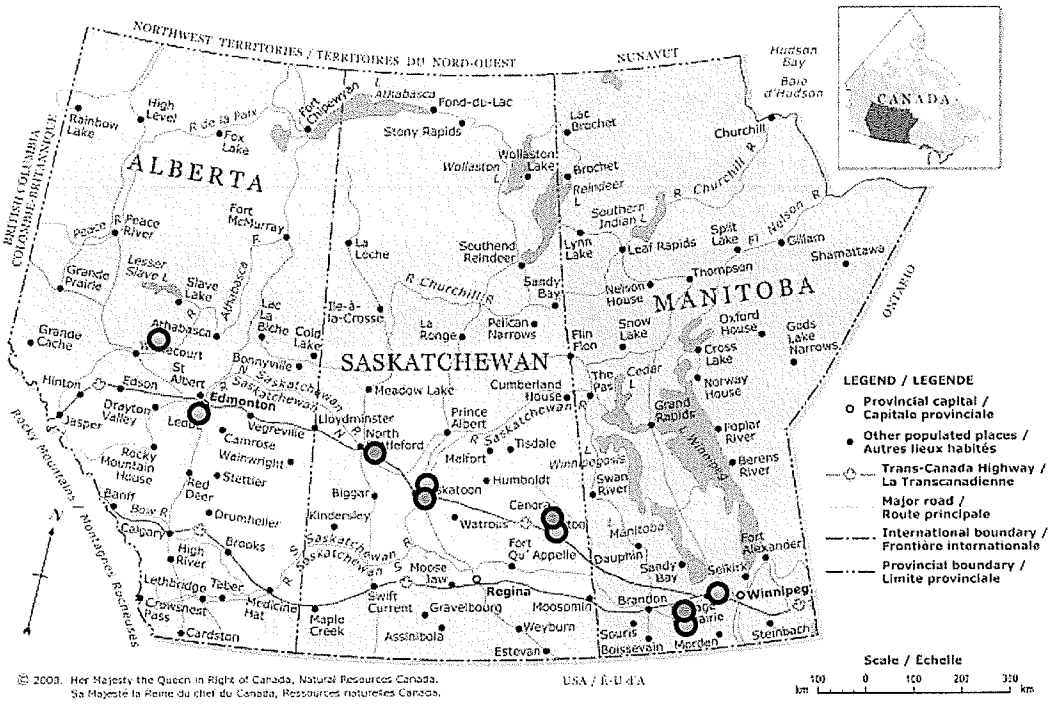

Figure 2.13: Trial sites in western Canada for 2007 ○ and 2008 ●

The variety of sites used covered the canola growing area well and minimized the risk of losing multiple sites due to environmental factors such as drought, flooding or hail. The variety of sites provided a good sample of environments across western Canada and permitted assessment of environmental effects versus genotype effect. In particular, we were interested in looking at how average daily temperature highs, precipitation and season length affected the oil and protein content in the seed.

3.3 Experimental Design

Each replicated yield trial consisted of nineteen entries, including six Monsanto-developed *ogu* INRA CMS F_1 hybrids, their 10 (B-line and R-line) inbred parents and three currently-grown commercial open pollinated canola cultivars. The trials used a randomized complete block (RCB) design with three replications. Six row plots 5 m long with 20 cm row spacing were used in all yield trials. Due to the possibility of competition from the more vigorous hybrids over the inbred parents or open pollinated check cultivars, the treatments were sub-divided into two groups prior to randomization. The six hybrids made up one group while the inbreds and open pollinated cultivars made up the other. Treatments were randomized within the groups as well as the groups themselves were also randomized (Figure 2.14).

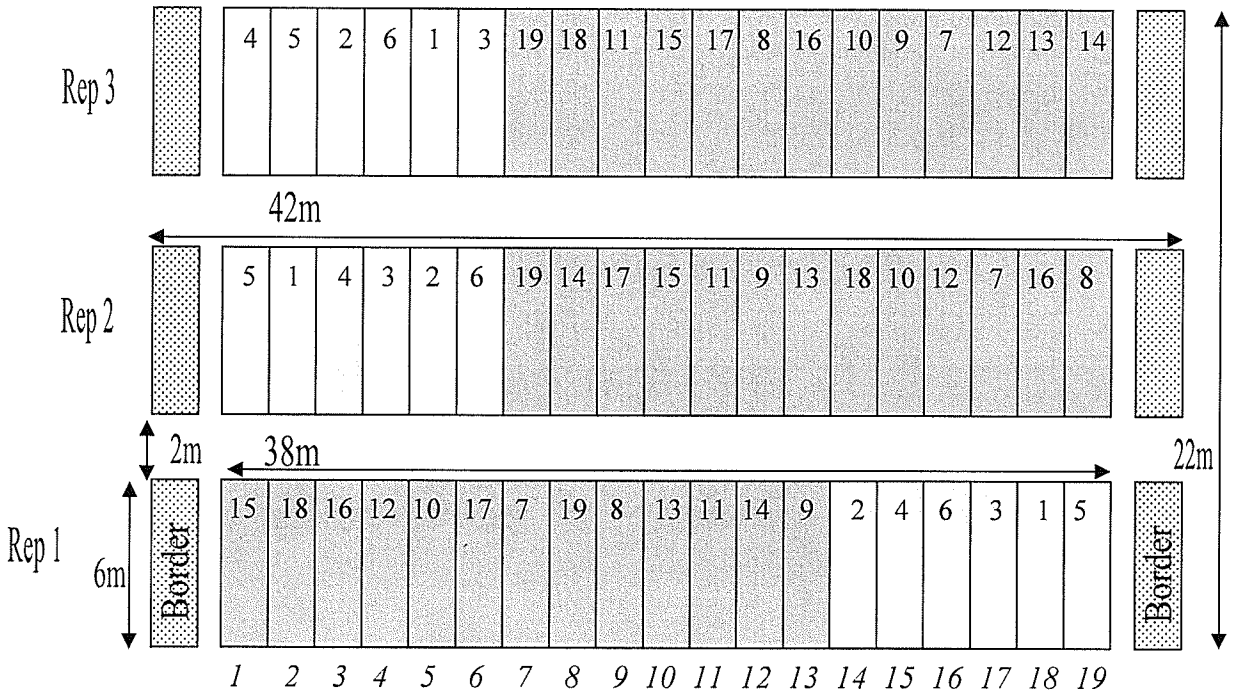

Figure 2.14: Field trial layout randomizations with 19 treatments and 3 replicates. Hybrid plots are grouped together shown in yellow while inbred parent and check plots are grouped together shown in green. Oakville 2007 site randomizations shown.

3.4 Seeding

The plots were mechanically seeded using an eight row R-Tech belt cone plot seeder. The eight rows include six plot rows 20 cm apart with two border rows set 30 cm outside the six row plot on each side. All entries in the yield trials were seeded at 1800 seeds per plot live seed or 1,875,000 seeds per hectare. Six metre plots were seeded initially with a metre cut back after emergence to ensure uniform plot length. The total area seeded in the plot was 6 m x 1.2 m = 7.2 m^2 (0.00072 ha). Seeding discs were set to place the seed between 1.5 and 2.5 centimetres depth depending on the soil conditions.

The seed of all entries was treated with Helix Xtra (containing the insecticide thiamethoxam and the fungicides difenoconazole, metalaxyl-M and fludioxonil) to control flea beetles (*Phyllotreta crucifera Goeze* and *P. striolata F.*) and seedling diseases on canola. Fertilizer was applied using field scale equipment prior to seeding by the grower. Fertilizer rates varied from site to site depending on soil test results; post-application soil fertility was that to produce a crop yield of 3 ton/hectare.

3.5 Site Maintenance

Weeds were controlled by herbicides, with 1 to 2 applications used depending on weed pressure. Since some of the inbred lines were not resistant to Roundup herbicide, the Roundup Ready system could not be utilized. Instead the trials were sprayed with a tank mix of Poast Ultra (Sethoxydim, emulsifiable concentrate at 450 g/L), Muster (Ethametsulfuron methyl, dry flowable at 75% concentration) and Lontrel (clopyralid, soluble concentrate at 260 g/L) to control the weeds. In 2008 intense weed pressure caused the abandonment of our trial at Vanscoy, Saskatchewan. There was also strong weed pressure in 2008 at the North Battleford site; the weeds were eventually eliminated; however, plant stand and size was decreased. The site was retained despite the damage.

Tillage operations were also utilized if necessary around the perimeter of the trial and through the alleyways between replicates. This was performed using a small plot cultivator about two metres wide.

3.6 Additional Site Information

3.6.1 Oakville - 2007

The Oakville, Manitoba research farm, located directly south west of Oakville, was seeded on May 10, 2007. The seed was placed into moisture at about 2 cm depth. The ground was still quite moist due to the 2.5 cm of rain that had fallen 5 days earlier. The site had been seeded to wheat the previous year.

3.6.2 Swan Lake – 2007

Swan Lake was seeded on May 10, 2007 at a depth of 2 cm. The previous crop grown on this land was oats. The site location was located one mile east of Swan Lake. Due to the cold Manitoba spring of 2007, the trial at Swan Lake was slow to reach rosette stage.

3.6.3 Yorkton – 2007

The Yorkton, Saskatchewan site was seeded on location at the research farm, located 1 km south east of Yorkton, on May 12, 2007. The seed was placed into moisture at about 2 cm depth. The site had been summerfallow in 2006. During seeding, two plots were interchanged placing a plot from rep two into rep three and subsequently a plot from rep three into rep two. The error was documented and the adjustment was carried through until harvest without further complications.

3.6.4 Saskatoon – 2007

The Saskatoon Saskatchewan research site was seeded on the research farm, located approximately 7 km north east of Saskatoon, on May 14, 2007. The seed was placed 2.5 cm into the soil which was down to moisture as it had rained over 1 cm earlier in the week. The site had been seeded to wheat the previous year.

3.6.5 Leduc – 2007

The Leduc Alberta trial site was seeded at a satellite site located directly north of the Edmonton airport on May 12, 2007. The seed was placed 2.5 cm into the soil which was down to moisture. The site had been seeded to wheat the previous year.

3.6.6 Barrhead – 2007

The Barrhead Alberta site was located 5km north of town and seeded on May 21, 2007. Seeding depth was about 2.5 cm down to soil moisture.

3.6.7 Swan Lake – 2008

The Swan Lake site was seeded on May 15 at a depth of 2.5 cm. The previous crop was oats. The site was located one mile north west of Swan Lake.

3.6.8 Yorkton – 2008

The Yorkton site in 2008 was located about 1 mile north of the previous site and 2 miles east of Yorkton on highway 10. The site was seeded on May 19 at a depth of 2.5 cm, into soil moisture.

3.6.6 Saskatoon – 2008

The Saskatoon site in 2008 was located adjacent to the previous year's site on the Monsanto Saskatoon research farm. The site was seeded on May 20 at a depth of 2.5 cm, into soil moisture.

3.6.6 North Battleford – 2008

The North Battleford site in 2008 was located about 3 miles north west of Denholm, half a mile north of highway 16. The site was seeded on May 22 at a depth of 2 cm, into soil moisture.

3.7 Agronomic Traits

Selected agronomic traits were measured on hybrid, parent and open pollinated lines at all locations. These agronomic traits included: vigour, number of days to 50% flower, uniformity, plant height, number of days to maturity and preharvest lodging.

Plant vigour was rated at the 4 to 5 leaf stage of growth on a scale from 1-9 (1 being low, 9 being high) and is a subjective measurement. Size and height of the plants as well as ground area cover was taken into consideration. Rows which consisted of large plants covering a large ground area were rated as 9 while rows which consisted of small plants were rated as 1.

Plot uniformity was a general subjective measurement made to check that all the treatments emerged simultaneously and distributed evenly throughout the plot. This rating also made it possible to note if weed pressure had hindered growth in some areas of the plot resulting in poor uniformity.

Days to 50% flower was measured from planting to when 50% of the plants in a plot had started flowering. This rating requires constant attention as the variation in initiation of flower across the treatments can be high. This measurement is similar to days to first flower, however, is less biased as it takes into account an average of the plants in a particular plot.

Plant height was measured after flowering was completed. For each plot, five plants were randomly selected and measured and an average of the five was taken. The heights were taken using a two metre long stick with measured increments every centimetre.

Plant maturity was measured as the number of days from planting to when half of the plants in the plot were physiologically mature. Maturity is measured subjectively using the common method of seed colour change. When 30-40% of the seed on the main raceme has changed colour from green to brown or black, the plant is considered to be physiologically mature.

Lodging resistance was taken just prior to harvest on a scale of 1 to 9; 9 being no lodging and 1 being completely flat. Canola is known to 'lean', meaning all the plants in a given area may be somewhat on a slant. We did not identify this common occurrence as lodging.

In 2007, the trials were swathed using an R-Tech swather at maturity and left to ripen prior to being combined. Unfortunately, due to logistics and time, each treatment could not be swathed individually at optimum time thus all were cut when the majorities of the plots were physiologically mature. In 2008 the trials were not swathed. Each plot was desiccated

using Reglone at physiological maturity and straight-cut harvested a week later using a Wintersteiger combine harvester. Fifty gram seed samples for each treatment were taken for seed quality analyses during harvest using the sample collector on the combine.

3.8 Seed Quality

The seed quality traits, oil, protein, sum of oil and protein and glucosinolate content as well as saturated fatty acid content in the oil were measured on a 50 g sub-sample of each plot. Oil, protein and glucosinolate content was measured using near infrared reflectance spectroscopy (NIR) using a Foss 6500 system (Daun et al., 1994, DeClercq, 2005). The measurements were calibrated to 0% moisture. The sum of oil and protein content was calculated through the addition of the NIR results. Saturated fatty acid content was determined through gas chromatography of methyl esters of fatty acids (DeClercq, 2005) using Monsanto laboratories in which all three reps per trial were tested. The results from these tests were used to calculate mid-parent, high-parent and commercial heterosis for each trait.

3.9 Data Analysis

Data on nine agronomic traits (days to emergence, vigour, plot uniformity, days to 50% flower, days to maturity, plant height and pre-harvest lodging) and five seed quality characteristics (oil content, protein content, sum of oil and protein content, moisture content and glucosinolate content) were collected on all entries at all sites using both NIR and wet chemistry techniques. Protein content was calculated by the percent of protein in the meal, excluding oil content. The sum of oil and protein content was calculated by taking the total oil content and adding to the percent protein in the meal. The seed

analyses were done at Monsanto laboratories in Winnipeg, MB. The design of the yield trails permitted the determination of mid-parent, high-parent and commercial heterosis for all agronomic and seed quality traits.

3.9.1 Statistical Analysis

Trial data was collected and entered into Microsoft Excel spreadsheets compatible with the statistical analysis software program SAS (Statistical Analysis Software). The trial data included agronomic and seed quality traits from a total of 10 sites collected over two years. Individual trials were analyzed using a randomized complete block (RCB) design in SAS using a mixed model method. In our analysis, each site year is considered its own environment, resulting in 10 separate environments and thus being labeled individually as locations. This approach was taken since many sites were not repeated over the two years, and most of those which were, were over three kilometers away. Saskatoon was the only true site which had trials for grown in both years.

Because a mixed model was used in the study, we selected to use the mixed procedure (Proc Mixed) in SAS. A mixed model was selected due to the study containing both fixed and random effects. There are a few notable benefits to using Proc Mixed over the traditional Proc GLM (general linear model). For instance, Proc Mixed is able to handle missing values; the data in our study was incomplete, missing entire fields of data at a particular location. Proc Mixed is also known to be better at handling multiple locations. In addition, Proc Mixed accommodates for unequal or heterogeneous variances, which negates the second assumption of ANOVA. Unlike Proc GLM which uses least of sum of squares residuals to fit data to a model, Proc Mixed uses maximum

likelihood which offers a way of tuning the free parameters of the model to provide a good fit.

The statistical model:

$$Y_{ikj} = \mu + Entry_i + Rep_k + Location_j + (Variety \times Location) + e_{ikj}$$

Where:

Y_{ikj} = trait
μ = population mean
$Entry_i$ = 19 entries for each environment
Rep_k = replications within a environment
Location = replications of the basic design

e_{ikj} = residual

Within the model, variables were grouped as being fixed (variety, year and breed which sorted between hybrid, inbred and commercial open pollinated) or being random (rep, location and variety by location).

This particular study was designed to determine heterosis between *B. napus* hybrids and their inbred parent lines. To assess heterosis, significant difference between the mean of the hybrids over the mean of the parents, least squares means were calculated producing single degree of freedom estimates that compared the hybrid group with the inbred group, the hybrid group with the commercial open pollinated group, and the inbred group with the commercial open pollinated group. These estimates were also used to calculate mid-parent heterosis between each hybrid and its particular inbred parents. Due to high-parent and commercial heterosis only using the better of the two parents,

different methods were used to calculate statistical difference. Individual hybrids were manually compared to their better inbred parent using the least squared means calculated by SAS. The p-value of each test assessed significant difference of each comparison.

