A Comparison of Pedigree Selection and Doubled Haploid Line Breeding Methods for Cultivar Development in High Erucic Acid Rapeseed

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Plant Science
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Abstract

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Major Professor: Dr. P.B.E. McVetty, Department of Plant Science

A plant breeder is constantly driven to develop new cultivars, and is only as good as their last cultivar developed, therefore, being able to determine the most efficient breeding method to use would be advantageous. Two contrasting breeding methods are currently used at the University of Manitoba to develop high erucic acid rapeseed cultivars, a pedigree selection breeding method and a doubled haploid line breeding method. This study compared the agronomic and quality performance of 60 pedigree selection families and 60 doubled haploid lines for three distinct crosses, in field trials, conducted at two locations in 2000. The agronomic characteristics days to first flower, plant height, days to maturity, and seed yield and the quality characteristics oil concentration, protein concentration, sum of oil and protein concentration, erucic acid concentration and glucosinolate concentration were investigated in this study. Neither breeding method was found to be superior in this study, however, more traits showed improvement using the doubled haploid line breeding method than the pedigree selection breeding method. The relative efficiency of the two breeding methods was also addressed by determining the number of families or lines that showed significant breeding advancements compared to standard selection criteria. When the selection criteria were set to equal performance of

the in-house Neptune BX check, the pedigree selection breeding method produced 45 desirable families while the doubled haploid line breeding method produced only 37 desired lines. When the selection criterion was set to produce a 10% increase in seed yield over Neptune BX, both breeding methods developed 20 desirable families/lines. When the selection criteria were set to produce a 20% increase in seed yield over Neptune BX, both breeding methods developed nearly equal numbers of desirable families/lines, with 12 for the pedigree selection breeding method and 14 for the doubled haploid line breeding method. This again suggests that there is little difference in the efficiency of the two breeding methods used, for these crosses, grown in these environments. Finally, resource requirements and relative costs associated with each breeding method were determined to distinguish if differences between methods occurred in this area. Considering total expenses incurred from the initial cross to completion of advanced yield trials for a single cross, there was a difference of approximately $18,000, between the pedigree selection breeding method and the doubled haploid line breeding method. There was also a savings of 61 days by using the doubled haploid line breeding method. There are more space requirements for the doubled haploid line breeding method, due to the laboratory space required to perform necessary microspore tissue culture techniques. Comparison of each breeding method to the marginal cost and marginal benefits showed that if the breeder performs over 10 crosses a year, and each cross made developed a registerable cultivar, and the maximum cultivar royalty is paid, a tiny marginal benefit will be seen. Direct cultivar development cost are substantially higher than the direct benefits that can be achieved, which is supported by the large negative net present value (NPV).

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1.0 Introduction

Brassica oilseed crops play an important role in Canada's economy. The ability of plant breeders to tailor the oil characteristics of Brassica species has lead to the development of many different kinds of Brassica oilseed types filling niche markets (National Research Council, 1992). High erucic acid rapeseed (HEAR) is one of these. HEAR oil has long been known for its ability and desirability as an industrial oil lubricant, and through market development, has been established as a reliable source of industrial vegetable oil in Canada's economy.

In Canada, HEAR oil is produced from two Brassica species, *B. napus* (Argentine rape or oilseed rape) and *B. rapa* (Polish rape or turnip rape). *B. napus* HEAR cultivars have high levels of erucic acid in the oil and low levels of glucosinolates in the meal, but these generally have lower seed yields than current *B. napus* canola cultivars.

Brassica oilseeds have become an important crop in Canada, because of their adaptation to temperate climate found in most of Canada. Brassica oilseed are also amenable to wide range of breeding techniques including traditional and modern ones. Traditional breeding methods use selection over several generations to not only incorporate new traits but also to make advances in agronomic and quality characteristics (Poehlman and Sleper, 1995). A contrasting approach to breeding is the development of doubled haploid lines that are homozygous for all loci in one generation (Poehlman and Sleper, 1995).

Brassica oilseed breeders can use many breeding methods to develop new improved cultivars. One objective of this project was to compare the pedigree selection

breeding method and the doubled haploid line development breeding method, to determine differences in the progeny for improved agronomic and quality traits.

The real success of a breeding method is the relative performance of individual progeny from each cross. Thus, a comparison of the number of superior performing families or doubled haploid lines (as determined by standard selection criteria used to screen for potentially registerable lines) in each of the three crosses grown at two locations in 2000, will address this issue and help determine differences between the two breeding methods.

The final objective is to compare the efficiency of resource utilization of pedigree selection breeding method and doubled haploid line breeding method, when developing families or lines, using economic analysis.

2.0 Literature Review

2.1 HISTORY

2.1.1 Rapeseed

The domestication of rapeseed (Brassica species) occurred concurrently with the adaptation of other economically important weeds, with direct references in the Chinese literature from 500 to 200BC and Indian Sanskrit in 2000 to 1500BC (Downey, 1983). During these ancient times, the oil from these domesticated plants was used as fuel for lamps and eventually for cooking purposes (Robbelen, 1991). It was later found that rapeseed oil had a unique ability to cling to water-washed and steam-washed metal and as a result, during the steam power era, rapeseed oil became a desired commodity (Canola Council of Canada, 2000). During World War II, a shortage of rapeseed oil lubricant used in steam powered ships, promoted the development of rapeseed production in Canada (Stefansson, 1983). In 1952, Dr. B. R. Stefansson, of the University of Manitoba, initiated an oilseed breeding program with *Brassica napus L.* summer rape strains introduced from Sweden. In these early years, the improvement of agronomically important traits was the main emphasis of this breeding effort.

2.1.2 Oilseed Brassicas

The genus Brassica belongs to the Cruciferae family. There is a common genetic

relationship that exists between the six agronomically important Brassica species. *B. napus* (oilseed rape), *B. juncea* (mustard), and *B. rapa* (turnip rape) are commonly known as the oilseed Brassicas, and have been demonstrated to be closely related through cytological studies. These three species have also been linked to three other agronomically important Brassicas species; *B. nigra* (black mustard), *B. carinata* (Abyssinian mustard) and *B. oleracea* (broccoli, cabbage, cauliflower, and kale).

In Canada, the two commonly used Brassica species used for canola/rapeseed oil, are *B. napus* (Argentine rape or oilseed rape) and *B. rapa* (syn. *B. campestris L.*) (Polish rape or turnip rape). The seed from these species contains over 40% oil, while the meal contains 36% to 44% protein. This protein rich meal provides a secondary market outlet for canola/rapeseed seed, which is used as a highly nutritive animal feed (Kimber and McGregor, 1995).

2.1.3 Genomic Relationships

Morinaga in 1934, (as cited in Olsson and Ellerstorm, 1980), demonstrated that a relationship existed among cultivated Brassica species, and strong cytological evidence showed that they all originated from a common ancestor with a basic haploid chromosome number of x = 5 or 6 (Heyn, 1977). The species with lower chromosome numbers (n = 8 to 12) apparently developed into separate monogenomic species through a process of autoploidy followed by a chromosomal loss and structural differentiation. The three species with higher chromosome complements appear to have resulted from natural crosses between the monogenomic species, with chromosome doubling occurring

before, during or after the interspecific cross to produce fertile amphidiploids (Singh, 1958 and Heyn, 1977). *B. napus* resulted from a natural interspecific hybridization between *B. oleracea* (2n = 18) and *B. rapa* (2n = 20), followed by chromosome doubling to produce a self-fertile amphidiploids (2n = 4x = 38) that was viable (Downey, 1983). This also occurred between *B. nigra* (2n = 2x = 16) and *B. rapa* or *B. campestris* (2n = 2x = 20) to produce *B. juncea* (2n = 36), and between *B. nigra* (2n = 2x = 16) and *B. olreacea* (2n = 2x = 18) to produce *B. carinata* (2n = 34). This genomic relationship is shown diagrammatically in the triangle of U, which was named after the Japanese scientist U who first illustrated this relationship (U, 1935).

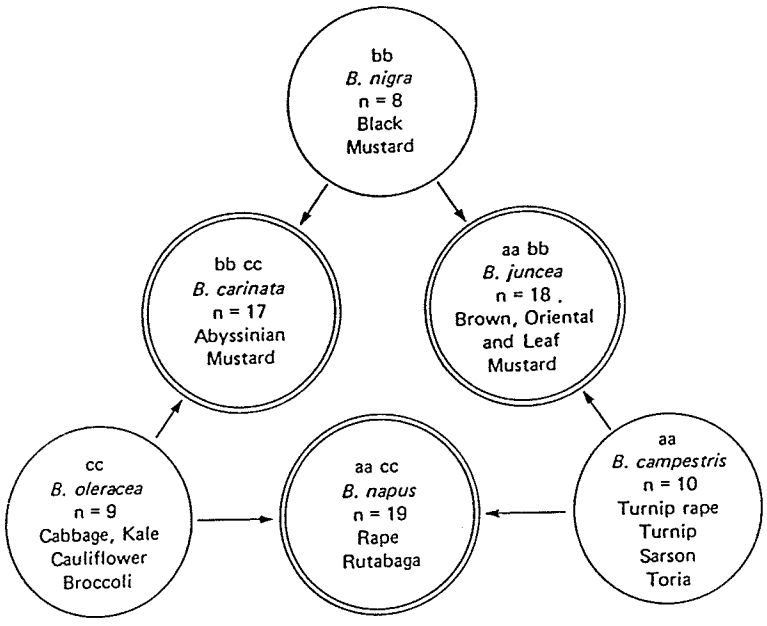

Figure 2.1: Triangle of U - genomic and chromosome relationship of Brassica species (Downey, 1983)

Knowledge of the genetic inter-relationships among Brassica species has enabled researchers to transfer useful characteristics from one species to another (Liu, 1985).

2.1.4 Growth Habits, Climatic Requirements and Reproductive Biology

Brassica species have two main growth habits, summer and winter, which can be divided into six main growth stages. Therefore, management of this crop makes reference to these growth stages. The following six main growth stages have been documented (Harper and Berkenkamp, 1975) and described below (Oplinger et al., 1989):

1. Stage 0 is the pre-emergence stage, which lasts 4 to 10 days from seeding.
2. Stage 1 is the seedling stage, when the young plants have emerged from the soil.
3. Stage 2 is the rosette stage, when a rapid increase in leaf number occurs. This stage lasts for several weeks for summer varieties.
4. Stage 3 is the bud stage or stem elongation.
5. Flowering begins stage 4 which lasts anywhere from 14 to 28 days in summer varieties.
6. Stage 5 is the pod/seed ripening stage, where the petals fall and pod filling occurs. This stage lasts about 35 to 45 days after initial flowering in summer varieties.

In summer varieties of oilseed rape, maturation occurs between 74 to 140 days after seeding (Oplinger et al., 1989). Oilseed rape is widely adapted, particularly to the

cooler climates of the temperate zones of the world. Oilseed rape grows best on medium textured, well-drained soil, with a soil pH as low as 5.5 (Oplinger et al., 1989).

Plant breeding practices rely heavily on the breeder's understanding of the reproductive biology of the species under development. Oilseed rape exhibits an indeterminate growth habit (Thompson and Hughes, 1986) that results in production of unrestricted numbers of lateral organs. The inflorescence of oilseed rape is racemose with no terminal flowers (Downey, 1983). The flowers are regular, bisexual and hypogynous with four free sepals in two whorls, median and transverse, and four free diagonally placed petals (Downey and Rakow, 1986). The flower is radial with four erect, prominent sepals and four alternating petals (Downey, 1983). The petal color is normally yellow, however, variations include several shades of yellow, orange and white, which suggests that several genes are associated with petal color development (Morice, 1960). Flowering first starts from the lowest bud on the main terminal raceme, resulting in the developing buds being positioned above the open flowers. Flowering of primary branches occurs subsequently. Flowers open first at the base of the branches and then open progressively upwards as the branches elongate, with three to five flowers per day opening. Flower initiation on secondary racemes occurs 3 days after floral initiation. There can be as many as 60 to 70, or as few as 32 to 36, flowers on the main terminal stem, and as many as 20 or as few as 8 to 12 flowers on the primary branches (Downey, 1983).

Oilseed rape is approximately eighty percent self-pollinated and twenty percent cross-pollinated, as a result of wind and insects (Downey, 1983). The fruit that develops following pollination is quite unique as the pod is a siliqua. The pod is made up of two carpels, which are separated by a false septum, thus providing two chambers. The number

of seeds in each pod can vary, but is generally in the 15 to 40 seeds per pod range. At maturity, the two carpels are easily split from the false septum, thus releasing the seeds (Appelqvist and Ohlson, 1972).

The seeds of *B. napus* rape are small (4 to 8 grams per 1000 seeds), but are larger than those of *B. rapa* and *B. juncea*. There are also differences between summer and winter oilseed rape varieties, with summer variety seed size being considerably smaller than winter variety seed size. The seed color of oilseed rape is mainly dark brown to black, but variability for seed color exists (Appelqvist and Ohlson, 1972).

2.1.5 Heritability

Heritability is an attribute of a quantitative trait in a population that expresses how much of the total phenotypic variation is due to genetic variation. In the broad sense, heritability is the degree to which a trait is genetically determined, and is expressed as the total genetic variance to the phenotypic variance (V_G/V_P). In the narrow sense, heritability is the degree to which a trait is transmitted from parent to offspring, and is expressed as the ratio of the additive genetic variance to the total phenotypic variance (V_A/V_P). This concept of additive genetic variance makes no assumption concerning the mode of gene action. Additive genetic variation responses to selection in a predictable way, providing information about how a trait will evolve. The predictability associated with narrow sense heritability makes this a better choice then broad sense heritability for quantitative agronomic and quality traits.

2.1.6 Agronomic Traits

2.1.6.1 Days to First Flower

Flowering is the end result of numerous physiological processes, biochemical sequences, and gene action. Due to their complexity and numerous interactions, the information on flowering available, is general in nature (Murfet, 1977). There is considerable variation in flowering response of *B. napus* cultivars under different environments (Thurling and Vijendra Das, 1979a) and it has been documented that under certain environments days to first flower can be highly heritable (Thurling and Vijendra Das, 1979b). However, a conservative estimate of the narrow sense heritability for days to first flower is low at 14.0% (Singh and Yadav, 1980). Partial dominant gene action decreases the number of days to first flower (Thukral and Singh, 1987). Additive gene effects are also important in governing the number of days to flowering (Thukral and Singh, 1987).

Time to first flower indirectly affects seed yield and is an important component for overall yield improvements. Appropriate number of days to first flower to the climate in which the variety is grown will result in noticeable seed yield increase (Thurling and Vijendra Das, 1979c), however, there is no documented evidence indicating the extent to which time to flowering should be reduced to maximize yield potential (Thurling and Kaveeta, 1992).