3.9.2 Heterosis Calculations

Heterosis is traditionally calculated using three different methods, mid-parent, high-parent and commercial. Mid-parent, also known as relative heterosis is used to assess basic heterosis of a particular trait. We hypothesized that the individual hybrid would have a positive mid-parent heterosis for a particular individual trait such as oil content over the mean of its inbred parents. This was done by comparing the phenotypic value of the hybrid to the average of the phenotypes of the parents. In this study both positive and negative heterosis was calculated; positive heterosis referred to the hybrid(s) being higher than that of the inbreds, negative heterosis being the opposite. Positive heterosis in traits such as oil and protein content can be beneficial to human production; however, negative heterosis in traits such as saturated fatty acid content or glucosinolate content could also be beneficial. Mid-parent heterosis was calculated using the formula:

$$\frac{\overline{F_1} - \overline{MP}}{\overline{MP}} \times 100 = \% \text{ MP Heterosis}$$

F_1 = hybrid combination
MP = mean of the two parental cultivars/lines of the F_1 hybrid combination

High-parent is used to assess if the hybrid exceeded both of the parents for a particular trait. This is done by comparing the value of the hybrid to the best of its parents. If a hybrid does in fact exhibit high parent heterosis its performance exceeds the performance of the best parent thus being a step forward in plant improvement. High-parent heterosis can be calculated by using the formula:

$$\frac{\overline{F_1} - \overline{HP}}{\overline{HP}} \times 100 = \% \text{ HP Heterosis}$$

F_1 = hybrid combination
HP = mean of the better of the two parental cultivars/strains of the F_1 hybrid combination
*(when dealing with days to 50% flower and days to maturity the earlier parent was used)

Commercial heterosis, also known as standard heterosis, is not a genetic based calculation. It is used to compare a hybrid with a current commercial standard, such as a leading open pollinated variety. In this study, the commercial check used was specific to the trait in question; the highest performing variety for the trait was selected to act as the commercial check. Highest performing is dependent on the desirable direction of the trait (e.g. increase in oil content or decrease in saturated fatty acid content). Commercial heterosis was calculated using the formula:

$$\frac{\overline{F_1} - \overline{OPP}}{\overline{OPP}} \times 100 = \% \text{ Commercial Heterosis}$$

F_1 = hybrid combination
OPP = the best OPP cultivar for that specific trait

Results and Discussion 4.0

4.1 Agronomic Traits

4.1.1 Seedling Vigour

Hybrid vigour accurately describes the vigour seen in these trials. It was easy to distinguish between hybrids and inbreds in observations of plants from emergence to the rosette stage (Figure 4.1). The hybrids H1, H2, H3, H4 and H5 were all different from the mean of their parental lines (Table 4.1). Hybrid H6 was not different in vigour from the mean of its parental lines because its female parent line had extremely strong vigour at 6.5. The hybrid mean, was 6.7 on a scale of 1 to 9 with 1 being poor and 9 being excellent vigour. Collectively, the hybrid group mean vigour rating of 6.7 was greater than the inbred parent's mean vigour rating of 5.1. The hybrid with the strongest vigour was H3 with a mean of 7.0, while the H4 was the weakest hybrid at 6.2. The inbreds on the other hand had a maximum vigour rating of 6.5 (H6P1) and a minimum vigour rating of 4.3 (H1P1). The open pollinated varieties had a mean vigour rating of 6.2, intermediate between the hybrids and inbreds with 32-75 the highest at 6.5 and 34-65 the lowest at 6.0.

Hybrid H4, which has the lowest vigour rating of all the hybrids, also had low vigour rating inbred parents, 2 of the lowest 3. However, the parents of the most vigourous hybrid for vigour, H3, were not top performing inbred lines for vigour ratings. In fact, H3P1 and H3P2 were low vigour rating inbred lines as well. Consequently, H3 had extremely strong mid-parent and high-parent heterosis for vigour ratings, 41.70% and 36.45% respectively. In contrast, H6 had strong performing inbred parents for vigour

ratings and in which it also displayed strong vigour (6.8). Since H6P1 (6.5) and H6P2 (5.25) both had excellent vigour ratings, heterosis for vigour ratings for H6 was restricted to 16.26% and 5.80% for mid-parent and high-parent respectively (Figure 4.2). Therefore, inbred parents with high vigour ratings do not ensure that high levels of heterosis for vigour ratings will be observed in their derived hybrids. It is curious to note that H6 shares its male parent with H1 and H2 which, along with H6 perform equally well for vigour ratings (6.8, 6.9, 6.8). Each of their female parents, however, is quite different for vigour ratings, 4.3, 5.3 and 6.5 for H1P1, H2P1 and H6P1 respectively. Observations suggest that there may be a trend between male parent vigour and hybrid vigour. Vigour was significantly correlated to days to 50% flower (-0.76)*** (Appendix Table A2). The early flowering could be the result of strong early season vigour in the hybrids; allowing these plants to access resources faster and thus speeds up their phenology.

The open pollinated check cultivars used in this study had excellent vigour ratings, 6.5, 6.1 and 6.0 for 32-75, 34-55 and 34-65 respectively. The open pollinated check cultivars were statistically similar to the hybrids in vigour ratings. Therefore, the levels of commercial heterosis for vigour ratings for the hybrids were quite small (Table 4.1).

Figure 4.1: Heterosis for vigour ratings. Parent H3P1 (left), Hybrid H3 (middle), Parent H3P2 (right). Photographs also provide information on ground area coverage and uniformity between treatments. Rep 1, Barrhead, 2007.

Environmental factors play a large role in seedling vigour and establishment. Cool, dry springs result in low vigour ratings in contrast with warm, wet springs aiding in the seedlings early growth and resulting in high vigour ratings. Lippman and Zimir (2007) state the following regarding heterosis for vigour ratings, genotype and environment, "magnitude of effect and significance of heterosis will vary between years, given the many phenotypes and loci involved and the influence on them of the environment".

Vigour ratings compared across environments were higher in Alberta than in other environments in 2007 (Table 4.2). Seedling vigour ratings are subjective and are a product of soil temperature, soil moisture and available nutrients early in the growing season. However, early season rainfall and temperature between environments in 2007 did not vary substantially between environments preventing the synthesis of an explanation for the differing plant vigour ratings.

Table 4.1: Mean seedling vigour ratings, mid-parent, high-parent and commercial heterosis in 10 locations in 2007 and 2008[+]

Cultivar	Mean Vigour Rating (1-low, 9-high)	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	6.8	1.9	41.8**	29.3**	11.3	-1.8704	-4.03	<.0001
H2	6.9	1.7	30.1**	29.1**	12.8	-0.9537	-2.06	0.0417
H3	7.0	1.8	41.7**	36.5**	14.8	-1.7963	-3.87	0.0002
H4	6.2	2.1	37.1**	31.0*	1.2	-1.2315	-2.65	0.0089
H5	6.5	2.1	27.4**	25.0*	5.9	-1.0926	-2.35	0.0199
H6	6.8	1.7	16.3	5.1	12.0	-0.1204	-0.26	0.7957
Average	6.7	1.9	32.4	26.0	9.6			
Inbreds								
H1P1	4.3	2.2						
(H1, H2, H6) P2	5.3	1.9						
H2P1	5.3	2.0						
H3P1	5.1	1.8						
H3P2	4.8	2.0						
H4P1	4.3	2.3						
H4P2	4.7	2.0						
H5P1	5.2	2.1						
H5P2	5.0	2.2						
H6P1	6.5	1.6						
Average	5.1	2.0						
Open Pollinated								
32-75	6.5	1.8						
34-55	6.1	1.8						
34-65	6.0	1.9						
Average	6.2	1.8						
Totals								
HY vs OP						0.5606	2.03	0.0572
HY vs IB						1.6917	8.41	<.0001

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

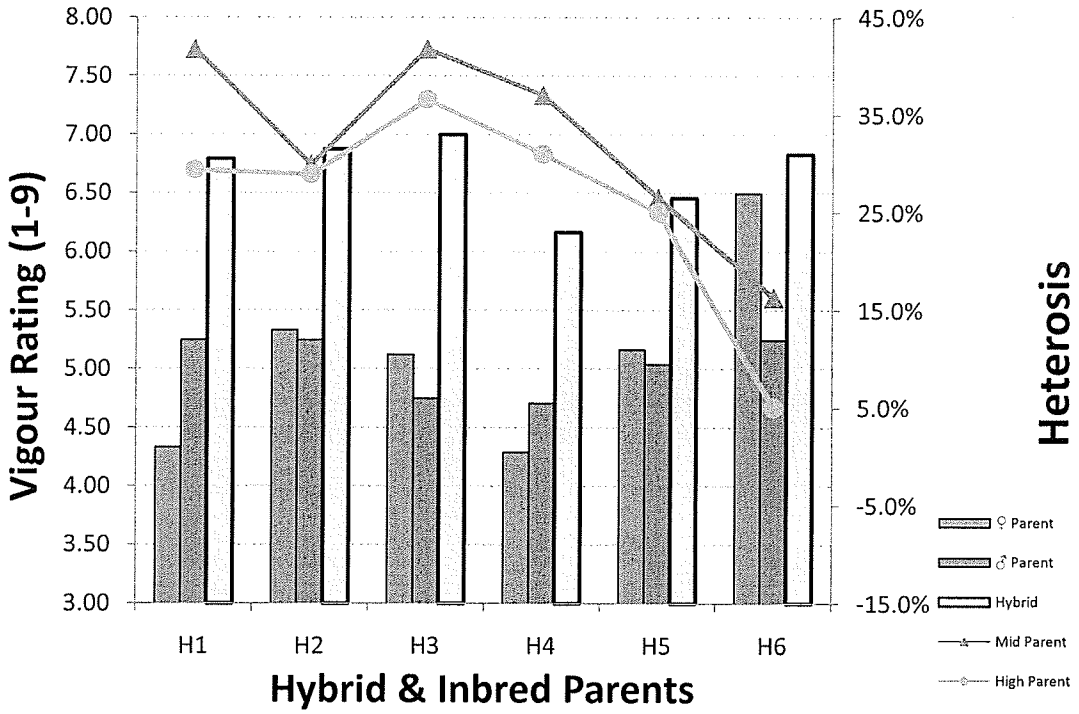

Figure 4.2: Mean vigour rating of inbred and hybrid means for each treatment (histogram) and mid-parent and high-parent heterosis (line graph).

Table 4.2: Location mean temperature and total rainfall (May to August) effects on vigour

Year	Location	Vigour	Rain (mm)	Temp. (°C)
2007	Barrhead	8.6	237.8	15.0
2007	Leduc	7.6	219.0	14.1
2007	Saskatoon		216.5	15.8
2007	Yorkton	2.9	238.3	15.8
2007	Swan Lake	4.4	282.4	16.8
2007	Oakville	5.9	228.9	15.8
2008	N. Battleford	5.7	272.6	15.1
2008	Saskatoon	4.8	183.0	15.3
2008	Yorkton	.	304.5	14.8
2008	Swan Lake	6.1	255.8	14.4

4.1.2 Uniformity

Uniformity is a rating style measurement used to compare treatments within the experiment to identify intraplot and interplot consistency. Often, non-uniform plots will most likely have emergence or plant stand issues which the uniformity rating can capture. The *B. napus* hybrids displayed higher uniformity on average (7.6), on a scale of 1 to 9 with 1 being poor and 9 being excellent uniformity, compared to the inbreds (6.7) and open pollinated varieties (7.4) (Table 4.3). There was mid-parent heterosis for uniformity at $\alpha = 0.01$ and high-parent heterosis for uniformity displayed by hybrids H1-H5 (at $\alpha =$ 0.05 or 0.01) (Figure 4.3). These results are most likely due to the high vigour and rapid emergence observed for the hybrids resulting in the apparent uniformity. Therefore, uniformity was strongly correlated to vigour (0.92)*** (Appendix Table A2). The hybrids ranged from a minimum of 7.4 (H4) to a maximum of 7.8 (H1) for uniformity ratings while the inbreds ranged from a minimum of 6.4 (H5P1) to a maximum of 7.2 (H6P2) for uniformity ratings.

Environment (α=0.05) and environment by variety (α=0.01) were both significant effects for uniformity. Uniformity was also positively correlated to oil content (0.46)* and negatively correlated to days to 50% flower (-0.58)**. These results suggest that F_1's with higher hybrid vigour may also display heterosis for early flowering and oil content.

Table 4.3: Mean uniformity ratings and mid-parent, high-parent and commercial heterosis estimates for hybrids, inbreds and OP cultivars grown in 10 environments in 2007 and 2008[+]

Cultivar	Mean Uniformity Rating (1-low, 9-high)	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	7.8	1.4	17.24**	14.02**	5.65	-1.8704	-4.03	<.0001
H2	7.6	1.1	9.64**	8.33*	2.82	-0.9537	-2.06	0.0417
H3	7.7	1.1	17.09**	14.91**	4.52	-1.7963	-3.87	0.0002
H4	7.4	1.2	11.95**	10.56**	0.56	-1.2315	-2.65	0.0089
H5	7.5	1.3	14.87**	13.29**	1.13	-1.0926	-2.35	0.0199
H6	7.5	1.2	7.74*	5.23	2.26	-0.1204	-0.26	0.7957
Average	7.6	1.2	13.1	11.1	2.8			
Inbreds								
H1P1	6.5	1.6						
(H1, H2, H6) P2	6.8	1.5						
H2P1	7.0	1.4						
H3P1	6.5	1.6						
H3P2	6.7	1.5						
H4P1	6.5	1.5						
H4P2	6.7	1.6						
H5P1	6.4	1.5						
H5P2	6.6	1.7						
H6P1	7.2	1.3						
Average	6.7	1.5						
Open Pollinated								
32-75	7.4	1.2						
34-55	7.5	1.2						
34-65	7.4	1.3						
Average	7.4	1.2						

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

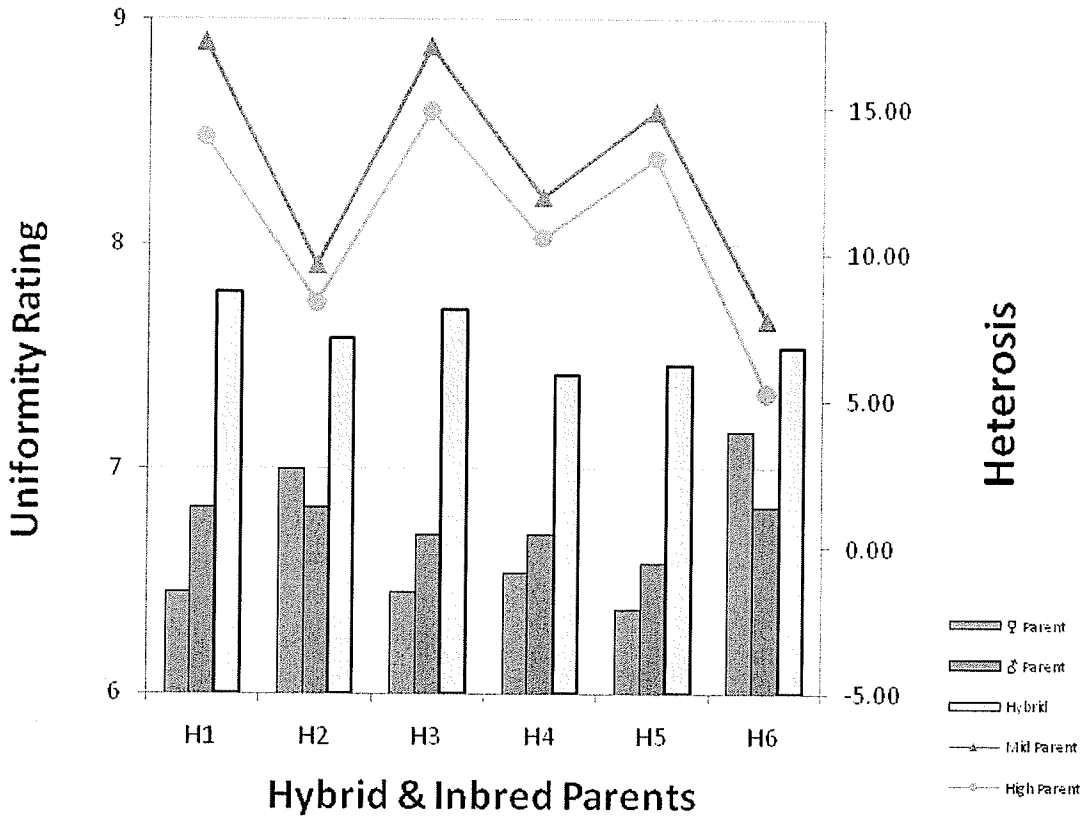

Figure 4.3: Mean uniformity ratings for inbred and hybrid treatments (histogram) and mean mid-parent and high-parent heterosis (line graph).

4.1.3 Plant Height

The hybrids were taller on average (105.9 cm) than the inbreds which averaged 98.5 cm (Table 4.4). The tallest hybrid was H4 with a mean height of 113.1 cm; the lowest was H2 with a mean height of 100.3 cm. The mean height of the inbreds was skewed higher because of tall inbred lines such as H4P2 which had the maximum height of any entry in these trials at 114.7 cm. Inbred line H2P1 was the shortest height entry in these trials at only 90.4 cm which created a large variance for height within the inbred line group. This is understandable as inbred lines are often selected for particular traits

while others such as height are ignored. Only H3 and H4 did not show significant mid-parent heterosis for height; which was most likely due to both H3 and H4 having one or more particularly tall parents. That being said, although heterosis can be observed for plant height (Figure 4.4), tall height does not confer to heterosis for seed yield or other agronomic or seed quality traits.

Mid-parent and high-parent heterosis for height was found within these parent-hybrid combinations although it was most likely not selected for. Even though tall, vigorous, plants do have 'curb appeal' that attracts growers, shorter plants may have higher resistance to lodging. Breeders select for seed yield, disease resistance and other high priority traits and do not focus on plant height.

Environment effect was significant for plant height at $\alpha = 0.05$. Variety by environment or VxE was significant at $\alpha = 0.0001$, suggesting a strong VxE effect. The variability of the means was quite high, especially for the hybrids (13.8 cm) which was greater than observed for the inbreds (11.9 cm). The open pollinated cultivars had the largest variation in height of 15.4 cm which could be due to their heterogeneous genetic makeup affecting height. Because inbred lines and hybrids developed from these inbred lines have theoretically homogeneous genotypes, their individual phenotypes should also be identical given the environment is constant. Open pollinated cultivars are populations and differ slightly in genotype. When comparing plant height levels to rainfall and average temperature, no trend appears evident (Table 4.5). Height also had no trend to geography from east to west.

Table 4.4: Mean plant height (cm) and mid-parent, high-parent and commercial heterosis estimates for hybrids, inbreds and OP cultivars grown in 10 environments in 2007 and 2008[+]

Cultivar	Mean Plant Height (cm)	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	106.3	14.4	10.34*	5.63*	14.66	-1.8704	-4.03	<.0001
H2	100.3	13.6	9.86*	8.89*	8.11	-0.9537	-2.06	0.0417
H3	105.3	14.1	4.33*	-0.66	13.58	-1.7963	-3.87	0.0002
H4	113.1	14.4	3.04	-1.42	21.92	-1.2315	-2.65	0.0089
H5	103.1	12.5	6.68*	5.69*	11.18	-1.0926	-2.35	0.0199
H6	107.4	14.0	11.30*	6.42*	15.81	-0.1204	-0.26	0.7957
Average	105.9	13.8	7.6	4.1	14.2			
Inbreds								
H1P1	100.7	14.8						
(H1, H2, H6) P2	92.1	11.9						
H2P1	90.4	12.1						
H3P1	95.9	12.8						
H3P2	106.0	14.6						
H4P1	104.8	15.3						
H4P2	114.7	15.3						
H5P1	97.6	13.0						
H5P2	95.2	10.9						
H6P1	100.9	11.4						
Average	98.5	11.9						
Open Pollinated								
32-75	92.7	15.0						
34-55	108.2	15.8						
34-65	107.7	15.3						
Average	102.9	15.4						
Totals								
HY vs OP						3.0308	2.21	0.0287
HY vs IB						6.0938	6.09	<.0001

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at $Pr < 0.05$
**Significantly higher at $Pr < 0.01$

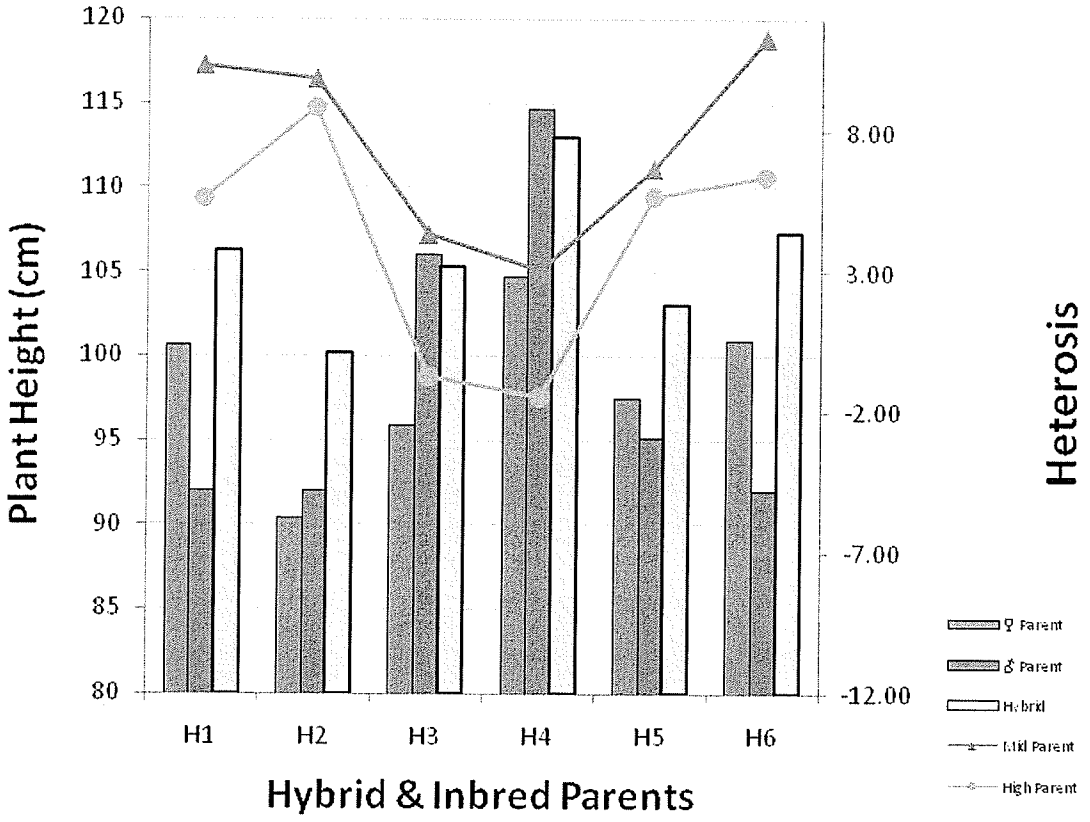

Figure 4.4: Mean plant heights of inbreds and hybrids for each treatment (histogram) and mid-parent and high-parent heterosis (line graph).

Table 4.5: Location mean temperature and total rainfall (May to August) effect on plant height (cm)

Year	Location	Height (cm)	Rain (mm)	Temp. (°C)
2007	Barrhead	101.8	237.8	15.0
2007	Leduc	99.1	219.0	14.1
2007	Saskatoon	119.0	216.5	15.8
2007	Yorkton	114.8	238.3	15.8
2007	Swan Lake	97.5	282.4	16.8
2007	Oakville	.	228.9	15.8
2008	N. Battleford	81.8	272.6	15.1
2008	Saskatoon	93.9	183.0	15.3
2008	Yorkton	116.9	304.5	14.8
2008	Swan Lake	95.3	255.8	14.4

4.1.4 Days to 50% Flower

Hybrids on average flowered 51.8 days after seeding, with a range of 51.1 to 53.7 days. In contrast, the inbreds flowered slightly later, 51.3 days to 50% flower for the earliest line (H2P1) and 54.7 days for the latest (H4P1) average 53.1 days, later than the hybrids on average (Table 4.6). Cultivar 32-75, an early maturing commercial open pollinated cultivar, reached 50% flower in only 50.9 days, while 34-55 and 34-65 started flowering at 52.3 and 52.2 days respectively. Hybrids H1 to H5 flowered earlier than their parents, confirming the reports of Sernyk and Stefansson (1983) and Grant and Beversdorf (1985), who reported that hybrids flowered earlier than parents in their studies. Hybrids H1-H5 were earlier flowering than their parental lines, and displayed both mid-parent and high-parent heterosis for days to flower (Figure 4.5). Due to the OP check cultivar 32-75 being early to flower, no hybrids showed commercial heterosis. These findings are contradictory to some previous studies (Starmer et al., 1998) which found no significant difference in flowering date. In addition, they found the length of flowering (not conducted in our study) to be significantly longer in the hybrids than inbreds, resulting in the hybrids maturing later than the inbreds. Days to 50% flower was positively correlated with days to maturity (0.76)*** (Appendix Table A2) suggesting that the offset of time between seeding and flowering continued to maturity. Days to 50% flower was also positively correlated to glucosinolate content (0.54)*, as days to flower increased, glucosinolate content increased.

Environment effect for days to 50% flower was significant at $\alpha = 0.05$ and environment by variety was also significant at $\alpha = 0.0001$. Saskatoon 2007 fell outside

the range of the environment mean, 52.4 for days to 50% flower, taking an average of 67.3 days to flower (Table 4.7).

Table 4.6: Mean days to 50% flower, mid-parent, high-parent and commercial heterosis estimates for hybrids, inbreds and OP cultivars grown in 10 environments in 2007 and 2008[+]

Cultivar	Mean Days to 50% Flower	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	51.7	5.4	-3.49**	-1.48	1.68	-1.8704	-4.03	<.0001
H2	50.9	5.0	-1.84*	-0.72	0.18	-0.9537	-2.06	0.0417
H3	51.2	5.8	-3.39**	-1.67	0.76	-1.7963	-3.87	0.0002
H4	53.7	6.2	-2.24**	-1.80	5.57**	-1.2315	-2.65	0.0089
H5	51.1	5.5	-2.06*	-1.92	0.44	-1.0926	-2.35	0.0199
H6	52.0	5.5	-0.23	0.50	2.22*	-0.1204	-0.26	0.7957
Average	51.8	5.6	-2.2	-1.2	1.8			
Inbreds								
H1P1	54.7	6.9						
(H1, H2, H6) P2	52.5	5.8						
H2P1	51.3	5.4						
H3P1	52.1	5.4						
H3P2	54.0	6.2						
H4P1	55.2	6.3						
H4P2	54.7	6.8						
H5P1	52.1	6.0						
H5P2	52.3	5.2						
H6P1	51.7	4.9						
Average	53.1	5.9						
Open Pollinated								
32-75	50.9	5.0						
34-55	52.3	5.6						
34-65	52.2	5.7						
Average	51.8	5.4						
Totals								
HY vs OP						-0.01236	-0.05	0.9633
HY vs IB						-1.271	-6.5	<.0001

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

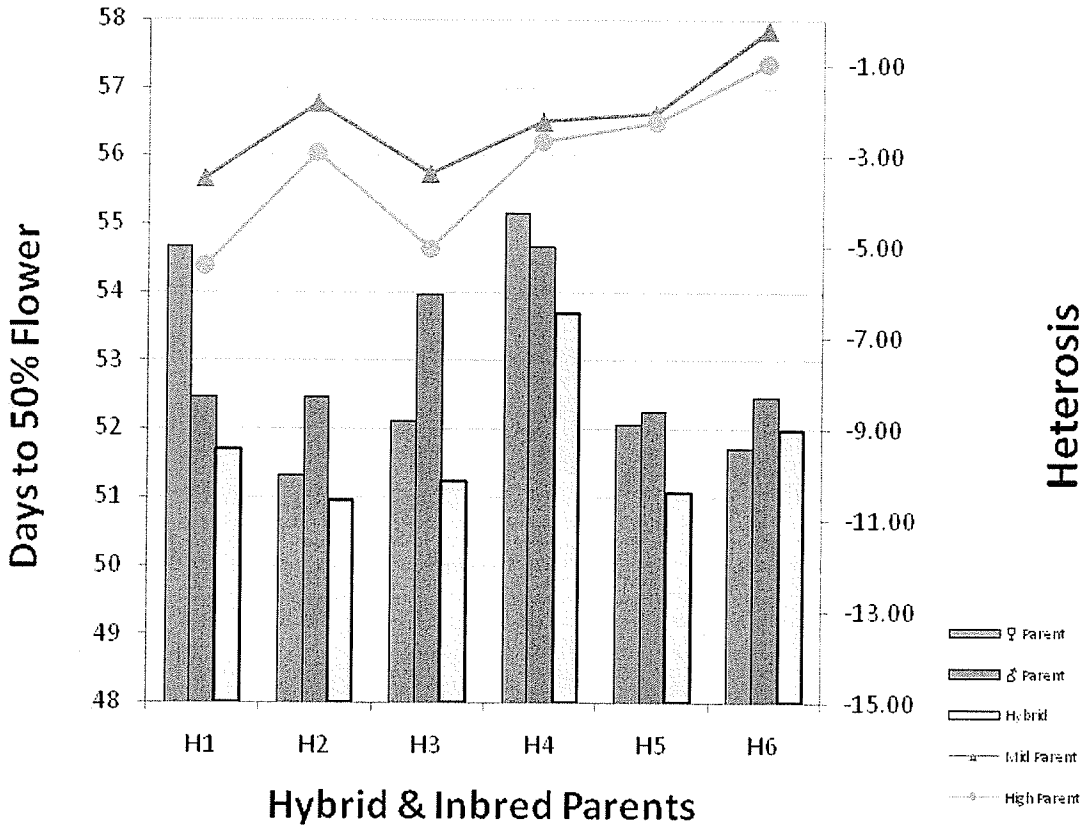

Figure 4.5: Mean days to 50% flower of inbred and hybrid for each treatment (histogram) and mid-parent and high-parent heterosis (line graph).

Table 4.7: Location mean temperature and total rainfall (May to August) effect on days to 50% flower

Year	Location	Days to Flower	Rain (mm)	Temp. (°C)
2007	Barrhead	47.3	237.8	15.0
2007	Leduc	50.5	219.0	14.1
2007	Saskatoon	67.3	216.5	15.8
2007	Yorkton	.	238.3	15.8
2007	Swan Lake	52.0	282.4	16.8
2007	Oakville	52.0	228.9	15.8
2008	N. Battleford	48.4	272.6	15.1
2008	Saskatoon	50.9	183.0	15.3
2008	Yorkton	50.9	304.5	14.8
2008	Swan Lake	52.7	255.8	14.4

4.1.5 Days to Maturity

Hybrids on average were earlier maturing (95.5 days) than their inbred parents (97.0 days) (Table 4.8). The earliest variety maturity was a hybrid, H2 (94.8 days) while H4 was the latest hybrid (96.7 days). The earliest of the inbred parents was H5P2 (94.9 days) while the latest was H4P2 (100.0 days). Hybrids H1, H2, H3 and H4 all showed mid-parent and high-parent heterosis while hybrids H5 and H6 did not show heterosis and were similar to their parents (Figure 4.6). Similarly to days to flower, 32-75, an early maturing commercial open pollinated cultivar reached maturity on average in just 94.9 days, slightly later than H2. This eliminated any chance of commercial heterosis. The other open pollinated varieties reached maturity at 96.7 and 97.6 days for 34-55 and 34-65 respectively. The means of the open pollinated varieties for days to maturity was not different from other hybrids or inbreds.

Days to maturity was significantly positively correlated to days to 50% flower (0.76)*** (Appendix Table A2), which agrees with the findings of Cuthbert (2006) and Grant and Beversdorf (1985). This correlation confirms that the time gained early in the season, between seeding and flowering continues through to maturity. Maturity was negatively correlated to vigour (-0.61)** relating higher vigour to early maturity. As well, maturity was positively correlated to height (0.51)*, as taller plants matured later.

Days to maturity had significant location, variety and location by variety effects. A noticeable trend between maturity and geographical region was apparent, generally latest trial sites maturing in the west, earliest in the east (Table 4.9). These results are

most likely due to the lack of growing degree days in central Alberta as compared to southern Manitoba. Differences in rainfall or temperature did not seem to have any effect.