2.1.6.2 Height

Height is an important agronomic trait that indirectly influences seed yield and thus it is an important trait to consider when breeding new varieties. Both additive and dominant gene action are significant for the inheritance for plant height (Govil et al., 1984). There is evidence that shorter plants improve seed yields since shorter plants are more resistant to lodging (Thompson and Hughes, 1986; Saindon et al., 1990). Lodging decreases seed yield by increasing the occurrence of shattered pods (Thompson and Hughes, 1986). Shorter plants also produced less straw material, which implies that the plant will place more emphasis on seed yield, than on growth of the plant (Thompson and Hughes, 1986), i.e. have a higher plant index. Thus, it is advantageous to develop shorter plants. Plant height in Brassica species can be manipulated using a combination of alleles at loci controlling stem termination and maturity (Saindon et al., 1990).

2.1.6.3 Days to Maturity

High seed yields have also been positively correlated with earliness (reduced days to maturity) and rapid development (Campbell and Kondra, 1978), thus breeding efforts to produce earlier maturing cultivars to increase seed yield could be worthwhile. Days to maturity has a high broad sense heritability (77%) and moderate narrow sense heritability (57%) (Ringdahl et al., 1986). There is overdominance gene action, which is the phenomenon of heterozygotes having more extreme phenotypes than either homozygosity, but it is uncertain if this is at a single locus or due to combinations of

favorable alleles over several loci (Thukral and Singh, 1987). Non-additive gene effects are important in governing the number of days to maturity (Thukral and Singh, 1987).

2.1.6.4 Seed Yield

The most important breeding goal in Brassica improvement is increased seed yield. Unfortunately, seed yield is also one of the most difficult traits to improve due to low heritability and due to the expense (large plots with considerable equipment requirements) and difficulty associated with yield measurements, especially with calculations of harvest index. Seed yield has considerable genotype and environment interactions, as well, seed yield is determined in part by the time to first flower, the overall height of the plants and by the time to maturity. The numerous interactions and the complex genetics associated with seed yield also contribute to the difficulties associated with seed yield, thus making seed yield the most difficult of all breeding objectives. Yield is measured with respect to standard checks. Low narrow sense heritability of 11.1% for seed yield required many locations and many years to provide estimates (Singh and Yadav, 1980).

2.1.7 Quality Traits

2.1.7.1 Oil Concentration

The seeds that are harvested from Brassica plants are crushed in order to obtain

the oil, the most valuable element contained in the seed. The portion remaining following oil extraction, the meal is worth approximately half the value of oil. About 80% of the seed oil is concentrated in the cells of the cotyledons, with the endosperm layer containing 7 to 12%, and the remainder contained in the seed coat (Downey et al. 1975).

Oil concentration is influenced by many factors. These include temperature and moisture during the development of the seed, nitrogen fertilization, and the species grown. Conditions that favor high oil concentrations in *B. napus* are cool, moist growing conditions, combined with moderate rates of nitrogen fertilization (Downey, 1983).

Oil concentration displays both additive and overdominance gene action (Govil et al., 1984). The narrow sense heritability for oil concentration is approximately 0.26 (Grami et al., 1977).

2.1.7.2 Protein Concentration

Approximately 20 to 40% of the weight of oilseeds is protein, and a large proportion of this is storage protein. These storage proteins are specifically produced as a source of amino acids used to reduce the levels of externally supplied nitrogen required during germination and early seed growth. There exist significant differences between both protein concentration and amino acid composition among Brassica species, and this is attributed to two principle factors, genotype and environment.

Proteins consist of water-soluble albumins and salt-soluble, water-insoluble globulins (storage proteins). The albumins contain the majority of metabolically active proteins, which are essential in every cell. These proteins are also responsible for the

biosynthesis and degradation of secondary proteins, the globulins, which constitute the bulk of the storage proteins and which serve as nitrogen reserves during germination (Lonnerdal and Janson, 1972). Storage proteins are sequestered in protein bodies in the storage cells of the seed, while albumin is located in the cystol of the cell. The total amount of storage protein is more substantial than the albumin proteins. Storage proteins are considered to be in two classes; 12S and 1.7S globulins, that exist in approximately 60% and 20% respectively in the seed (Norton, 1989). 12S globulins have molecular weight ranging from 300,000 to 350,000 at pI 7.25. The 12S globulins consist of six subunits arranged in a triagonal antiprism and have dissociation-association characteristics, thus under the correct conditions the hexameric molecule can dissociate into its subunits or monomers (Schwenke et al., 1981). The 1.7S or napin protein group is highly basic (pI ca. 11.0) with a molecular weight of 12,000 to 14,000 (Norton, 1989). Table 2.2 shows the amino acid composition of both 12S and 1.7S storage proteins in *B. napus*.

Table 2.1: Amino Acid Composition of Storage Proteins of *B. napus* Integer Value (Norton, 1989)

Amino acid	Cruciferin (12S globulin)[a]	Napin (1.7S protein) subunit [b]		
		Large	Small	Total
Lysine	81	4	3	7
Histidine	46	2	2	4
Arginine	144	3	3	6
Aspartic acid	270	2	-	2
Threonine	120	4	-	4
Serine	129	4	2	6
Glutamic acid	434	22	10	32
Proline	151	10	3	13
Glycine	257	5	3	8
Alanine	177	3	4	7
Valine	182	6	-	6
Isoleucine	139	3	1	4
Leucine	233	6	2	8
Tyrosine	58	1	-	1
Phenylalanine	121	1	2	3
Half-cystine	31	5	2	7
Methionine	44	1	1	2
Tryptophan	20	-	-	1

Data from: [a] Schwenke et al. (1981)

[b] Lönnerdal and Janson (1972). B_3 Napin only

A protein is series of polypeptides chains made up of varying lengths and of varying amounts of the twenty different amino acids. The pattern that the polypeptide chains configure and how they interact in a three dimensional way result in additional secondary and tertiary structures. The different configurations individualize each protein to perform unique tasks. Protein synthesis, in the most generic terms, involves transcription at the gene level, and translation where the genetic material is converted into the polypeptide chains.

It has been estimated that the narrow sense heritability for protein concentration is 0.26 (Grami et al., 1977). The low narrow sense heritability associated with protein

concentration indicates that genetic advancement can be difficult.

2.1.7.3 Sum of Oil and Protein Concentration

Oil and protein concentration considered together have a higher narrow sense heritability (0.33) than either trait alone (Grami et al., 1977). These traits are highly negatively correlated indicating that it is necessary to select for both oil and protein concentration to maximize the intrinsic value of the seed. (Robbelen, 1978).

2.1.7.4 Erucic Acid Concentration

Although the concentration of oil in a seed is meaningful, it is equally important to characterize the oil for fatty acid composition. Vegetable oil is made up primarily of triglycerides. A triglyceride consists of a glycerol backbone molecule with three fatty acid carbon chains of varying length, ranging from 12 to 24 carbons in length esterfied to it. The chains of carbon atoms can also exhibit varying amounts of saturation, i.e. the number of hydrogen atoms attached to each carbon atom in the chain. For example 12:0 indicates that there is a twelve-carbon chain and it is completely saturated with hydrogens, known as a saturated fatty acid. A fatty acid with the formula 18:1 has eighteen carbons with a double bond present, or two hydrogen atoms missing from each of two adjacent carbon atoms. This is a mono-unsaturated fatty acid. As the number of double bonds present increase, the fatty acids are termed poly-unsaturated fatty acids. The basis for the oil quality differences in Brassica species is largely due to the

differences in the fatty acid compositions of the oil. For example, in HEAR there is high percentage of erucic acid, C22:1 or *cis*-13-docosenoic acid, meaning that a 22 carbon long monounsaturated chain with one double bond present. It is the erucic acid concentration present in rapeseed oil that provides its superior ability to cling to steam and water washed metal surfaces. Table 2.2 present fatty acid composition information of 2 different Brassica types grown in Canada (Downey, 1990).

Table 2.2: Percent Fatty Acid composition of Canadian Oils (Downey, 1990)

Fatty Acid	Formula	Oilseed Rape (HEAR)	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%

Fatty acid composition of rapeseed oil is largely determined by the genetic make up of the developing embryo, rather than the maternal parent (Downey and Harvey, 1963; Thomas and Kondra, 1973), but the levels of polyunsaturated fatty acids are strongly influenced by the environment during oil deposition and seed maturation (Downey, 1983). For example, higher temperatures during oil deposition tend to reduce polyunsaturated fatty acid levels.

There are two major stages in plants where the biosynthesis of fatty acids occurs in plants; synthesis, where the carbon chains are attached to the glycerol molecule, and modification, when alterations are performed on the fatty acid chains. Synthesis occurs in

the chloroplasts, where esterification of fatty acids to the glycerol molecule also occurs. This is driven by photosynthetic energy. Modification of fatty acids occurs in the endoplasmic reticulum, and can include elongation, desaturation, and/or reduction. The general biochemical pathway for rapeseed fatty acid synthesis and modification is shown in Figure 2.2 (Stumpf, 1989).

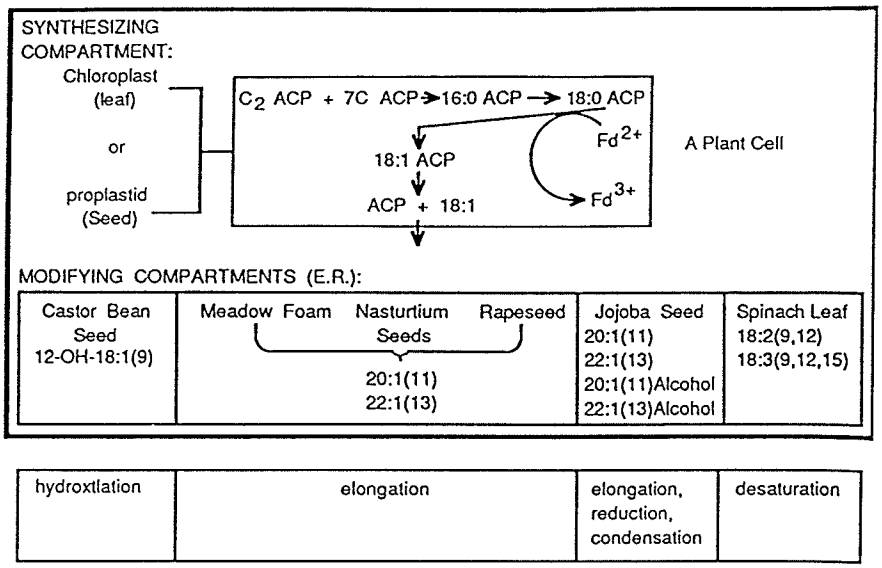

Figure 2.2: Biochemical Pathway of Major Fatty Acids (Stumpf, 1989)

Erucic acid (C22:1) biosynthesis has been shown to result from the addition of a two carbon fragment, to the carboxyl end of oleic acid (C18:1) to form eicosnoic acid (C20:1), followed by a second two carbon addition to form erucic acid (C22:1) (Jonsson, 1977). It has not been possible to obtain greater than 66% erucic acid concentration in the seed oil of Brassica species. This may be the result of the physical limitations of positioning the long C22 chain fatty acid in the middle position of the glycerol molecule

during the synthesis process in the chloroplast, not in the modification process in the endoplasmic reticulum (Tallent, 1972; Calhoun and Crane, 1975). Further increases in erucic acid concentration in *B. napus* would require the transfer of genes to enhance esterification of an additional erucic fatty acid chain on the number 2 carbon of the glycerol molecule (Taylor et al., 1992). Erucic acid concentration in rapeseed ranges generally from 0% to 55% (Scarth et al., 1992).

In *B. napus*, there are two genes, each with multiple alleles, acting in an additive manner, controlling erucic acid concentration (Grami and Steffansson, 1977; Harvey and Downey, 1964). With ongoing efforts to map genes using numerous RFLP markers, and quantitative trait loci (QTL's) controlling agronomically important traits, there have also been the localization of two erucic acid genes and three QTLs influencing seed oil concentration (Ferreira and Williams, 1994). There are at least five alleles that govern erucic acid concentration in *B. napus*, including e, E^a, E^b, E^c, and E^d which contribute <1%, 10%, 15%, 30% and 3.5% erucic acid respectively (Siebel and Pauls, 1989a). Thus, the manipulation of these genes can fix the concentration of erucic acid at values ranging from less than 1% to above 60% (Krzymanski and Downey, 1969). A single locus controlling erucic acid concentration has been mapped in *B. rapa*, but the positions of the loci in *B. napus* have not been reported yet (Thormann and Romero, 1996).

2.1.7.5 Glucosinolate Concentration

Rapeseed meal, the part that remains after extraction of the oil from the seed, has a well balanced amino acid composition, which makes it an excellent animal feed,

however, the use of rapeseed meal has been limited by the presence of a sulphur containing compounds called glucosinolates. Glucosinolates contain a central –S-C=N group with different aromatic and aliphatic side chains. When hydrolyzed by the enzyme myrosinase, present in all Brassica vegetative and seed tissues, thiocyanates, isothiocyanates or nitriles are released, resulting in bitter-tasting and toxic compounds (Figure 2.3).

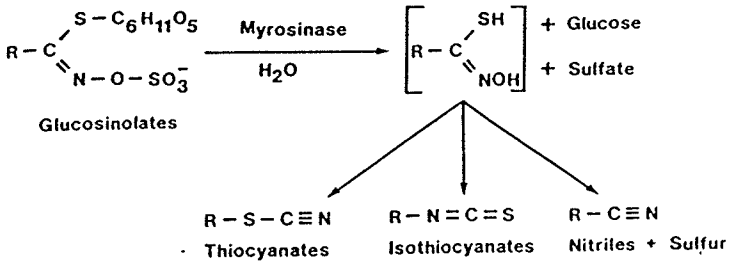

Figure 2.3: Chemical structure and enzymatic hydrolysis products of glucosinolates (Downey, 1983)

The myrosinase enzyme is heat inactivated as one of the initial steps in the oil extraction process to minimize the release of thiocyanates and other compounds. The concentration of glucosinolates in rapeseed meal is important for two reasons. First, the bitter taste imparted to the meal reduces palatability (Bowland et al., 1965) and can reduce intake by animals, thus reducing growth rate. The second, and more important reason, is that the by-product oxazolidinethione, has been shown to inhibit the function of the thyroid gland (Greer, 1950), thereby limiting the amount of rapeseed meal, which can be incorporated into the feed of non-ruminant animals such as pigs and poultry (Bell, 1977). The breeding objective is to develop cultivars with low concentrations of

glucosinolates (Downey and Robbelen, 1989). There are six major glucosinolates found in rapeseed, gluconapin, glucobrassicanapin, progoitrin, gluconapoleiferin, glucobrassicin, and neoglucobrassicin (Thompson and Hughes, 1986); but as many as ninety glucosinolates are known to exist in the Cruiferae family (Fenwick, 1982). As a result, rapeseed meal used in Canada and Europe as a high protein feed supplement must be "Canola grade meal". The canola grade meal is defined by having less than 20 micro-moles total glucosinolates per gram seed at 8.5% moisture, a definition adopted in Canada in 1997 (Canola Council of Canada, 2000). There are considerable differences in the amount of specific glucosinolates detected in various parts of the same plant – roots, stems, leaves, inflorescences, fruit, and seeds (Josefsson 1967, Downey, 1978) and these can even vary among the same organ with the highest concentration occurring during the period of most active growth (Paxman and Hill 1974, Jurges, 1978). Considerable research into seed glucosinolates has been done because of the significant economic interest. There are degrees of variation in the seed with respect the glucosinolate concentration, which have been correlated with the silique position on the plant (Josefsson, 1970).