Table 4.8: Mean days to maturity, mid-parent, high-parent and commercial heterosis in 10 environments in 2007 and 2008[+]

Cultivar	Mean Days to Maturity	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	95.0	3.0	-1.78	-1.46	0.16	-1.8704	-4.03	<.0001
H2	94.8	3.3	-1.69	-1.69	-0.08	-0.9537	-2.06	0.0417
H3	94.9	3.2	-2.75**	-0.51	0.00	-1.7963	-3.87	0.0002
H4	96.7	3.7	-2.14*	-0.99	1.99	-1.2315	-2.65	0.0089
H5	95.6	3.3	-0.23	0.74	0.78	-1.0926	-2.35	0.0199
H6	96.3	3.6	-0.12	-0.08	1.56	-0.1204	-0.26	0.7957
Average	95.5	3.4	-1.5	-0.7	0.7			
Inbreds								
H1P1	97.0	3.9						
(H1, H2, H6) P2	96.4	4.0						
H2P1	96.2	4.0						
H3P1	95.3	5.1						
H3P2	99.7	4.7						
H4P1	97.7	4.5						
H4P2	100.0	4.3						
H5P1	96.7	3.7						
H5P2	94.9	4.1						
H6P1	96.5	3.4						
Average	97.0	4.2						
Open Pollinated								
32-75	94.9	3.2						
34-55	97.6	4.0						
34-65	96.7	3.7						
Average	96.4	3.7						
Totals								
HY vs OP						-0.8487	-1.77	0.0933
HY vs IB						-1.5347	-4.39	0.0004

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

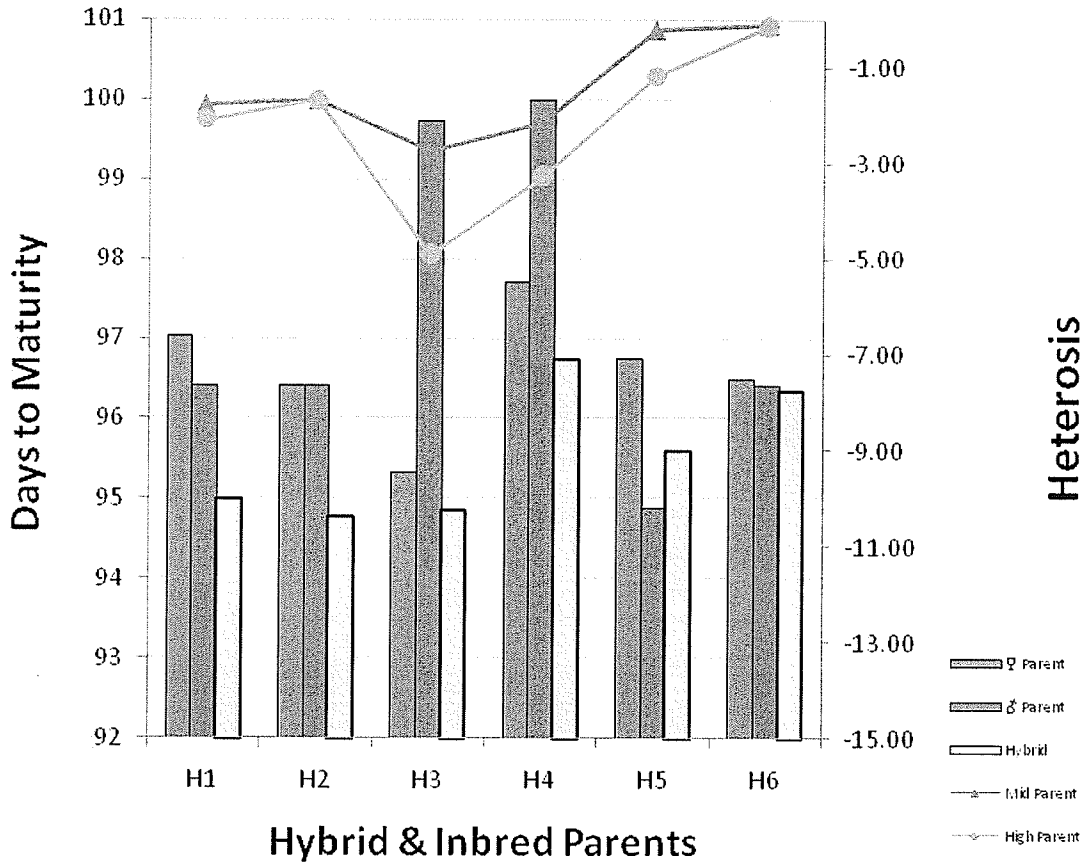

Figure 4.6: Mean days to maturity of inbred and hybrid for each treatment (histogram) and mid-parent and high-parent heterosis (line graph).

Table 4.9: Location mean temperature and total rainfall (May to August) effects on days to maturity

Year	Location	Days to Maturity	Rain (mm)	Temp. (°C)
2007	Barrhead	100.8	237.8	15.0
2007	Leduc	98.6	219.0	14.1
2007	Saskatoon	88.1	216.5	15.8
2007	Yorkton	94.4	238.3	15.8
2007	Swan Lake	95.4	282.4	16.8
2007	Oakville	-	228.9	15.8
2008	N. Battleford	98.1	272.6	15.1
2008	Saskatoon	98.3	183.0	15.3
2008	Yorkton	97.9	304.5	14.8
2008	Swan Lake	96.6	255.8	14.4

4.1.6 Preharvest Lodging

Preharvest lodging resistance ratings were taken at each site. At all 10 sites, no preharvest lodging was reported. These results could be a combination between the standability of these plants and the lack of environmental pressure. Some plots developed a 'lean', however, we did not categorize this as preharvest lodging as it did not affect crop performance or ease of harvest.

4.2 Seed Quality Traits

4.2.1 Oil Content

Mean oil content in the hybrids was higher than in the parental lines and significantly so for five of six hybrids (H1-H5). The ranges of oil contents were relatively small (σ =1.2), as the hybrids remained close to the average of 46.81% with a minimum of 45.74% in H6 to a maximum of 47.43% in H5. The parents had lower oil content on average than the hybrids at 45.1%, ranging from minimum of 43.72% (H3P1) to a maximum of 47.14% (H6P1) (Table 4.10). Mid-parent heterosis was high for hybrids H1 through H5 and high-parent heterosis was also higher for hybrids H1 through H4 (Figure 4.7). Hybrid H5, however, did not display high-parent heterosis for oil content since one of its parents averaged 47.17%, nearly a percentage point above the next closest inbred. The open pollinated varieties with a mean of 45.20% performed slightly higher than the inbred parents but significantly poorer than the hybrids. Commercial heterosis was evident for all hybrids and the hybrids averaged over 1.6% oil content compared to the open pollinated varieties.

These results clearly show heterosis for oil content. This contradicts studies by Grant and Beversdorf (1985) and Sernyk and Stefansson (1983) which reported no heterosis for oil content in *B. napus* hybrids in their studies. In contrast, Cuthbert (2006) and Shen et al. (2005) did report heterosis for oil content. Cuthbert (2006) found mid-parent values at 11.9% of parental lines for oil content, however, also identified a large number of negative high-parent heterosis hybrids. Both Cuthbert (2006) and Shen et al. (2005) also found a positive correlation between oil content and seed yield.

As predicted, oil content is negatively correlated to protein content (-0.58) (Appendix Table A2). This confirms the inverse relationship between oil and protein content reported by Sernyk and Stefansson (1983) and Grant and Beversdorf (1985).

Table 4.10: Mean seed oil content, mid-parent, high-parent and commercial heterosis in 10 environments in 2007 and 2008[+]

Cultivar	Mean Seed Oil Content (%)	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	47.34	2.64	4.15**	2.51*	4.19**	-1.8704	-4.03	<.0001
H2	47.24	3.25	5.43**	5.24**	3.98**	-0.9537	-2.06	0.0417
H3	46.07	2.80	4.11**	2.87**	1.40	-1.7963	-3.87	0.0002
H4	47.06	2.90	5.20**	3.49**	3.59**	-1.2315	-2.65	0.0089
H5	47.43	2.90	3.08**	0.62	4.41**	-1.0926	-2.35	0.0199
H6	45.74	2.84	0.97	-0.31	0.69	-0.1204	-0.26	0.7957
Average	46.81	2.89	3.8	2.4	3.0			
Inbreds								
H1P1	46.18	2.62						
(H1, H2, H6) P2	44.72	2.72						
H2P1	44.88	2.94						
H3P1	43.72	2.34						
H3P2	44.78	3.23						
H4P1	45.47	3.11						
H4P2	44.00	2.91						
H5P1	47.14	3.23						
H5P2	45.00	2.57						
H6P1	45.88	3.03						
Average	45.10	2.85						
Open Pollinated								
32-75	45.22	3.37						
34-55	45.43	2.65						
34-65	44.94	2.90						
Average	45.20	3.0						
Totals								
HY vs OP						1.6267	6.04	<.0001
HY vs IB						1.6378	8.33	<.0001

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

Oil content was affected by variety, environment and variety by environment effect. As with other studies, oil content differs between genotype ($\alpha = 0.001$) and environment ($\alpha = 0.001$). In addition, oil content had a significant genotype by environment effect meaning different genotypes preformed better or worse than others at

different locations. Genotype by environment effect in oil content can be seen with hybrid H2 and inbred H6P1. In Yorkton in 2007, H6P1 had higher oil content by 0.32%, however, in North Battleford in 2008 H6 had higher oil content by 2.86% (Table 4.11). This change in ranking between the two varieties in different locations is not solely due to genetics or environment, but also due to and interaction of the two. Without a detailed analysis of each environment and how particular genotypes react to that environment it is impossible to identify the causes of the interaction. The total rainfall over the summer had a negligible if any effect on oil content. Most sites received near historically average rainfall producing minimal variation in oil content between trials. The minimum rainfall was in Saskatoon in 2008 which received 183.0 mm, while the maximum rainfall was in Yorkton in 2008 which received 304.5 mm (Table 4.11). The lack of severe weather allowed the crop to grow and mature normally at all locations.

Average temperature had somewhat more of an effect on oil content. In 2007 average temperature was higher in the eastern region (Manitoba) and decreased moving west, which is consistent with the long term temperature averages for this region. Oil content across the sites increased moving from east to west, suggesting an inverse relationship with temperature. This observation ignores the oil content results from Swan Lake in 2007, where the trial was damaged by hail skewing results. In 2008, the average temperature patterns across the prairies were warmer than the long term average, nonetheless, oil content increased from east to west. The higher oil content in the west was most likely the result of lower daily temperatures, especially at seed fill resulting in a longer length of time to maturity (Alberta locations were often harvested weeks later than average prairie harvest date). These observations are based on Environment Canada data

taken near the sites. Detailed analysis of weather conditions correlating to oil content is outside the scope of this study. That being said, location across the prairies did seem to play a role in determining soil content.

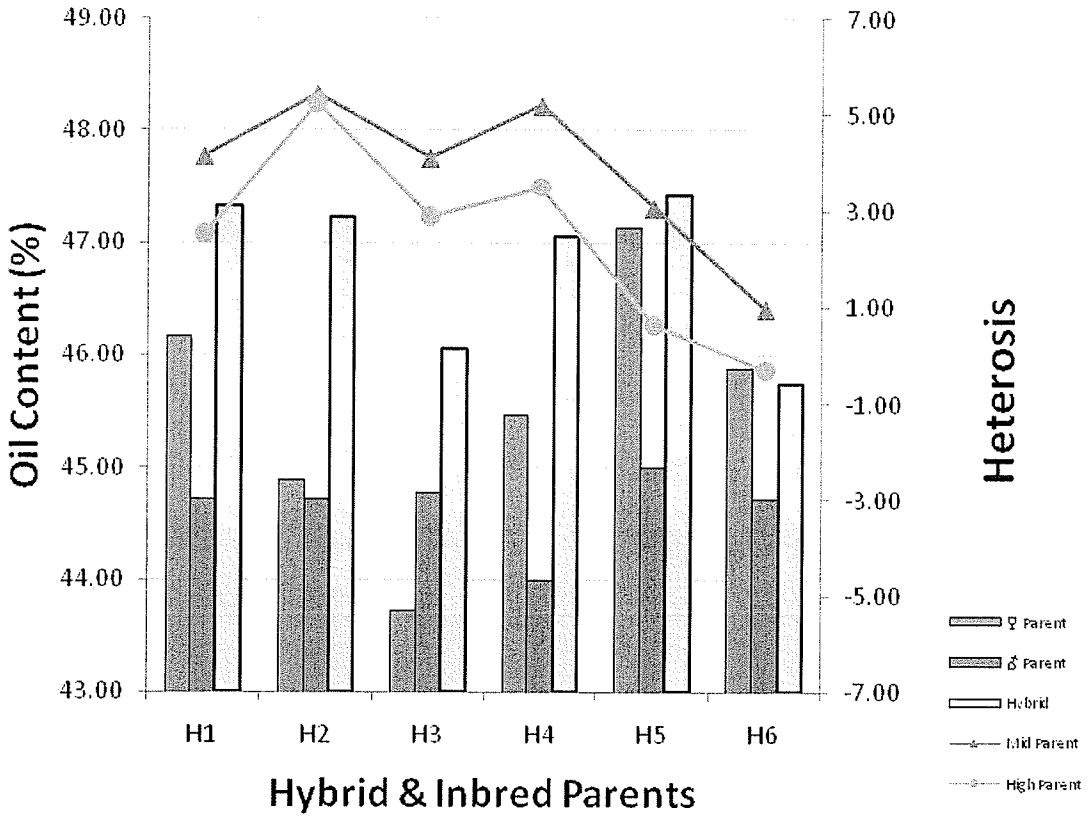

Figure 4.7: Mean oil content of inbred and hybrid means for each treatment (histogram) and mid-parent and high-parent heterosis (line graph).

Table 4.11: Location mean temperature and total rainfall (May to August) effects on oil content

Year	Location	% Oil Content	Rain (mm)	Temp. (°C)
2007	Barrhead	48.7	237.8	15.0
2007	Leduc	48.3	219.0	14.1
2007	Saskatoon	43.3	216.5	15.8
2007	Yorkton	42.6	238.3	15.8
2007	Swan Lake	43.6	282.4	16.8
2007	Oakville	41.0	228.9	15.8
2008	N. Battleford	48.7	272.6	15.1
2008	Saskatoon	47.6	183.0	15.3
2008	Yorkton	47.4	304.5	14.8
2008	Swan Lake	47.3	255.8	14.4

4.2.2 Seed Meal Protein Content

Protein content was lower for the mean of the hybrids compared to the mean of the inbreds. There was no difference, however, between the mean protein content for the hybrids and open pollinated mean or between the open pollinated mean and the inbred mean. The hybrid mean protein content was 46.85% with a maximum value of 49.07% (H6) and a minimum value of 45.64% (H1) (Table 4.12). Other than hybrid H6 being substantially higher than the other hybrids, the standard deviation of protein content for the hybrids remained quite low at 0.48%. The inbred lines had a protein content mean of 48.07 with a maximum of 51.71% and a minimum of 43.48%. Standard deviation for protein content for the inbred lines was 0.50%, suggesting little variability between reps and environments. Due to the protein content of the hybrids being lower to that of their inbred parents, positive heterosis was non-existent. In attempting to calculate heterosis, results consistently displayed negative values (Figure 4.8).

Protein content showed a significant variety effect, environment effect and variety by environment effect. As predicted, both genotype and environment affect protein content in the developing seed. The genotype or variety was significant at $\alpha = 0.001$ while environment was only significant at $\alpha = 0.05$ suggesting that genotype played more of a role in protein content. An example of genotype by environment effect can be seen with hybrid H3 and inbred H4P1. Their protein content means at Barrhead in 2007 were 46.24% and 50.44% while at Yorkton in 2008 the protein content means were 45.68% and 41.77%. The rank of each variety reversed between locations or environments, suggesting a genotype by environment effect.

Like oil content, protein content is affected by temperature. Due to oil and protein content's inverse relationship, protein content decreases from east to west across the prairies and oil content increased (Table 4.13). This trend is due to average daily temperature decreasing, and the longer length of time to harvest as one goes west. In addition, the higher maximum daily temperatures in the east contributed to plant stress, which encouraged protein deposition in the seed and not oil.

Table 4.12: Mean seed meal protein content, mid-parent, high-parent and commercial heterosis in 10 environments in 2007 and 2008[+]

Cultivar	Mean Seed Meal Protein Content (%)	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	45.64	0.57	-1.00	-6.32**	-4.66	-1.8704	-4.03	<.0001
H2	46.51	0.45	-3.16*	-4.52	-2.83	-0.9537	-2.06	0.0417
H3	46.84	0.39	-6.16**	-6.40**	-2.14	-1.7963	-3.87	0.0002
H4	46.36	0.55	-2.83*	-10.34**	-3.14	-1.2315	-2.65	0.0089
H5	46.69	0.41	-1.08	-3.95	-2.46	-1.0926	-2.35	0.0199
H6	49.07	0.52	-0.64	-1.98	2.52	-0.1204	-0.26	0.7957
Average	46.85	0.48	-2.5	-5.6	-2.1			
Inbreds								
H1P1	43.48	0.50						
(H1, H2, H6) P2	48.72	0.41						
H2P1	47.35	0.42						
H3P1	49.79	0.40						
H3P2	50.04	0.63						
H4P1	43.72	0.64						
H4P2	51.71	0.51						
H5P1	45.86	0.40						
H5P2	48.61	0.50						
H6P1	50.07	0.55						
Average	48.07	0.50						
Open Pollinated								
32-75	45.48	0.42						
34-55	47.16	0.54						
34-65	47.87	0.33						
Average	46.84	0.4						
Totals								
HY vs OP						0.00861	-0.02	0.9847
HY vs IB						-1.1235	-3.47	0.003

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

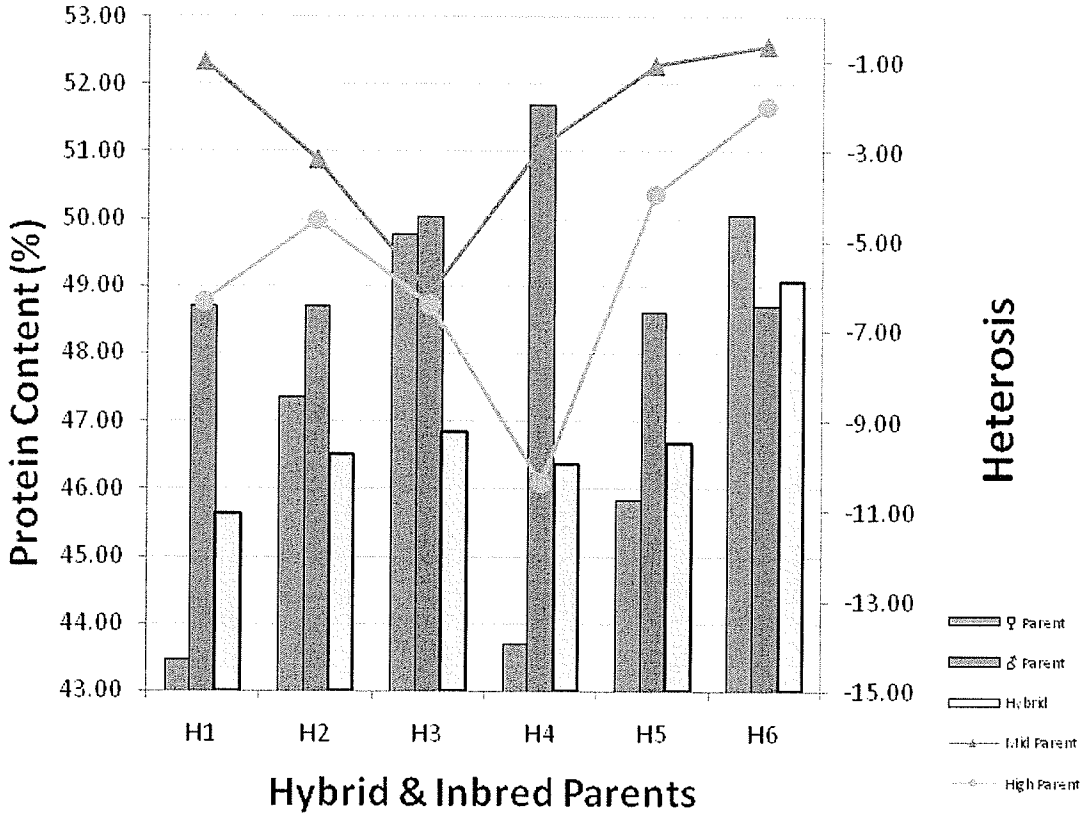

Figure 4.8: Mean protein content of inbred and hybrid means for each treatment (histogram) and mid-parent and high-parent heterosis (line graph).