Glucosinolates are derived from amino acids and are formed by a common biosynthetic pathway (Gander, 1976). The aglycone portion of the glucosinolate is derived from common amino acids (Kjaer, 1960) and then the C-2 atom and the nitrogen atom are incorporated through a serious of biochemical steps to produce a glucosinolate. Figure 2.4 shows the biochemical pathway used for the formation of a glucosinolate molecule using L-phenylalanine as the amino acid base (Gander, 1976).

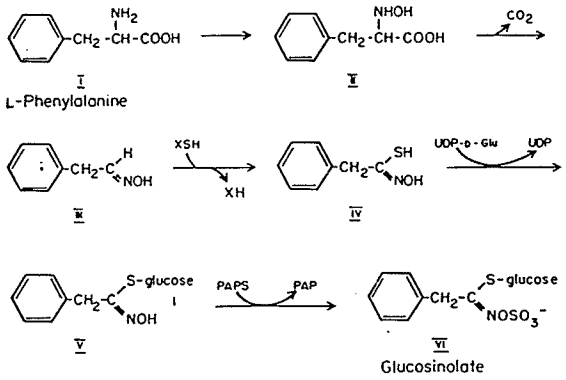

Figure 2.4: Pathway for the formation of a glucosinolate from L-phenylalanine (Gander, 1976)

Genetic differences in glucosinolate concentration are relatively small within Brassica species (Josefsson, 1970; Lein, 1970; Nami et al., 1972,; Nami and Hosoda, 1975). Genetic studies (Kondra and Downey, 1969; Krzymanski, 1970; Lien, 1970) indicated that there are three to five gene loci involved in the inheritance of glucosinolate concentration, depending on the type of glucosinolate. Gluconapin has three loci controlling the concentration, while glucobrassicanapin was determined by four or five loci with overdominance (Robbelen et al., 1989). Through reciprocal crossing, it was determined that glucosinolate concentration is determined by the maternal genotype rather than the embryonic genotype (Stefansson, 1983). The gene systems controlling the three major glucosinolates do not segregate independently of each other, which suggest these genes are control in the early stages in the biosynthetic pathway (Stefansson, 1983).

2.1.8 Herbicide Tolerance to the Broadleaf Herbicide, Bromoxynil

The high erucic acid rapeseed (HEAR) materials used in this research have an additional value-added component, i.e. they contain an herbicide tolerance transgene to the broadleaf herbicide bromoxynil. A transgenic plant is defined as plant that has had its genetic material altered in a way which does not naturally occur by mating to enhance pest control, improve agronomic performance, increase product quality and/or improve crop nutrition (Staniland, 1997). In this case, a single, dominant, herbicide tolerance transgene was introduced that confers very high levels of transgene resistance to an herbicide called bromoxynil (Freyssinet et al., 1989). Bromoxynil or 3,5-dibromo-4-hydroxybenzonitrile has a molecular formula of $C_7H_3Br_2NO$ and is used to control post emergent broad leaf species (Herbicide Handbook, 1994). It is considered to be a group 6 herbicide based on its mode of action (Crop Protection Guide, 2000). Group 6 herbicides contain photosynthetic inhibitors – nitriles/benzothiadiazoles (Crop Protection Guide 2000), which allows the herbicide to attack the broadleaf weeds at the leaf level. There are no known broadleaf weeds that have developed resistance to bromoxynil, thus making the insertion of this herbicide tolerance gene in HEAR cultivars advantageous.

2.2 HIGH ERUCIC ACID INDUSTRIAL OIL RAPESEED

2.2.1 History

A breeding program to develop high erucic acid, low glucosinolate rapeseed was initiated in 1969 at the University of Manitoba. This program utilized existing genetic

stocks, which had desirable agronomic traits. This initial work, conducted on two oilseed Brassica species, *B. napus* and *B. rapa,* focused on reciprocal crosses between high erucic and low glucosinolate parents. This breeding approach succeeded in increasing the concentration of erucic acid, desired for industrial purposes while achieving low levels of glucosinolates in the meal, producing new cultivars desirable for both industrial oil markets and animal feed markets (Calhoun and Crane, 1975).

2.2.2 Rapeseed and Canola

There are two types of rapeseed in use in the Canadian markets, those produced for edible oil products, and those produced for industrial oil products. The edible oil varieties, commonly known as canola, contain low concentrations of erucic acid, while the industrial oil varieties, commonly known as High Erucic Acid Rapeseed (HEAR), or non-edible oils, contain high concentrations of erucic acid. Regulations in Canada state that canola must contain less than 1% erucic acid in extracted seed oil (Eskin et al., 1996), while HEAR oil destined for industrial applications must have erucic acid concentration in the seed oil exceeding 46% (Kramer and Sauer, 1983). Canola meal must contain less than 20 umol glucosinolate /g seed at 8.5% moisture by Canadian government regulations (Eskin et al., 1996). Through conventional breeding methods, HEAR cultivars of *B. napus* rapeseed have been developed which have over 50% erucic acid in the oil and less than 20 umol glucosinolates /g seed at 8.5% moisture (Scarth and McVetty, 1991).

2.2.3 Uses Of High Erucic Acid Rapeseed

High erucic acid rapeseed oil, or HEAR oil, has established its place in history, from oil for lamps in ancient time to lubricants for steam powered ships in 20th century wartime. In the last 50 years, HEAR has expanded to new markets and new uses. A common use in North America of HEAR oil since 1961 is as a thickener and stabilizer component in peanut butter. Fully hydrogenated HEAR oil has been used for this purpose, but only at a maximum concentration of 2% of the weight of the finished peanut butter, thereby not causing health issue (Federal Register, 1977). A partial list of products in which HEAR oil is used includes: surfactants - hair conditioners, fabric softeners, detergents, lubricants – industrial oils, hydraulic fluids, photography reagent as silver behenate, pour point depressants, food emulsifiers, water repellents, plasticizers, waxes and resins, surface-active agents, fuel and fuel extenders, engineering thermoplastics, nylon 1313, synthetic fats, and cosmetics (Luhs and Friedt, 1993).

HEAR products have also made inroads in environmentally friendly lubricant markets, in which vegetable oil based lubricants replace the more commonly used petroleum based lubricants. As Canada is one of the world's chief HEAR oil exporters, it makes good economic sense to promote the potential of non-mineral oil based lubricants as a possible replacement, or substitute, for resource-limited petroleum based lubricants. In Europe, the emphasis on "green" products, i.e. products compatible with mankind and the environment, has encouraged increased interest in producing biodegradable lubricants, as well as other vegetable oil based lubrication products (Luhs and Friedt, 1993).

2.3 BREEDING METHODS

2.3.1 Background

Successful strategies for breeding and selection include three elements; breeding program methods or approach, operational experience, and breeders personal style. Of these, operational experience and style may contribute more to the success than the breeding methods or approach (Jensen, 1988). Breeding methods or approaches place emphasis on the type of population available to them. In an open pollinated population, such as *B. napus*, there is the presence of different homozygous genotypes and heterozygous genotypes. A single plant has the potential to produce a pure line, or new population, different from the original population. It is also assumed that homozygous genes (AA or aa) will remain homozygous, while heterozygous genes (Aa) will segregate, producing in equal portions, homozygous and heterozygous progeny, through successive generation of self pollination. Thus, through each successive generation in a self-pollinated population, heterozygosity will be reduced by half. Heterozygous plants may arise either through cross-pollination between different genotypes, or through mutations.

When the breeding goal is to developing new cultivars from a population, the initial step is to make selections from the available germ plasm, which requires identification of the available genotypes (Poehlman and Sleper 1995). It is important that the genes of interest, contributing to enhancement of agronomic or quality characteristics, be present in the source population, to develop future superior populations or lines.

Selection is one procedure used in breeding programs, and is a key component in crop improvement advancement methods, however, this requires identification and propagation of individuals, or groups of genotypes. Selection can lack effectiveness because phenotypic variability is usually a result of environmental influences as well as genetic ones.

Hybridization is another breeding procedure, which uses cross-pollination between genetically different parents to obtain gene recombination in the derived progeny. This can result in new gene combinations, hopefully producing types that fall outside of the range of the parents used. These are called transgressive segregates.

Hybridization and selection form the bases for breeding new cultivars of *B. napus* high erucic acid rapeseed at the University of Manitoba. There are several breeding methods available that incorporate both practices of hybridization and selection including bulk population, single seed decent, pedigree breeding, and doubled haploid line development. Pedigree breeding and the doubled haploid line development breeding methods are reviewed in detail below.

2.3.2 Pedigree Selection Breeding Method

The pedigree selection breeding method is a classical approach to breeding, and is defined by Poehlman and Sleper in 1995 as a procedure used in segregating populations, in which the progenies of the selected F2 plants are reselected in successive selfing derived generations, until near genetic purity is reached.

The pedigree selection method starts with an initial cross between two varieties,

which will be denoted as Parent 1 and Parent 2. These parents are from different genetic backgrounds, which is important when making genetic advancement of traits through creation of transgressive segregates. These parent lines are crossed, usually in the greenhouse, using hand emasculation and pollination techniques to produce viable seed. Approximately 50 to 100 F1 seeds are produced. When summer habit *B. napus* is used, this generation will take four months to complete, but this time is very dependent on the species used and its habit. This F1 seed are grown, under greenhouse conditions, denoted as the F1 generation. This generation starts with random selection of F1 seeds that will be grown, known as bulk planting. Individual plants are self-pollinated and the end result will be F2 seed. This generation will have a four-month duration as well. The F2 seed will then be planted in individual pots, to allow individual plants to be assessed. Usually 200 to 250 F2 plants are grown. At this stage, superior plants having superior desired traits will be harvested and selected. The selected superior trait F3 families will be grown in pots, so that, again, individual plants can be assessed. This breeding method will continue until the end of the F5 generation, and will have produced approximately 25 to 50 F5 families. The identity of plants and rows is maintained, and superior traits of the plants have been recorded. The seed is now transferred from the controlled environments of the greenhouse to the field for field trials, which will be the following generations.

For the field grown generations, several measurements are made to assess agronomic characteristics such as number of days to flowering, number of days to maturity, lodging, height of plants, and seed yield. Disease nurseries are grown at the same time as the yield trials to assess for disease resistance or susceptibility of the field grown generation. Quality analysis on the harvested seed is also conducted to determine

oil and protein concentration. Selections are based on the breeder's objectives, and only the superior lines or families advance to the next growing season.

After the selection is complete, the families chosen will enter preliminary yield trials. This will usually consist of 1 location in a multiple replications, in a multi-row plot test. Plants, as well as the seed are analyzed in a similar fashion to the previous nursery trials. The selected seed from each entry is usually combined or bulked and used for the advanced yield trials. Advanced yield trials are usually planted at several locations consisting of multiple replication of a multi-row plot test. Once again screening at the plant and seed stage is done. Once families have been selected, they are ready for the first and second years of official registration tests. Successful families or lines will be supported for registration and then can be used to begin pedigree seed production. Several years will be needed to increase the amount of pedigreed seed available. The total amount of time required to develop a cultivar using the pedigree selection breeding method can be 8 or 9 years, excluding commercial registration trials. A flow chart of the pedigree selection breeding method is shown in Figure 2.5.

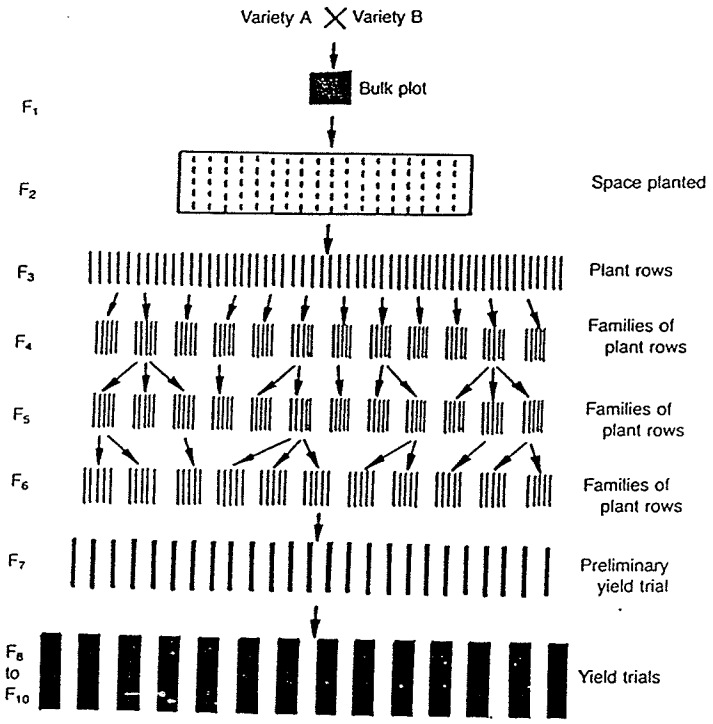

Figure 2.5: Pedigree Selection Breeding Method (Poehlman and Sleper, 1995)

There are many advantages and disadvantages to the pedigree selection breeding method of cultivar development. The most important advantage is that the costs associated with development are manageable. Another advantage is that by using progeny families or lines, only those plants with desirable characteristics are carried forward to the next generation, thus permitting the collection of self supporting genetic information. With respect to the disadvantages, this breeding method is quite labor intensive, and there is a need for detailed record keeping, but the most important drawback is time as this breeding method can take up to 12 years to develop new cultivars. The time needed can be decreased through the use of greenhouses for the beginning generations, and/or by using counter season breeding sites to achieve two field-based breeding generations per year.

2.3.3 Doubled Haploid Line Development Breeding Method

As previously stated, one of the major drawbacks associated with the pedigree selection method is the time required to reach homozygous lines. The development of a reliable and efficient microspore tissue culture system has provided rapeseed breeders with an alternative breeding method to achieve homozygosity in a single generation, compared to the five or six generations required for the pedigree selection breeding method, therefore, the second method to be considered involves the development of doubled haploid lines, using microspore tissue culture techniques.

Haploidy in plants was documented more than 60 years ago (Blakeslee et al., 1922; Kostoff, 1934). Haploids are individuals that contain half of the chromosome number of the normal diploid (2n), thus haploids (n) are individuals whose genomic constitution is the gametic chromosome number of that species, a trait with great potential usefulness. Generally, haploid plants are used in breeding programs because their chromosomes can be doubled; hence the name doubled haploid, creating lines that are completely homozygous at all loci (Poehlman and Sleper, 1995). Genetic segregation is simplified because allele masking is eliminated and transfer of genetic material is detected immediately, thus decreasing the number of plants required to be carried forward, producing savings in time and money.