Table 4.13: Location mean temperature and total rainfall (May to August) effect on seed meal protein content

Year	Location	% Protein Content	Rain (mm)	Temp. (°C)
2007	Barrhead	47.1	237.8	15.0
2007	Leduc	46.8	219.0	14.1
2007	Saskatoon	49.2	216.5	15.8
2007	Yorkton	48.9	238.3	15.8
2007	Swan Lake	44.8	282.4	16.8
2007	Oakville	51.3	228.9	15.8
2008	N. Battleford	44.8	272.6	15.1
2008	Saskatoon	46.8	183.0	15.3
2008	Yorkton	46.2	304.5	14.8
2008	Swan Lake	49.4	255.8	14.4

4.2.3 Sum of Oil and Seed Meal Protein Contents

The sum of oil and protein contents was another variable used to calculate hybrid advantage. Because oil content was higher and protein content was lower in the hybrids, the consideration of each alone gives an incomplete picture as to what is going on. Selection based on these two traits simultaneously may be advantageous since their combined heritability is higher than their individual heritabilities. This was noted by Grami and Stefansson (1977) who suggested simultaneous selection for oil and protein content. The mean sum of oil and protein for the hybrids (93.79%) was greater than for the inbred lines with a mean of (93.29%) ($\alpha = 0.05$) and the open pollinated cultivars with a mean of (92.16%) ($\alpha = 0.0001$) for sum of oil and protein content (Table 4.14). Hybrid H6 performed the best of the hybrids (94.94%), while H3 was the poorest (93.03%). The inbred lines varied more than the hybrids, with a maximum sum of oil and protein content of 96.07% (H5P2) and a minimum of 89.32% (H4P1). Five out of six hybrids had mean values higher than the mean of their two parents; however, this was a significant difference in only one hybrid (H1). Similarly, no significant high parent heterosis or commercial heterosis the sum of oil and protein content were found in this study (Figure 4.9).

Variety, environment and variety by environment were all significant effects. This was to be expected since it was observed for oil and protein content separately. When looking across the trial sites from east to west, as oil increased, protein content decreased offsetting each other. In 2007, the sum of oil and protein tended to increase from the east (eg. Swan Lake – 88.6%) to west (Barrhead – 95.5%) (Table 4.15). As with oil content,

these results are most likely the effect of less heat stress on the plant and a longer duration between flowering and harvest at the western locations allowing the plant to develop fully filled seed.

Table 4.14: Mean sum of oil and protein content, mid-parent, high-parent and commercial heterosis in 10 environments in 2007 and 2008[+]

Cultivar	Mean Sum of Oil and Protein Content (%)	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	93.10	2.12	1.55*	-0.50	0.18	-1.8704	-4.03	<.0001
H2	93.88	2.71	0.98	0.34	1.02	-0.9537	-2.06	0.0417
H3	93.03	2.13	-1.33	-2.02	0.11	-1.7963	-3.87	0.0002
H4	93.55	2.30	1.05	-2.38	0.66	-1.2315	-2.65	0.0089
H5	94.25	2.54	0.87	0.55	1.42	-1.0926	-2.35	0.0199
H6	94.94	2.49	0.13	-1.18	2.16	-0.1204	-0.26	0.7957
Average	93.79	2.38	0.5	-0.9	0.9			
Inbreds								
H1P1	89.78	2.67						
(H1, H2, H6) P2	93.56	2.81						
H2P1	92.36	2.99						
H3P1	93.63	2.50						
H3P2	94.95	2.85						
H4P1	89.32	4.00						
H4P2	95.83	2.20						
H5P1	93.12	3.39						
H5P2	93.74	3.66						
H6P1	96.07	2.81						
Average	93.29	2.99						
Open Pollinated								
32-75	90.83	2.65						
34-55	92.72	2.14						
34-65	92.93	2.27						
Average	92.16	2.35						
Totals								
HY vs OP						1.6324	4.75	<.0001
HY vs IB						0.5543	2.21	0.0287

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

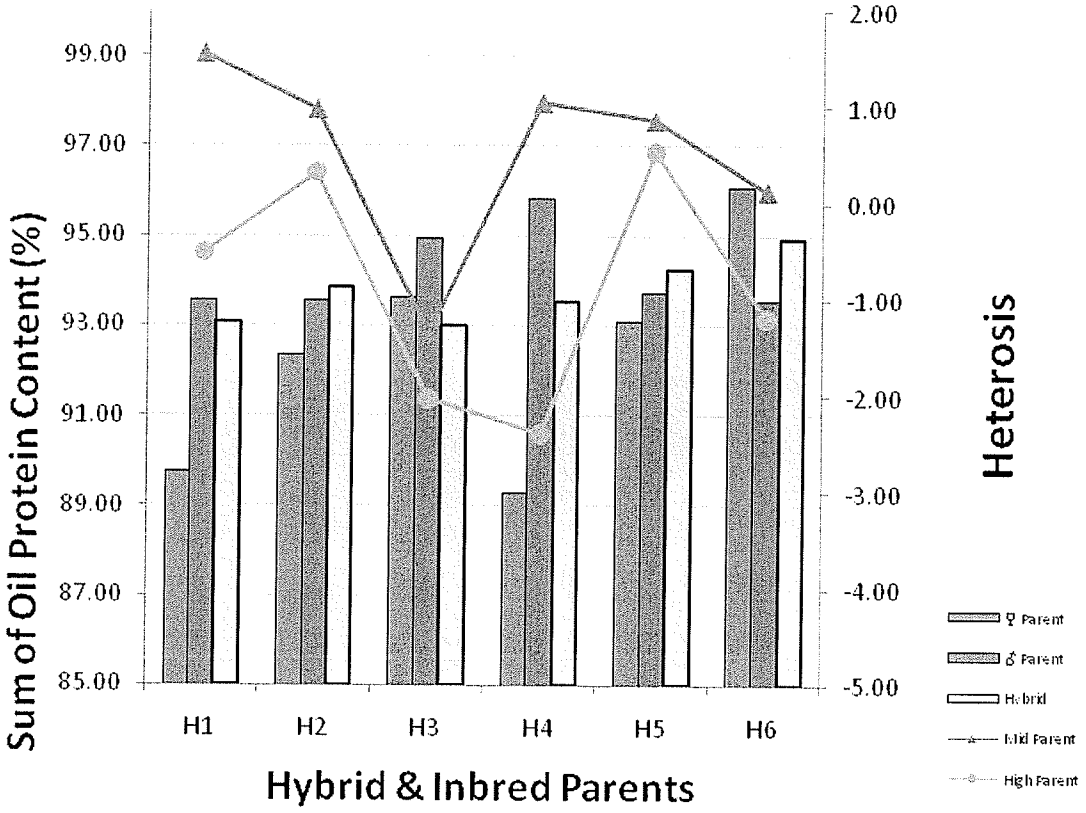

Figure 4.9: Mean sum of oil and protein content for inbred and hybrid treatments (histogram) and mean mid-parent and high-parent heterosis (line graph).

Table 4.15: Location mean temperature and total rainfall (May to August) effects on sum of oil and protein content

Year	Location	Sum Oil and Protein %	Rain (mm)	Temp. (°C)
2007	Barrhead	95.5	237.8	15.0
2007	Leduc	94.8	219.0	14.1
2007	Saskatoon	92.4	216.5	15.8
2007	Yorkton	91.4	238.3	15.8
2007	Swan Lake	88.6	282.4	16.8
2007	Oakville	92.5	228.9	15.8
2008	N. Battleford	93.1	272.6	15.1
2008	Saskatoon	94.4	183.0	15.3
2008	Yorkton	93.4	304.5	14.8
2008	Swan Lake	96.5	255.8	14.4

4.2.4 Saturated Fatty Acid Content in the Seed Oil

The mean saturated fatty acid (SFA) content of the hybrids was not different from the mean SFA content of the inbred parents. However, three of six hybrids included H1, H4 and H5 were lower in SFA content than the average of their parents. H3 was significantly higher in SFA content than the average of its parents. Hybrids had an average SFA content of 7.14% while the inbreds averaged 7.20% (Table 4.16). The standard deviation of the hybrids and inbreds was not different, suggesting that SFA content did not vary between varieties; however, the varieties themselves had a significant affect on SFAs. The open pollinated cultivar mean for SFA content was higher than both hybrids and inbreds. Mid-parent and high-parent heterosis was nearly non-existent as none of the hybrids had significantly positive values (Figure 4.10). In addition, negative commercial heterosis, was found by four of the six hybrids due to 32-75 having a low SFA content (6.82). Heterosis in SFA content is not desirable; in fact, these contents of SFA content could prove detrimental not only for health reasons but also variety registration. The maximum level of SFA content in canola, according to the Canola Council of Canada, is 7.1% (Canola Council of Canada, 2008).

Saturated fatty acid content was significantly affected by environment and variety by environment. The genotype by environment interaction can be seen when looking at hybrid H6 and inbred H5P1 when comparing Swan Lake in 2007 and North Battleford in 2008. Hybrid H6 had a mean SFA content of 7.84% at Swan Lake in 2007 while inbred parent H5P1 had a mean SFA content of 8.16%. This trend was reversed in North Battleford in 2008 as H6's mean was 6.98% and H5P1's SFA content mean was 6.93%.

There were no trends observed between rain, temperature or location and SFA content (Table 4.17).

Table 4.16: Mean saturated fatty acid content, mid-parent, high-parent and commercial heterosis in 10 environments in 2007 and 2008[+]

Cultivar	Mean SFA Content (%)	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	7.19	0.47	-2.32**	-1.36	5.44**	-1.8704	-4.03	<.0001
H2	7.41	0.47	0.08	0.42	8.70**	-0.9537	-2.06	0.0417
H3	7.09	0.42	1.57	5.75	4.02**	-1.7963	-3.87	0.0002
H4	6.97	0.46	-2.47**	-1.08	2.30	-1.2315	-2.65	0.0089
H5	6.89	0.47	-1.85**	-0.01	1.01	-1.0926	-2.35	0.0199
H6	7.28	0.36	-0.25	-1.15	6.79**	-0.1204	-0.26	0.7957
Average	7.14	0.44	-0.9	0.9	4.7			
Inbreds								
H1P1	7.29	0.48						
(H1, H2, H6) P2	7.43	0.46						
H2P1	7.38	0.66						
H3P1	6.71	0.30						
H3P2	7.26	0.50						
H4P1	7.25	0.58						
H4P2	7.05	0.40						
H5P1	7.14	0.59						
H5P2	6.89	0.46						
H6P1	7.17	0.35						
Average	7.16	0.48						
Open Pollinated								
32-75	6.82	0.42						
34-55	7.69	0.38						
34-65	7.36	0.56						
Average	7.29	0.5						
Totals								
HY vs OP						-0.1514	-3.52	0.0024
HY vs IB						-0.01744	-0.56	0.5855

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at Pr < 0.05
**Significantly higher at Pr < 0.01

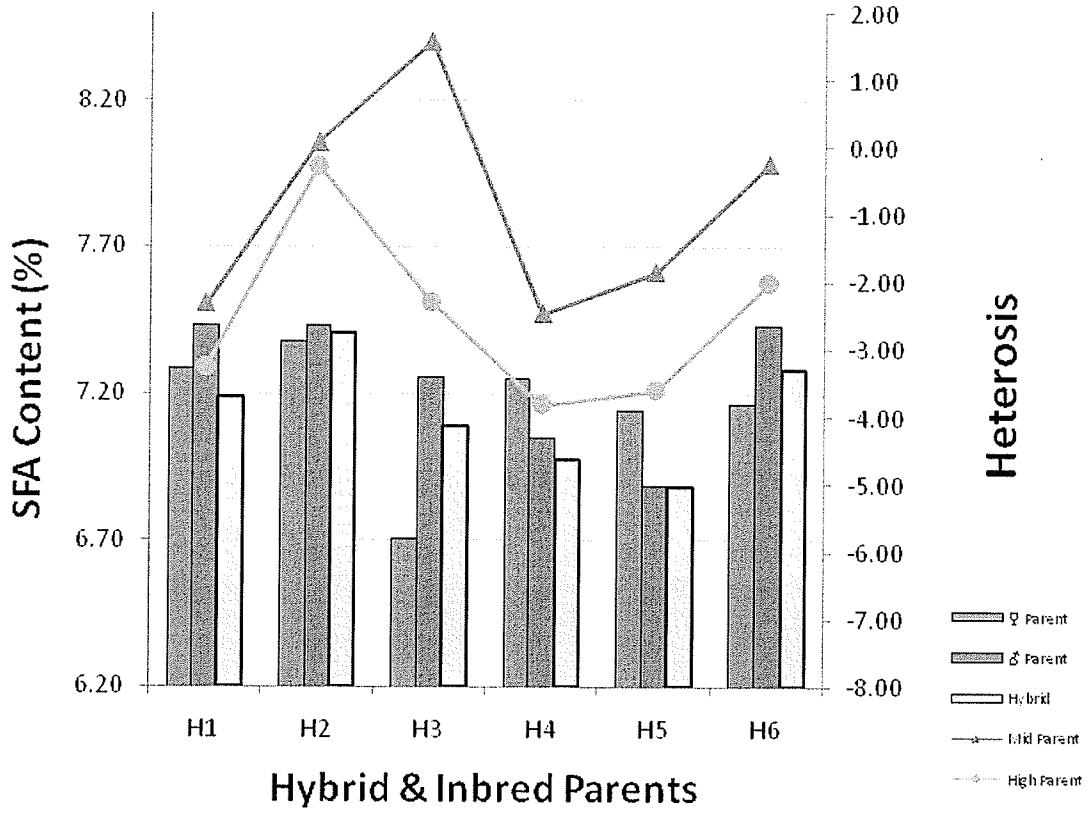

Figure 4.10: Mean saturated fatty acid content for inbred and hybrid treatments (histogram) and mean mid-parent and high-parent heterosis (line graph).

Table 4.17: Location mean temperature and total rainfall (May to August) effect on SFA content

Year	Location	% SFA Content	Rain (mm)	Temp. (°C)
2007	Barrhead	6.5	237.8	15.0
2007	Leduc	-	219.0	14.1
2007	Saskatoon	7.6	216.5	15.8
2007	Yorkton	7.5	238.3	15.8
2007	Swan Lake	7.8	282.4	16.8
2007	Oakville	-	228.9	15.8
2008	N. Battleford	6.9	272.6	15.1
2008	Saskatoon	7.1	183.0	15.3
2008	Yorkton	7.1	304.5	14.8
2008	Swan Lake	6.8	255.8	14.4

4.2.4 Glucosinolate Content

Glucosinolate content was lower in the hybrids as a group compared to the inbreds as a group or the open pollinated varieties as a group. The hybrids had a mean glucosinolate content of 11.71 μmol g^{-1} seed, while the inbred mean glucosinolate content was 12.65 μmol g^{-1} seed (Table 4.18). Glucosinolate content had a large range with a standard deviation of 1.31 for the hybrids and 1.82 for the inbred parents. The minimum glucosinolate content values for the hybrids was 10.21 μmol g^{-1} seed while the maximum was 13.77 μmol g^{-1} seed; the inbred values had a minimum glucosinolate content of 9.49 μmol g^{-1} seed and a maximum of 14.91 μmol g^{-1} seed. The open pollinated varieties had a large range with a standard deviation of 1.98 with an average glucosinolate content of 12.43 μmol g^{-1} seed and 10.97 μmol g^{-1} seed and 14.69 μmol g^{-1} seed for a minimum and maximum respectively. Two hybrids had negative mid-parent and high-parent heterosis for glucosinolate content (Figure 4.11). Therefore, unlike traits like vigour and oil content, glucosinolate content remained within the range of the parents but below the mean of the parents.