The development of a cultivar using the doubled haploid line development breeding method is quite different from the pedigree selection breeding method. Again, the initial crosses are made between two parents to produce F1 seeds, taking 4 months in the greenhouse setting. The F1 seed are bulked. There will be random selection of the F1

seeds to be grown, consisting of at least 20 plants. The immature anthers are harvested from each plant. The microspores are released from the immature anthers and then undergo microspore tissue culture techniques, resulting in the development of an embryo which will ultimate developed into a mature plant. This process takes approximately 8 to 12 months to complete, consisting of both greenhouse and laboratory work. These plants, doubled haploid lines or doubled portions of haploid plants, will have two exact copies of the original haploid chromosomes, thus all loci are homozygous. It is unnecessary, therefore, to grow the segregating generations as in the pedigree selection breeding method. The doubled haploid line development breeding procedures must ultimately result in a plant that will produce sufficient seeds for use in the field. This seed is denoted as DH1 seed. If there is insufficient seed amount obtained, the DH1 plants are regrown in an additional four-month generation in the greenhouse to produce DH2 seed. Further generations do not change in genetic status since all traits are homozygous, the only advantage is to increase the seed quantity. This seed, either the DH1 or DH2, will go to field trials. These consist of single nursery rows 3 to 5 meters in length and follow the same type of analysis as in the nursery trials of the pedigree selection breeding method. After four months in the field, the seed is harvested and undergoes quality analysis same as described in the pedigree selection breeding method. The selected seed is denoted as DH3 seed, and is ready for preliminary yield trials, which will be done at 1 location in a four rep, 6-row plot test. The plants, as well as the seed are analyzed for oil content, protein content, fatty acid composition, glucosinolate concentration and the agronomically important traits mention under the pedigree selection breeding method section. The selected seed is now denoted as the DH4 seed that will be used for the

advanced yield trials. The seed is planted at up to 5 locations consisting of 4 reps of a 6-row plot test. Once again screening at the plant and seed stage is done. Once DH5 lines are selected, these will be ready for the two years of official registration tests. Successful entries or DH lines will be supported for registration and then can be used to begin pedigree seed production. Several years are needed to increase the amount of pedigreed seed available. The total amount of time required to develop a DH-line-based cultivar is 8 to 9 years from initial cross to commercial registration. The doubled haploid line development breeding method is shown in Figure 2.6.

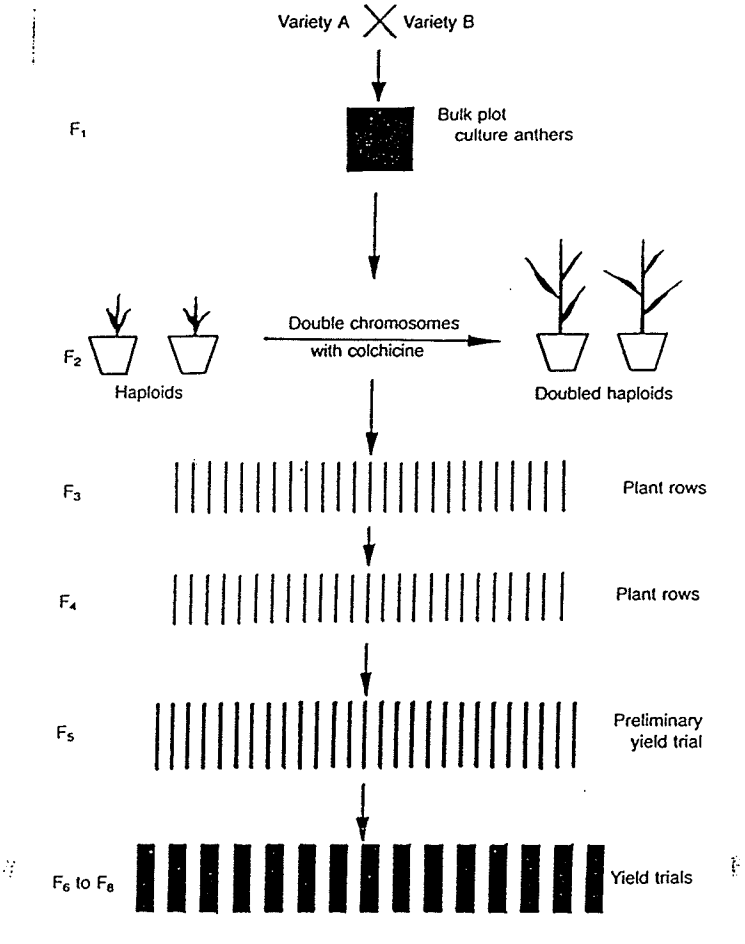

Figure 2.6: Doubled Haploid Line Breeding Method (Poehlman and Sleper, 1995)

As in the pedigree selection breeding method, there are also advantages and disadvantages associated with the use of the doubled haploid line development breeding method. The most obvious advantage is that the number of generations required to complete the process from initial cross to registration may be less than in the pedigree selection breeding method. When doubling occurs the plants become homozygous at all loci, therefore eliminating the differential expression that can arise with allele interaction. Doubled haploid lines are desirable for genetic studies because the homozygous genotypes can be propagated indefinitely by sexual means. The use of homozygous genotypes in replicated experiments facilitates the estimation of genetic and environmental components of phenotypic variation (Siebel and Pauls, 1989b). A final advantage is the use of doubled haploids can significantly reduce the number of plants that must be screened. When dealing with recessive traits, they will not be masked, and genetic ratios are simplified (Henderson and Pauls, 1992). Disadvantages of this method include the costs associated with the development of a new cultivars using the doubled haploid line breeding method. In addition, this method is quite difficult to perform and as a result trained personnel are required to understand the methodology. Another limitation of this method is that some species are recalcitrant to in vitro embryo production.

2.4.3.1 Microspore tissue culture techniques

In the Brassicas, DH lines are usually produced through the culture of male gametes or microspores (Ferrie and Keller, 1995), which was first demonstrated in 1982 by Lichter. The basic procedures are to culture the microspores to induce cell division

and embryo formation, culture the embryo and double the chromosomes to produce a fertile doubled haploid. To ensure doubling, the haploid embryos are subjected to a chemical called colchicine, which inhibits the spindle fiber apparatus resulting in doubled chromosome numbers. This application causes the pollen to mitotically divide symmetrically. (Mathias and Robbelen, 1991). Since each microspore has the potential of embryogenetic development into a plant, all genetic variation of the population of the microspores is potentially available (Ferrie et al., 1994) and should sample the whole gametic array with distinct genetic contributions from both parents (Powell et al., 1986). This involves selecting the buds containing the late uninucleate to early binucleate microspores which have not undergone the first pollen mitosis (Keller et al., 1975; Pechan and Keller, 1988). At this stage, microspores can be characterized by cytological analysis (Kott et al., 1988) or by using a flow cytometry (Fuchs and Pauls, 1992). Buds are then macerated to release the microspores. These will be cultured in liquid medium to recover the haploid embryos, which will then be exposed to chemicals to ensure chromosome doubling. The newly doubled haploid embryos will be grown under different media to produce callus, a mass of undifferentiated cells. Further propagation using auxins and cytokins, is used to develop plantlets that will ultimately grow and produce viable seed.

There are numerous factors that can affect the success of microspore embryogenesis, including ones that affect the plant such as genotype, donor plant or pollen, culture methods such as pretreatments and media components as well as environmental conditions (Keller et al., 1987; Huang and Keller, 1989). The frequency of embryo production and viability will depend on whether or not the above conditions are

optimal. For the purpose of this study, the efficiency of microspore tissue culture will be measured, as well as the time required to obtain finished DH lines i.e. length of the breeding cycle and the genetic gain obtained per breeding cycle (Charne and Beversdorf, 1991).

2.4 SELECTION PROTOCOLS

The legal right to commercialize a newly bred variety in any country usually depends on the results of official registration testing, thus when a developing new variety, it is essential to consider all facets of this area. Official registration testing provides information to help ensure that the agronomic performance, disease resistance and seed quality traits are suitable of a new variety for farmers and merchants. In Canada, the Variety Registration Office of the Canadian Food Inspection Agency (CFIA), based on the evaluations and procedures determined by the Western Canadian Canola/Rapeseed Recommending Committee Inc. (WCC/RRC), registers Brassica oilseed crop varieties for use in western Canada.