Table 4.18: Mean glucosinolate content, mid-parent, high-parent and commercial heterosis in 10 environments in 2007 and 2008[+]

Cultivar	Mean Gluc. Content ($\mu mol\ g^{-1}$) seed	Standard Deviation	Mid-Parent Heterosis (%)	High-Parent Heterosis (%)	Commercial Heterosis (%)	Estimate	t - Value	Pr > |t|
Hybrids								
H1	12.35	0.47	-1.11	9.70	12.50*	-1.8704	-4.03	<.0001
H2	10.21	0.47	-11.13**	-9.32	-7.00	-0.9537	-2.06	0.0417
H3	12.22	0.42	-17.17**	-15.12**	11.40*	-1.7963	-3.87	0.0002
H4	13.77	0.46	-3.63	0.78	25.47**	-1.2315	-2.65	0.0089
H5	10.75	0.47	-5.92	13.35*	-2.01	-1.0926	-2.35	0.0199
H6	10.96	0.36	-6.69	-2.58	-0.09	-0.1204	-0.26	0.7957
Average	11.71	0.44	-7.6	-0.5	6.7			
Inbreds								
H1P1 (H1, H2, H6)	13.71	0.48						
P2	11.25	0.46						
H2P1	11.71	0.66						
H3P1	14.40	0.30						
H3P2	15.11	0.50						
H4P1	13.66	0.58						
H4P2	14.91	0.40						
H5P1	9.49	0.59						
H5P2	13.76	0.46						
H6P1	12.24	0.35						
Average	12.65	0.48						
Open Pollinated								
32-75	14.69	0.42						
34-55	11.63	0.38						
34-65	10.97	0.56						
Average	12.43	0.5						
Totals								
HY vs OP						-0.7195	-2.4	0.0173
HY vs IB						-1.3168	-6.03	<.0001

[+]Barrhead AB 2007, Leduc AB 2007, Oakville MB 2007, Saskatoon SK 2007 and 2008, Yorkton SK 2007 and 2008, Swan Lake MB 2007 and 2008 and North Battleford SK 2008.
*Significantly higher at $Pr < 0.05$
**Significantly higher at $Pr < 0.01$

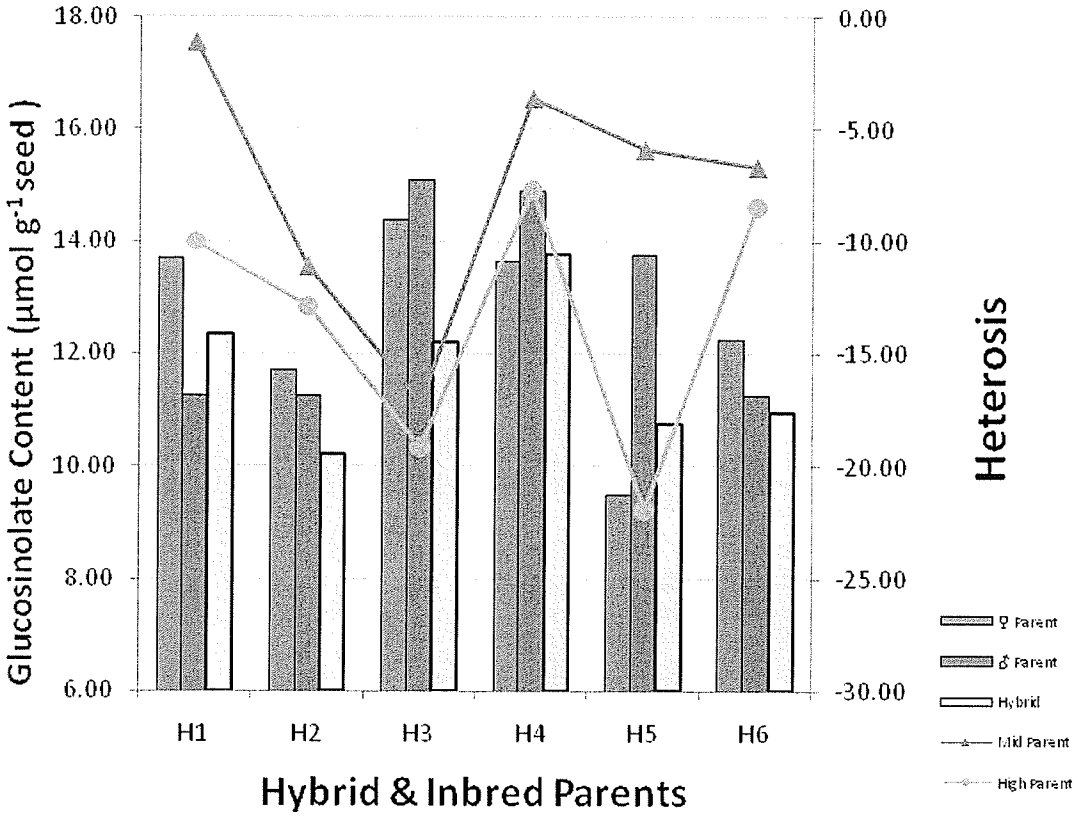

Figure 4.11: Mean glucosinolate content of inbred and hybrid means for each treatment (histogram) and mid-parent and high-parent heterosis (line graph).

Variety, location and variety by location had significant effects on glucosinolate content. The genotype or variety was significant at $\alpha = 0.001$ while location was only slightly significant at $\alpha = 0.05$ suggesting that genotype played more of a role in glucosinolate content. In addition the variety by location was also significant at $\alpha = 0.001$. There was no specific trend observed between rain, temperature or geography and glucosinolate content observed in this study (Table 4.20).

Table 4.19: Location mean temperature and total rainfall (May to August) effects on glucosinolate content

Year	Location	Glucosinolate Content ($\mu mol\ g^{-1}$ seed)	Rain (mm)	Temp. (°C)
2007	Barrhead	13.2	237.8	15.0
2007	Leduc	13.3	219.0	14.1
2007	Saskatoon	10.2	216.5	15.8
2007	Yorkton	12.8	238.3	15.8
2007	Swan Lake	12.0	282.4	16.8
2007	Oakville	15.3	228.9	15.8
2008	N. Battleford	12.3	272.6	15.1
2008	Saskatoon	13.6	183.0	15.3
2008	Yorkton	9.9	304.5	14.8
2008	Swan Lake	12.6	255.8	14.4

Conclusions 5.0

Very few studies extensively report on both agronomic and seed quality components in *Brassica napus* yield trials. In addition, this study was original as it focused on commercial or near-commercial hybrids and their parents, with hybrids produced using the *ogu* INRA CMS system. The hybrids in question came from established breeding programs and were commercial products or nearing registration, they were top performers from previous small plot and field scale evaluations. The disease resistance of the hybrids used in this study was not evaluated. However, all hybrids were either commercialized products or late stage experimental hybrids, being evaluated for registration and all hybrids and parents and OP checks had a minimum of a 'MR' rating for blackleg.

5.1 Mid-Parent Heterosis

Inbred line derived hybrids, on average, displayed mid-parent heterosis for vigour, uniformity, days to 50% flower, days to maturity, height, seed oil content, sum of oil and protein content and glucosinolate content. 'Negative mid-parent heterosis' – in which there was a decrease from the parental mean, was observed for seed protein content was expected since oil and protein content have an inverse relationship in the seed. Mean saturated fatty acid content was similar in both hybrids and their parents, although the hybrids had a lower mean. Mid-parent heterosis was observed in six of six hybrids for uniformity, five of six hybrids for seedling vigour, days to 50% flower and seed oil content, four of six hybrids for height and saturated fatty acid content, three of six hybrids for days to maturity, two of six hybrids for sum of oil and protein content and

glucosinolate content. The hybrids in this study were superior for most traits compared to the average of their inbred line parents including oil content, which confirms our hypothesis that *ogu* INRA *B. napus* hybrids display mid-parent heterosis for oil content and vigour. The data also provided evidence of mid-parent heterosis for other agronomic and seed quality traits. In addition, the fact that mean protein content in the hybrids was less than the mean protein content of the inbred parents confirms our hypothesis that negative mid-parent heterosis was evident for protein content in the *B. napus* lines used in this study.

5.2 High-Parent Heterosis

High-parent heterosis is identified as the hybrid being superior to the better of its two parents. This observation places the hybrid phenotypes outside the phenotypic range of the parents, which suggests a synergistic interaction between genes. Positive high-parent heterosis is classified as higher than the better parent for traits in which greater is better, such as for vigour and oil content. However, positive heterosis is not always advantageous; in traits such as days to 50% flower and days to maturity negative high-parent heterosis occurred when the hybrid reached the stage earlier than the better parent. High-parent heterosis was identified in six of six hybrids for uniformity, five of six hybrids for vigour, four of six hybrids for height and seed oil content and one of six hybrids for glucosinolate content. Although mid-parent heterosis is a common calculation for measuring heterosis, high-parent heterosis is essential for harnessing and utilizing this genetic phenomenon in the development of new canola cultivars.

By analyzing high-parent heterosis, we ranked the hybrids for each trait. Using this approach, instead of mean values, we were able to isolate the genetic contribution to heterosis from each parental pair to their hybrid (Table 5.1). Hybrid H3 performed exceptionally well for high-parent heterosis in agronomic traits, and showed average performance for seed quality. Hybrid H1 also preformed well with a strong showing overall. Hybrid H2 did not perform well agronomically; however, it had excellent heterosis for seed quality traits. The next step would be to assess the parental lines in an attempt to capture the genetic contribution for heterosis for specific traits to make new parental combinations. For example, a breeder could pair H3P1 together with H2P2, in an attempt to capture high heterosis for both agronomic and seed quality traits.

5.3 Commercial Heterosis

Commercial heterosis is identified as a hybrid being superior to that of the current commercial equivalent open pollinated variety. 32-75, 34-55 and 34-65 were used to assess the performance of the six hybrids. For many of the traits, the hybrids did show commercial heterosis over the best of the three open pollinated commercial varieties; this display of heterosis was strongly displayed in uniformity, height, vigour and oil content. Higher uniformity can be attributed to the F1 hybrids sharing the same genotype as their parents are inbreds while the open pollinated varieties contain much more genetic variability. The difference in height between the hybrids and open pollinated varieties can be attributed to both the display of heterosis and many of the inbred parents being tall. Both vigour and oil content can solely be attributed to the display of heterosis.

5.4 Agronomic Traits

Hybrids with strong early season vigour were expected since this has been reported previously (Sernyk and Stefansson, 1983); however, few studies in *B. napus* refrain to comment about vigour as it is apparently taken for granted. The enhanced vigour of the hybrids used in this study seemed to enhance uniformity, while the inbred lines struggled with emergence and derived uniformity. Days to 50% flower happened earlier in the hybrids compared to the inbred parents as confirmed by Sernyk and Stefansson (1983) and Grant and Beversdorf (1985). Days to 50% flower was strongly correlated to days to maturity, days to maturity also showed heterosis for earliness in the hybrids. When breeding for shorter season climates, such as in western Canada, the use of hybrids which display heterosis for earliness could prove to be extremely beneficial.

Table 5.1: High-parent heterosis and rankings for agronomic and seed quality traits for hybrids H1 to H6

Hybrid		H1	H2	H3	H4	H5	H6
Seedling Vigour	Mean	6.8	6.9	7.0	6.2	6.5	6.8
	HPH	29.3	29.1	36.5	31.0	25.0	
Uniformity	Mean	7.8	7.6	7.7	7.4	7.5	7.5
	HPH	14.02	9.64	17.09	11.95	13.29	0.00
Days to Flower	Mean	51.7	50.9	51.2	53.7	51.1	52.0
	HPH	0.00	0.00	0.00	0.00	0.00	0.00
Days to Maturity	Mean	95.0	94.8	94.9	96.7	95.6	96.3
	HPH	0.00	0.00	0.00	0.00	0.00	0.00
Height (cm)	Mean	106.3	100.3	105.3	113.1	103.1	107.4
	HPH	5.63	9.86	0.00	0.00	5.69	6.42
Oil Content (%)	Mean	47.34	47.24	46.07	47.06	47.43	45.74
	HPH	2.51	5.24	2.87	3.49	0.00	0.00
Protein Content (%)	Mean	45.64	46.51	46.84	46.36	46.69	49.07
	HPH	-6.32	0.00	-6.40	-10.34	0.00	0.00
Oil and Pro Content (%)	Mean	93.10	93.88	93.03	93.55	94.25	94.94
	HPH	-0.50	0.34	-2.02	-2.38	0.55	-1.18
SFA Content (%)	Mean	7.19	7.41	7.09	6.97	6.89	7.28
	HPH	-1.36	0.42	5.75	-1.08	-0.01	1.58
Gluc. Content ($\mu mol\ g^{-1}$ seed)	Mean	12.35	10.21	12.22	13.77	10.75	10.96
	HPH	0.00	0.00	-15.12	0.00	13.35	0.00

*HPH – High-parent heterosis

5.5 Seed Quality Traits

The large majority of previous studies reported no significant heterosis for seed oil content in *B. napus* (Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985; Lefort-Buson et al., 1987; Brandle and McVetty, 1990; Banks and Beversdorf, 1994). However, a minority of researchers have reported heterosis for seed oil content (Starmer et al., 1998; Shen et al., 2005; Cuthbert, 2006). Higher levels of heterosis for agronomic traits compared to seed quality traits were observed in this study. These differences are likely due to agronomic traits such as number of seeds, pods, seed size which are all components of yield, are correlated to the plants reproductive success. Shen et al. 2005

demonstrated that these seed yield related agronomic traits were controlled by both additive and non-additive gene effects whereas the seed quality traits were mainly affected by additive gene action. This may explain the higher heterosis levels observed for seed yield related traits. In addition, most seed quality traits may not be beneficial for plant growth and thus are controlled by many recessive genes, limiting their level of heterosis (Fu, 2000).

What is not discussed in previous studies is the possible genetic interaction between the *B. napus* nucleus while using the *ogu* INRA CMS hybrid system which consists of *B. napus* chloroplasts and radish (*Raphanus sativus*) mitochondria. Many CMS systems produce a biological cost (McVetty, 1998), which was originally evident in the *ogu* CMS system. However, after the protoplast fusion producing a cybrid in which only the mitochondria were of radish origin, these biological costs disappeared. Is there some synergistic genetic effect between the *ogu* INRA hybrid cytoplasm and the *B. napus* nucleus that produces or enhances heterosis for seed quality traits such as oil content? A definitive answer is not yet available; however, experiments that have found heterosis for oil content in *B. napus* have not used the *ogu* INRA CMS system (Starmer et al., 1998; Shen et al., 2005; Cuthbert, 2006). In addition, recent work by Djordjevic (personal communication) showed oil content increases in *B. napus* hybrids using both *ogu* INRA CMS and hand cross of materials in the *nap* cytoplasm.

Protein content showed negative heterosis, as predicted and reported in previous studies (Sernyk and Stefansson, 1983 and Grant and Beversdorf, 1985), due to the inverse relationship of protein content with oil content. To further explore seed quality trait

heterosis, the sum of oil and protein content was analyzed to observe whether the hybrids filled more of the seed with oil and protein or just offset increased oil production with reduced protein production. Five out of six hybrids had numerically higher sum of oil and protein contents than the parental mean, however, only one hybrid was significantly different from its parents for this trait. That being said, the majority of the oil content increase comes from a corresponding decrease in protein content. Breeders wishing to increase both oil and protein content in hybrid *B. napus* cultivars must use simultaneous selection to maximize both constituents.

Glucosinolate content is an undesirable component in the *B. napus* seed; which, during the last 30 years has been reduced or nearly eliminated through breeding. The hybrids in this study, on average, were lower than the inbred parental lines in glucosinolate content. While all six hybrids that were numerically lower than their mid-parent values, only two hybrids were significantly lower in glucosinolate content. Reducing glucosinolate content further is just another benefit breeding *B. napus* canola hybrids which have lower glucosinolate content and higher meal quality.

Saturated fatty acid content, like glucosinolate content, are also undesirable. The Canola Council of Canada reports that all canola has a SFA content of 7.1% or less. *B. napus* is known to be higher in oil content than *B. rapa* and is frequently at or above 7% SFA content. Three of the six hybrids used in this study showed negative MPH for saturated fatty acid content. The mean SFA content of the hybrids was not different from the mean of their parental lines, however, and they did average slightly over 7% (7.14%).

Therefore, there was a slight increase in oil quality in the hybrids compared to the parents (7.16%) as well as the commercial check cultivars (7.29%).

Genotype, location and genotype by location had a significant effect on all traits. Each hybrid performed somewhat differently than each other hybrid, comprising the genotype or variety effect. The environmental effect is most likely due to the temperature and rainfall differences between sites and years. The genotype by location interaction could have been caused by a wide number of effects, for example, one particular hybrid performs well in hot, dry climates and worse in cool wet climates as compared to another hybrid.

Based on the results of this study, further research into heterosis of seed quality traits such as oil content in *ogu* INRA CMS hybrid canola cultivars appears justified. Currently, hybrid canola breeders are focusing primarily on seed yield; however, using inbred lines with high combining abilities for oil content to exploit heterosis for oil content would be beneficial. Before this strategy can move forward, however, more research must be completed to understand the factors behind heterosis for oil content in order to exploit it efficiently. As well, the industry must further shift towards rewarding growers for high oil content seed.

Additional research continuing from this project would include an expansion of the original project with more hybrids, more parents, more locations and more traits such as seed yield and oil yield. As well, an additional project could focus on comparing heterosis of hybrid canola produced using the *ogu* INRA cytoplasm to *nap* cytoplasm hybrids in an effort to discover possible biological benefit in cybrids. The environment

and geographic locations and their effects on each of the traits observed in this study could produce another interesting study.

This study has identified heterosis for both agronomic and seed quality traits in *ogu* INRA CMS *B. napus* canola hybrids. In the future, this understanding of the benefits of heterosis can be utilized to develop more productive *B. napus* hybrid cultivars which display not only increased yield, but increased vigour, increased plant height, earliness and higher oil content.

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Appendix 7.0

7.1 SAS Statistical Analysis Software Calculations

7.1.1 Mixed Model SAS Code used to calculate ANOVA, estimates and least squared means – Oil content variable shown

```
Proc Mixed Data=One covtest;
                    *variables;
                    Classes Rep Variety Breed Location Year;
                    *oil variable tested using mixed model, fixed variables, degrees of freedom computed by the
satterthwaite formula;
                    Model Oil = Variety / DDFM = satterth residual outp=res;
                    *random variables;
                    Random Rep(location) location variety*location;
                    *hybrid vs. OP estimate, hybrid vs. inbreed estimate, OP vs. inbred estimate;
                    Estimate 'hyb vs. conv' variety 0.16667 0.16667 0.16667 0.16667 0.16667 0.16667 0 0 0 0 0 0 0 0 0
0 -0.33333 -0.33333 -0.33333;
                    Estimate 'hyb vs. inb' variety 0.166667 0.166667 0.166667 0.166667 0.166667 0.166667 -0.1 -0.1 -
0.1 -0.1 -0.1 -0.1 -0.1 -0.1 -0.1 -0.1 0 0 0;
                    Estimate 'conv vs. inb' variety 0 0 0 0 0 0 -0.1 -0.1 -0.1 -0.1 -0.1 -0.1 -0.1 -0.1 -0.1 -0.1 0.33333
0.33333 0.33333;
                    *estimates between each hybrid and its 2 parents;
                    Estimate 'H1 vs. P1 P2' variety 1 0 0 0 0 0 -0.5 -0.5 0 0 0 0 0 0 0 0 0 0;
                    Estimate 'H2 vs. P1 P2' variety 0 1 0 0 0 0 0 -0.5 -0.5 0 0 0 0 0 0 0 0 0;
                    Estimate 'H3 vs. P1 P2' variety 0 0 1 0 0 0 0 0 0 -0.5 -0.5 0 0 0 0 0 0 0;
                    Estimate 'H4 vs. P1 P2' variety 0 0 0 1 0 0 0 0 0 0 0 -0.5 -0.5 0 0 0 0 0;
                    Estimate 'H5 vs. P1 P2' variety 0 0 0 0 1 0 0 0 0 0 0 0 0 -0.5 -0.5 0 0 0;
                    Estimate 'H6 vs. P1 P2' variety 0 0 0 0 0 1 0 -0.5 0 0 0 0 0 0 0 -0.5 0 0;
                    *removal of boundary constraints on covariance parameters;
                    parms/nobound;
                    *least squared means output;
                    lsmeans variety/pdiff;
Run;
Proc Univariate normal plot data=One; *test for normality;
        Var oil;
Run;
Proc Univariate normal plot data = res; *test residuals;
        Var Resid;
Run;
Proc Print data=res; *print residual plot;
Run;
proc sort data= one; *sorts by breed (HY, IB, OP);
 by breed;
run;
Proc means data = one; *Means for oil by breed;
 var oil;
 by breed;
run;
```

7.1.2 Mixed Model SAS Output including ANOVA, estimates and least squared means – Oil content variable shown

Rep	3	1 2 3
Variety	19	HyH1 HyH2 HyH3 HyH4 HyH5 HyH6 IpH1P1 IpH1P2H2 IpH2P1 IpH3P1 IpH3P2 IpH4P1 IpH4P2 IpH5P1 IpH5P2 IpH6P1 OP32-75 OP34-55 OP34-65
Breed	3	Hy Ib Op
Location	10	BH01 LD01 NB02 OK01 SK01 SK02 SW01 SW02 Y001 Y002
Year	2	2007 2008

Dimensions

Covariance Parameters	4
Columns in X	22
Columns in Z	230
Subjects	1
Max Obs Per Subject	570

Number of Observations

Number of Observations Read	570
Number of Observations Used	570
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	2624.17023344	
1	1	1732.35943059	0.00000000

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The Mixed Procedure

Convergence criteria met.

Covariance Parameter Estimates

Cov Parm	Estimate	Standard Error	Z Value	Pr Z
Rep(Location)	0.02933	0.02071	1.42	0.1565
Location	5.5267	2.7970	1.98	0.0482
Variety*Location	0.8677	0.1226	7.08	<.0001
Residual	0.6764	0.05042	13.42	<.0001

Fit Statistics

-2 Res Log Likelihood	1732.4
AIC (smaller is better)	1740.4
AICC (smaller is better)	1740.4
BIC (smaller is better)	1746.0

PARMS Model Likelihood Ratio Test

DF	Chi-Square	Pr > ChiSq
3	891.81	<.0001

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	18	162	11.72	<.0001
Year	1	8	4.07	0.0783

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
hyb vs. conv	1.6159	0.2338	162	6.91	<.0001
hyb vs. inb	1.6346	0.1707	162	9.57	<.0001
conv vs. inb	0.01875	0.2177	162	0.09	0.9314
H1 vs. P1 P2	1.8853	0.4049	162	4.66	<.0001
H2 vs. P1 P2	2.4345	0.4049	162	6.01	<.0001
H3 vs. P1 P2	1.8170	0.4049	162	4.49	<.0001
H4 vs. P1 P2	2.3253	0.4049	162	5.74	<.0001
H5 vs. P1 P2	1.3610	0.4049	162	3.36	0.0010

The Mixed Procedure

Estimates

Label	Estimate	Standard Error	DF	t Value	Pr > |t|
H6 vs. P1 P2	0.4402	0.4049	162	1.09	0.2787

Least Squares Means

Effect	Variety	Estimate	Standard Error	DF	t Value	Pr > |t|
Variety	HyH1	47.6431	0.8284	11.1	57.51	<.0001
Variety	HyH2	47.5461	0.8284	11.1	57.39	<.0001
Variety	HyH3	46.3751	0.8284	11.1	55.98	<.0001
Variety	HyH4	47.3694	0.8284	11.1	57.18	<.0001
Variety	HyH5	47.7414	0.8284	11.1	57.63	<.0001
Variety	HyH6	46.0517	0.8284	11.1	55.59	<.0001
Variety	IpH1P1	46.4847	0.8284	11.1	56.11	<.0001
Variety	IpH1P2H2	45.0307	0.8284	11.1	54.36	<.0001
Variety	IpH2P1	45.1924	0.8284	11.1	54.55	<.0001
Variety	IpH3P1	44.0281	0.8284	11.1	53.15	<.0001
Variety	IpH3P2	45.0881	0.8284	11.1	54.43	<.0001
Variety	IpH4P1	45.7804	0.8284	11.1	55.26	<.0001
Variety	IpH4P2	44.3077	0.8284	11.1	53.48	<.0001
Variety	IpH5P1	47.4487	0.8284	11.1	57.28	<.0001
Variety	IpH5P2	45.3121	0.8284	11.1	54.70	<.0001
Variety	IpH6P1	46.1924	0.8284	11.1	55.76	<.0001
Variety	OP32-75	45.5294	0.8284	11.1	54.96	<.0001
Variety	OP34-55	45.7387	0.8284	11.1	55.21	<.0001
Variety	OP34-65	45.2477	0.8284	11.1	54.62	<.0001

Differences of Least Squares Means

Effect	Variety	_Variety	Estimate	Standard Error	DF	t Value	Pr > |t|
Variety	HyH1	HyH2	0.09700	0.4676	162	0.21	0.8359
Variety	HyH1	HyH3	1.2680	0.4676	162	2.71	0.0074
Variety	HyH1	HyH4	0.2737	0.4676	162	0.59	0.5592
Variety	HyH1	HyH5	-0.09833	0.4676	162	-0.21	0.8337
Variety	HyH1	HyH6	1.5913	0.4676	162	3.40	0.0008
Variety	HyH1	IpH1P1	1.1583	0.4676	162	2.48	0.0143
Variety	HyH1	IpH1P2H2	2.6123	0.4676	162	5.59	<.0001
Variety	HyH1	IpH2P1	2.4507	0.4676	162	5.24	<.0001
Variety	HyH1	IpH3P1	3.6150	0.4676	162	7.73	<.0001
Variety	HyH1	IpH3P2	2.5550	0.4676	162	5.46	<.0001
Variety	HyH1	IpH4P1	1.8627	0.4676	162	3.98	0.0001
Variety	HyH1	IpH4P2	3.3353	0.4676	162	7.13	<.0001

The Mixed Procedure

Differences of Least Squares Means

Effect	Variety	_Variety	Standard Estimate	Error	DF	t Value	Pr > |t|
Variety	HyH1	IpH5P1	0.1943	0.4676	162	0.42	0.6783
Variety	HyH1	IpH5P2	2.3310	0.4676	162	4.99	<.0001
Variety	HyH1	IpH6P1	1.4507	0.4676	162	3.10	0.0023
Variety	HyH1	OP32-75	2.1137	0.4676	162	4.52	<.0001
Variety	HyH1	OP34-55	1.9043	0.4676	162	4.07	<.0001
Variety	HyH1	OP34-65	2.3953	0.4676	162	5.12	<.0001
Variety	HyH2	HyH3	1.1710	0.4676	162	2.50	0.0133
Variety	HyH2	HyH4	0.1767	0.4676	162	0.38	0.7061
Variety	HyH2	HyH5	-0.1953	0.4676	162	-0.42	0.6767
Variety	HyH2	HyH6	1.4943	0.4676	162	3.20	0.0017
Variety	HyH2	IpH1P1	1.0613	0.4676	162	2.27	0.0245
Variety	HyH2	IpH1P2H2	2.5153	0.4676	162	5.38	<.0001
Variety	HyH2	IpH2P1	2.3537	0.4676	162	5.03	<.0001
Variety	HyH2	IpH3P1	3.5180	0.4676	162	7.52	<.0001
Variety	HyH2	IpH3P2	2.4580	0.4676	162	5.26	<.0001
Variety	HyH2	IpH4P1	1.7657	0.4676	162	3.78	0.0002
Variety	HyH2	IpH4P2	3.2383	0.4676	162	6.93	<.0001
Variety	HyH2	IpH5P1	0.09733	0.4676	162	0.21	0.8354
Variety	HyH2	IpH5P2	2.2340	0.4676	162	4.78	<.0001
Variety	HyH2	IpH6P1	1.3537	0.4676	162	2.89	0.0043
Variety	HyH2	OP32-75	2.0167	0.4676	162	4.31	<.0001
Variety	HyH2	OP34-55	1.8073	0.4676	162	3.87	0.0002
Variety	HyH2	OP34-65	2.2983	0.4676	162	4.92	<.0001
Variety	HyH3	HyH4	-0.9943	0.4676	162	-2.13	0.0350
Variety	HyH3	HyH5	-1.3663	0.4676	162	-2.92	0.0040
Variety	HyH3	HyH6	0.3233	0.4676	162	0.69	0.4903
Variety	HyH3	IpH1P1	-0.1097	0.4676	162	-0.23	0.8149
Variety	HyH3	IpH1P2H2	1.3443	0.4676	162	2.88	0.0046
Variety	HyH3	IpH2P1	1.1827	0.4676	162	2.53	0.0124
Variety	HyH3	IpH3P1	2.3470	0.4676	162	5.02	<.0001
Variety	HyH3	IpH3P2	1.2870	0.4676	162	2.75	0.0066
Variety	HyH3	IpH4P1	0.5947	0.4676	162	1.27	0.2053
Variety	HyH3	IpH4P2	2.0673	0.4676	162	4.42	<.0001
Variety	HyH3	IpH5P1	-1.0737	0.4676	162	-2.30	0.0229
Variety	HyH3	IpH5P2	1.0630	0.4676	162	2.27	0.0243
Variety	HyH3	IpH6P1	0.1827	0.4676	162	0.39	0.6966
Variety	HyH3	OP32-75	0.8457	0.4676	162	1.81	0.0724
Variety	HyH3	OP34-55	0.6363	0.4676	162	1.36	0.1754
Variety	HyH3	OP34-65	1.1273	0.4676	162	2.41	0.0170
Variety	HyH4	HyH5	-0.3720	0.4676	162	-0.80	0.4274
Variety	HyH4	HyH6	1.3177	0.4676	162	2.82	0.0054
Variety	HyH4	IpH1P1	0.8847	0.4676	162	1.89	0.0603
Variety	HyH4	IpH1P2H2	2.3387	0.4676	162	5.00	<.0001
Variety	HyH4	IpH2P1	2.1770	0.4676	162	4.66	<.0001
Variety	HyH4	IpH3P1	3.3413	0.4676	162	7.15	<.0001
Variety	HyH4	IpH3P2	2.2813	0.4676	162	4.88	<.0001

The Mixed Procedure

Differences of Least Squares Means

Effect	Variety	_Variety	Standard Estimate	Error	DF	t Value	Pr > |t|
Variety	HyH4	IpH4P1	1.5890	0.4676	162	3.40	0.0009
Variety	HyH4	IpH4P2	3.0617	0.4676	162	6.55	<.0001
Variety	HyH4	IpH5P1	-0.07933	0.4676	162	-0.17	0.8655
Variety	HyH4	IpH5P2	2.0573	0.4676	162	4.40	<.0001
Variety	HyH4	IpH6P1	1.1770	0.4676	162	2.52	0.0128
Variety	HyH4	OP32-75	1.8400	0.4676	162	3.94	0.0001
Variety	HyH4	OP34-55	1.6307	0.4676	162	3.49	0.0006
Variety	HyH4	OP34-65	2.1217	0.4676	162	4.54	<.0001
Variety	HyH5	HyH6	1.6897	0.4676	162	3.61	0.0004
Variety	HyH5	IpH1P1	1.2567	0.4676	162	2.69	0.0080
Variety	HyH5	IpH1P2H2	2.7107	0.4676	162	5.80	<.0001
Variety	HyH5	IpH2P1	2.5490	0.4676	162	5.45	<.0001
Variety	HyH5	IpH3P1	3.7133	0.4676	162	7.94	<.0001
Variety	HyH5	IpH3P2	2.6533	0.4676	162	5.67	<.0001
Variety	HyH5	IpH4P1	1.9610	0.4676	162	4.19	<.0001
Variety	HyH5	IpH4P2	3.4337	0.4676	162	7.34	<.0001
Variety	HyH5	IpH5P1	0.2927	0.4676	162	0.63	0.5323
Variety	HyH5	IpH5P2	2.4293	0.4676	162	5.20	<.0001
Variety	HyH5	IpH6P1	1.5490	0.4676	162	3.31	0.0011
Variety	HyH5	OP32-75	2.2120	0.4676	162	4.73	<.0001
Variety	HyH5	OP34-55	2.0027	0.4676	162	4.28	<.0001
Variety	HyH5	OP34-65	2.4937	0.4676	162	5.33	<.0001
Variety	HyH6	IpH1P1	-0.4330	0.4676	162	-0.93	0.3558
Variety	HyH6	IpH1P2H2	1.0210	0.4676	162	2.18	0.0304
Variety	HyH6	IpH2P1	0.8593	0.4676	162	1.84	0.0679
Variety	HyH6	IpH3P1	2.0237	0.4676	162	4.33	<.0001
Variety	HyH6	IpH3P2	0.9637	0.4676	162	2.06	0.0409
Variety	HyH6	IpH4P1	0.2713	0.4676	162	0.58	0.5625
Variety	HyH6	IpH4P2	1.7440	0.4676	162	3.73	0.0003
Variety	HyH6	IpH5P1	-1.3970	0.4676	162	-2.99	0.0032
Variety	HyH6	IpH5P2	0.7397	0.4676	162	1.58	0.1156
Variety	HyH6	IpH6P1	-0.1407	0.4676	162	-0.30	0.7639
Variety	HyH6	OP32-75	0.5223	0.4676	162	1.12	0.2656
Variety	HyH6	OP34-55	0.3130	0.4676	162	0.67	0.5042
Variety	HyH6	OP34-65	0.8040	0.4676	162	1.72	0.0874
Variety	IpH1P1	IpH1P2H2	1.4540	0.4676	162	3.11	0.0022
Variety	IpH1P1	IpH2P1	1.2923	0.4676	162	2.76	0.0064
Variety	IpH1P1	IpH3P1	2.4567	0.4676	162	5.25	<.0001
Variety	IpH1P1	IpH3P2	1.3967	0.4676	162	2.99	0.0033
Variety	IpH1P1	IpH4P1	0.7043	0.4676	162	1.51	0.1339
Variety	IpH1P1	IpH4P2	2.1770	0.4676	162	4.66	<.0001
Variety	IpH1P1	IpH5P1	-0.9640	0.4676	162	-2.06	0.0408
Variety	IpH1P1	IpH5P2	1.1727	0.4676	162	2.51	0.0131
Variety	IpH1P1	IpH6P1	0.2923	0.4676	162	0.63	0.5327
Variety	IpH1P1	OP32-75	0.9553	0.4676	162	2.04	0.0427
Variety	IpH1P1	OP34-55	0.7460	0.4676	162	1.60	0.1126

The Mixed Procedure

Differences of Least Squares Means

Effect	Variety	_Variety	Standard Estimate	Error	DF	t Value	Pr > |t|
Variety	IpH1P1	OP34-65	1.2370	0.4676	162	2.65	0.0090
Variety	IpH1P2H2	IpH2P1	-0.1617	0.4676	162	-0.35	0.7300
Variety	IpH1P2H2	IpH3P1	1.0027	0.4676	162	2.14	0.0335
Variety	IpH1P2H2	IpH3P2	-0.05733	0.4676	162	-0.12	0.9026
Variety	IpH1P2H2	IpH4P1	-0.7497	0.4676	162	-1.60	0.1108
Variety	IpH1P2H2	IpH4P2	0.7230	0.4676	162	1.55	0.1240
Variety	IpH1P2H2	IpH5P1	-2.4180	0.4676	162	-5.17	<.0001
Variety	IpH1P2H2	IpH5P2	-0.2813	0.4676	162	-0.60	0.5482
Variety	IpH1P2H2	IpH6P1	-1.1617	0.4676	162	-2.48	0.0140
Variety	IpH1P2H2	OP32-75	-0.4987	0.4676	162	-1.07	0.2878
Variety	IpH1P2H2	OP34-55	-0.7080	0.4676	162	-1.51	0.1319
Variety	IpH1P2H2	OP34-65	-0.2170	0.4676	162	-0.46	0.6432
Variety	IpH2P1	IpH3P1	1.1643	0.4676	162	2.49	0.0138
Variety	IpH2P1	IpH3P2	0.1043	0.4676	162	0.22	0.8237
Variety	IpH2P1	IpH4P1	-0.5880	0.4676	162	-1.26	0.2104
Variety	IpH2P1	IpH4P2	0.8847	0.4676	162	1.89	0.0603
Variety	IpH2P1	IpH5P1	-2.2563	0.4676	162	-4.83	<.0001
Variety	IpH2P1	IpH5P2	-0.1197	0.4676	162	-0.26	0.7983
Variety	IpH2P1	IpH6P1	-1.0000	0.4676	162	-2.14	0.0340
Variety	IpH2P1	OP32-75	-0.3370	0.4676	162	-0.72	0.4721
Variety	IpH2P1	OP34-55	-0.5463	0.4676	162	-1.17	0.2444
Variety	IpH2P1	OP34-65	-0.05533	0.4676	162	-0.12	0.9059
Variety	IpH3P1	IpH3P2	-1.0600	0.4676	162	-2.27	0.0247
Variety	IpH3P1	IpH4P1	-1.7523	0.4676	162	-3.75	0.0002
Variety	IpH3P1	IpH4P2	-0.2797	0.4676	162	-0.60	0.5506
Variety	IpH3P1	IpH5P1	-3.4207	0.4676	162	-7.32	<.0001
Variety	IpH3P1	IpH5P2	-1.2840	0.4676	162	-2.75	0.0067
Variety	IpH3P1	IpH6P1	-2.1643	0.4676	162	-4.63	<.0001
Variety	IpH3P1	OP32-75	-1.5013	0.4676	162	-3.21	0.0016
Variety	IpH3P1	OP34-55	-1.7107	0.4676	162	-3.66	0.0003
Variety	IpH3P1	OP34-65	-1.2197	0.4676	162	-2.61	0.0099
Variety	IpH3P2	IpH4P1	-0.6923	0.4676	162	-1.48	0.1406
Variety	IpH3P2	IpH4P2	0.7803	0.4676	162	1.67	0.0971
Variety	IpH3P2	IpH5P1	-2.3607	0.4676	162	-5.05	<.0001
Variety	IpH3P2	IpH5P2	-0.2240	0.4676	162	-0.48	0.6325
Variety	IpH3P2	IpH6P1	-1.1043	0.4676	162	-2.36	0.0194
Variety	IpH3P2	OP32-75	-0.4413	0.4676	162	-0.94	0.3467
Variety	IpH3P2	OP34-55	-0.6507	0.4676	162	-1.39	0.1660
Variety	IpH3P2	OP34-65	-0.1597	0.4676	162	-0.34	0.7332
Variety	IpH4P1	IpH4P2	1.4727	0.4676	162	3.15	0.0019
Variety	IpH4P1	IpH5P1	-1.6683	0.4676	162	-3.57	0.0005
Variety	IpH4P1	IpH5P2	0.4683	0.4676	162	1.00	0.3180
Variety	IpH4P1	IpH6P1	-0.4120	0.4676	162	-0.88	0.3796
Variety	IpH4P1	OP32-75	0.2510	0.4676	162	0.54	0.5921
Variety	IpH4P1	OP34-55	0.04167	0.4676	162	0.09	0.9291
Variety	IpH4P1	OP34-65	0.5327	0.4676	162	1.14	0.2563

The Mixed Procedure

Differences of Least Squares Means

Effect	Variety	_Variety	Standard Estimate	Error	DF	t Value	Pr > |t|
Variety	IpH4P2	IpH5P1	-3.1410	0.4676	162	-6.72	<.0001
Variety	IpH4P2	IpH5P2	-1.0043	0.4676	162	-2.15	0.0332
Variety	IpH4P2	IpH6P1	-1.8847	0.4676	162	-4.03	<.0001
Variety	IpH4P2	OP32-75	-1.2217	0.4676	162	-2.61	0.0098
Variety	IpH4P2	OP34-55	-1.4310	0.4676	162	-3.06	0.0026
Variety	IpH4P2	OP34-65	-0.9400	0.4676	162	-2.01	0.0461
Variety	IpH5P1	IpH5P2	2.1367	0.4676	162	4.57	<.0001
Variety	IpH5P1	IpH6P1	1.2563	0.4676	162	2.69	0.0080
Variety	IpH5P1	OP32-75	1.9193	0.4676	162	4.10	<.0001
Variety	IpH5P1	OP34-55	1.7100	0.4676	162	3.66	0.0003
Variety	IpH5P1	OP34-65	2.2010	0.4676	162	4.71	<.0001
Variety	IpH5P2	IpH6P1	-0.8803	0.4676	162	-1.88	0.0615
Variety	IpH5P2	OP32-75	-0.2173	0.4676	162	-0.46	0.6427
Variety	IpH5P2	OP34-55	-0.4267	0.4676	162	-0.91	0.3629
Variety	IpH5P2	OP34-65	0.06433	0.4676	162	0.14	0.8907
Variety	IpH6P1	OP32-75	0.6630	0.4676	162	1.42	0.1581
Variety	IpH6P1	OP34-55	0.4537	0.4676	162	0.97	0.3334
Variety	IpH6P1	OP34-65	0.9447	0.4676	162	2.02	0.0450
Variety	OP32-75	OP34-55	-0.2093	0.4676	162	-0.45	0.6550
Variety	OP32-75	OP34-65	0.2817	0.4676	162	0.60	0.5478
Variety	OP34-55	OP34-65	0.4910	0.4676	162	1.05	0.2953

The UNIVARIATE Procedure
Variable: Oil

Moments

N	570	Sum Weights	570
Mean	45.697614	Sum Observations	26047.64
Std Deviation	3.06360338	Variance	9.38566565
Skewness	-0.3385466	Kurtosis	-0.7152199
Uncorrected SS	1195655.44	Corrected SS	5340.44376
Coeff Variation	6.70407732	Std Error Mean	0.12832022

Basic Statistical Measures

Location		Variability	
Mean	45.69761	Std Deviation	3.06360
Median	46.11500	Variance	9.38567
Mode	46.59000	Range	13.66000
		Interquartile Range	4.75000

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	356.1217	Pr > |t|	<.0001
Sign	M	285	Pr >= |M|	<.0001
Signed Rank	S	81367.5	Pr >= |S|	<.0001

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.972946	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.076001	Pr > D	<0.0100
Cramer-von Mises	W-Sq	0.740237	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	4.323826	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	51.800
99%	50.830
95%	50.250
90%	49.515
75% Q3	48.100
50% Median	46.115
25% Q1	43.350

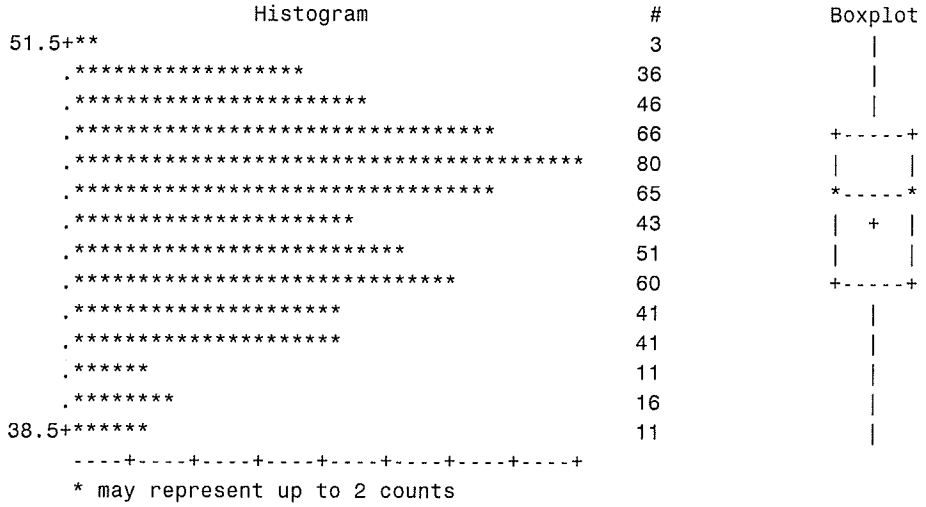
The UNIVARIATE Procedure
Variable: Oil

Quantiles (Definition 5)

Quantile	Estimate
10%	41.590
5%	40.450
1%	38.630
0% Min	38.140

Extreme Observations

-----Lowest----		----Highest----	
Value	Obs	Value	Obs
38.14	370	50.89	3
38.21	462	50.90	484
38.28	311	51.19	33
38.59	282	51.36	92
38.60	310	51.80	93



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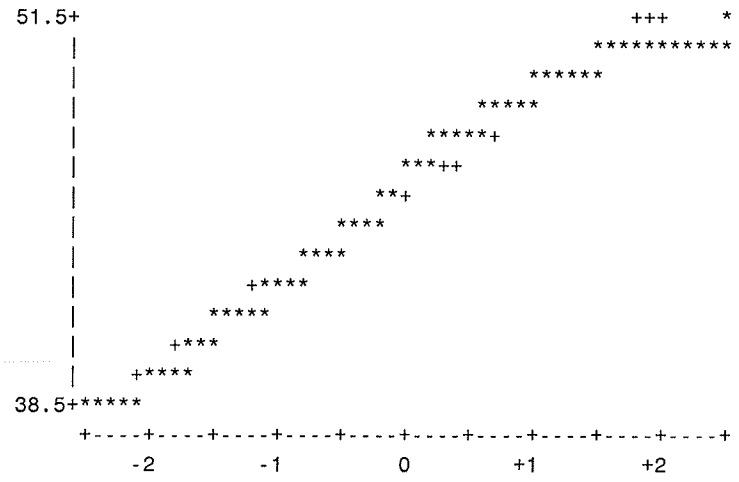
The UNIVARIATE Procedure
Variable: Oil

Normal Probability Plot



The UNIVARIATE Procedure
Variable: Resid (Residual)

Moments

N	570	Sum Weights	570
Mean	0	Sum Observations	0
Std Deviation	0.6933446	Variance	0.48072674
Skewness	0.08301147	Kurtosis	1.46974048
Uncorrected SS	273.533513	Corrected SS	273.533513
Coeff Variation	.	Std Error Mean	0.02904101

Basic Statistical Measures

Location		Variability	
Mean	0.000000	Std Deviation	0.69334
Median	0.027601	Variance	0.48073
Mode	.	Range	5.75897
		Interquartile Range	0.81982

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	6	Pr >= |M|	0.6450
Signed Rank	S	252.5	Pr >= |S|	0.9489

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.98646	Pr < W	<0.0001
Kolmogorov-Smirnov	D	0.046978	Pr > D	<0.0050
Cramer-von Mises	W-Sq	0.217349	Pr > W-Sq	<0.0050
Anderson-Darling	A-Sq	1.402873	Pr > A-Sq	<0.0050

Quantiles (Definition 5)

Quantile	Estimate
100% Max	3.0668316
99%	1.7348982
95%	1.1316726
90%	0.8416413
75% Q3	0.4043063
50% Median	0.0276013
25% Q1	-0.4155180

The UNIVARIATE Procedure
Variable: Resid (Residual)

Quantiles (Definition 5)

Quantile	Estimate
10%	-0.8664549
5%	-1.1436863
1%	-1.6550118
0% Min	-2.6921350

Extreme Observations

------Lowest-----		-----Highest-----	
Value	Obs	Value	Obs
-2.69213	147	1.75686	334
-2.18020	214	1.76788	215
-1.97086	370	2.22557	102
-1.92048	318	3.00883	316
-1.77599	120	3.06683	372

```
                Histogram                    #          Boxplot
3.25+

    .*                                       1             0
    .*                                       4             0
    .********                               32             |
    .*******************                    79             |
0.25+*******************************************  173      +--+--+
    .*****************************************    168      +-----+
    .******************                       69             |
    .********                               30             |
    .***                                    10             0
    .*                                       1             0
-2.75+*                                      1             0
     ----+----+----+----+----+----+----+----+----
     * may represent up to 4 counts
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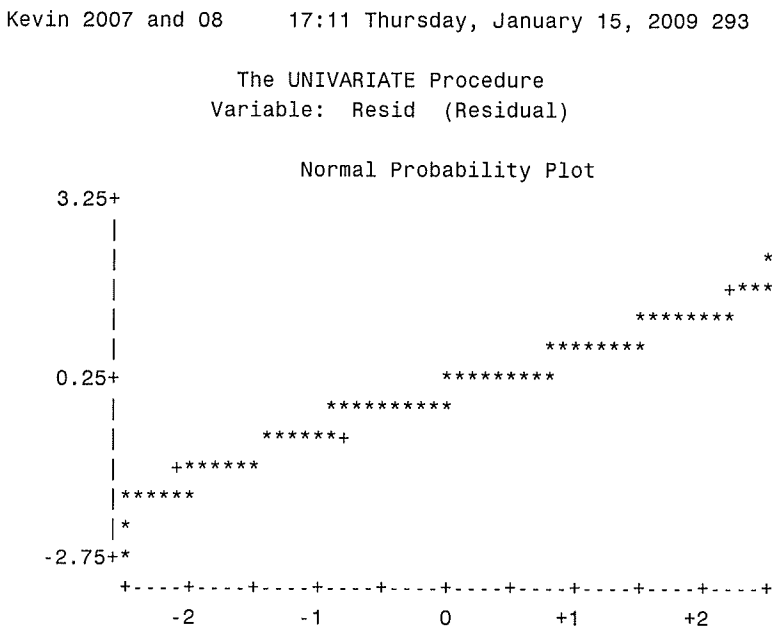

Appendix Table 1: Means for agronomic and seed quality traits for yield trials at 10 locations in western Canada in 2007 and 2008

Variable	Barrhead 2007	Leduc 2007	North Battleford 2008	Oakville 2007	Saskatoon 2007	Saskatoon 2008	Swan Lake 2007	Swan Lake 2008	Yorkton 2007	Yorkton 2008
Oil	48.31	47.92	48.25	41.12	43.18	47.66	43.75	47.13	42.51	47.14
Protein	47.15	46.85	44.83	51.34	49.20	46.78	44.82	49.37	48.88	46.22
Gluc.	13.23	13.29	12.31	15.27	10.19	13.62	11.96	12.63	12.78	9.89
SFA	6.54	-	6.91	-	7.65	7.06	7.84	6.76	7.53	7.08
Flower	47.26	50.53	48.42	51.96	67.26	50.93	52.04	52.67	-	50.91
Mature	100.84	98.60	98.14	-	88.05	98.30	95.44	96.58	94.37	97.91
Height	101.75	99.12	81.84	-	119.04	93.95	97.46	95.26	114.79	116.89
Vigour	8.58	7.58	5.67	5.89	-	4.77	4.42	6.09	2.88	-
Uniform	8.56	7.95	-	6.16	8.68	-	7.05	5.82	6.56	5.86