For the purpose of this thesis, the specialty and contract registration committee or SCRC, (part of the WCC/RRC Inc.) recommendation standards involving the use of one or more varieties designated as "check" against which the trait of the new entries or strains is compared. This will ensure the developing families or DH lines are on the path to being successful registered, denoted as acceptable families or lines (AFL).

2.4.1 High Erucic Acid Standard Check

The checks used by the SCRC of the WCC/RRC for high erucic acid rapeseed contract registration tests are Reston, Defender, Excel and Legacy. Defender, Excel and Legacy are canola quality checks used for blackleg resistance and maturity. Reston is a high erucic acid rapeseed used for yield, oil concentration, and protein concentration check. In this study, Neptune BX was used as the internal check because it contained the bromoxynil herbicide resistance gene of interest in this study. Neptune, the non-herbicide tolerant form of Neptune BX, was developed at the University of Manitoba and registered on January 20th 1995 by the Variety Registration Office of the CFIA. Neptune summer rape (*B. napus L.*) was a high erucic acid rapeseed cultivar with canola quality meal, with erucic acid concentration of approximately 53.5% and with 8.3 umol/g of aliphatic glucosinolates in the meal (McVetty et al., 1996). Other characteristics of this cultivar were a yield mean of 2263 kg/ha, a days to maturity mean of 105 days, a mean of 3.1 lodging on a scale from 1 to 5, a mean height of 108 cm, mean seed oil concentration of 458 g/kg, and mean meal protein concentration of 450 g/kg (McVetty et al., 1996). Neptune was evaluated for disease resistance to black leg and classified as moderately resistant. Neptune BX is six backcross derivative of Neptune containing the bromoxynil herbicide resistance gene, making it an excellent cultivar to be used as an internal check against the developed families and doubled haploid lines created in this study.

2.5 ECONOMICS ASSOCIATED WITH HEAR

Economics is defined as the study of efficient allocation of limited resources to produce goods and services used to meet societal demands (McConnell et al., 1999). In this project, the allocation of limited resources are the financial costs, space allocations and the time frames associated with two contrasting high erucic acid rapeseed breeding methods to ultimately produce superior new high erucic acid rapeseed families or lines that could be entered into official registration tests.

The diverse demand for the development of new varieties exists partly due to the diversity that exists in the numerous markets. Markets are defined as the organized exchange of commodities (goods, services, or resources) between buyers and sellers within a specific geographic area and during a given period of time; or more specifically buyers who want a good - the demand side of the market and sellers who have it - the supply side of the market (McConnell et al., 1999).

2.5.1 The Laws of Supply and Demand

Demand and supply concepts define the market for a product. The law of demand states that the higher the price, the less quantity is demanded, while the lower the price, the more quantity is demanded. This results in a downward sloping line on the price/quantity graph. There are numerous determinates which can alter the slope, such as, consumers taste and preference, income, consumer number, the expectations for the product, and most importantly the price of other similar products that exist.

The law of supply states that as the price rises, the quantity supplied rises, and as the price falls, the quantity supplied falls. Supply is defined as the amount of a product a producer is willing and able to produce or sell and in relation to price, results in an upward sloping line on the price/quantity graph. Changes in supply result from resource price, technology costs, taxes, price of other goods and the number of sellers in a market.

Graphically, where the two supply and demand lines meet is called a market equilibrium, as represented in Figure 2.7.

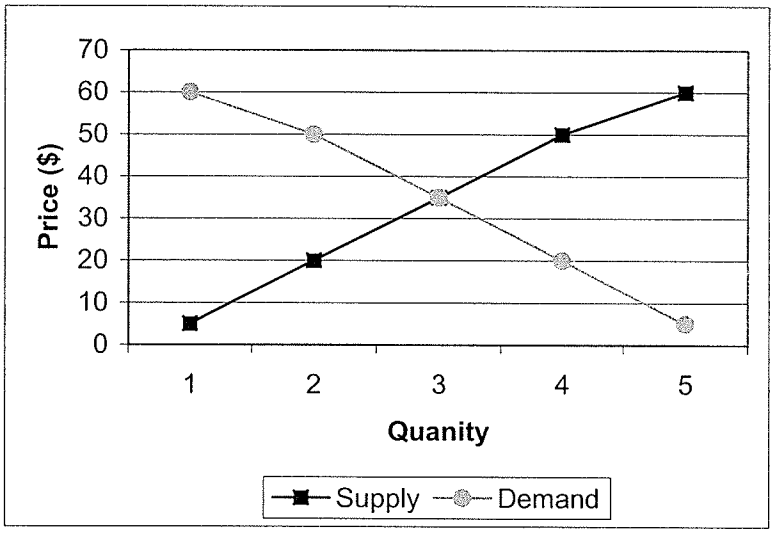

Figure 2.7: Supply and Demand: Market Equilibrium (McConnell et al., 1999)

As the primary focus of this project is on development of a product, cost factors can become very complex, especially with respect to the antagonistic relationship between supply and demand; thus, a few assumptions were made. For the cost analysis, demand was assumed to be constant or constantly increasing, therefore all calculated costs are considered solely on the supply side. A goal of the cost analysis was to

determine the efficiency levels. Consideration of the demand side was limited to an in-depth look at the markets and potential markets that exist in Manitoba.

2.5.2 Markets

Crops containing vegetable oil high in erucic acid are utilized as a raw material for many industrial purposes (Kramer and Sauer, 1983). In 1998, Manitoba farmers received approximately $2.2 million from high erucic acid rapeseed cultivar production, and a five fold increase in demand by 2002 is predicted (Gallowy, 1999). Total Brassica oilseed production in Canada in 1999 was estimated at $2.883 billion, oilseed processing contributed half billion dollars indirect value added, and over $1 billion in spin-off benefits in the economy (Statistics Canada, 2001).

High erucic acid rapeseed oil has found a place in a diverse set of markets. Opportunities at the seed level, or at a product level, exist for either retained use in Canada or for international export. For example, the United States imported 40 million pounds of HEAR oil in 1990 (Van Dyne et al., 1990). The opportunity for Canadian expansion of the industrial use of erucic acid simply to meet the demands of the United States has been quite significant, however, export markets of seed oils and products are not limited to the United State. It is suggested that this industry will only grow as the demand for raw and developed product increase, however, in Canada this industry is very proprietary, so availability of current information on annual HEAR acreage and HEAR oil exportation is very limited.

Market demands for canola oil seed have created export potential for industrial rapeseed oil and products. The Canadian oilseed industry is a leader when it comes to

standards and safety protocols. Canada strives to not only meet but also to exceed expectations in this area, thus allowing high erucic acid rapeseed oils and products to find a strong market place in a canola oil dominated industry. HEAR products and production exhibits diverse markets using an identity preserved system that ensures segregation from canola production and products. Investments in Canada's oilseed handling infrastructure have resulted in one of the most advanced oilseed handling and logistical systems. It is capable of handling, cleaning, delivering and separating identity preserved materials to all ports along the Pacific Ocean and the Great Lakes (Canola Council of Canada, 2000).

High erucic acid rapeseed oils can be utilized in several forms and they are a major player in many markets (Nieschlag and Wolff, 1971):

i) HEAR oil can be used raw, without further processing.

ii) Erucic acid can be obtained from the oil and then transformed into derivatives.

iii) Erucic acid can be cleaved at its unsaturated linkage to yield two different acids (brassylic acid (13C) and perlargonic acid (9C)) that can be further processed to provide many useful chemical products.

At the present time, high erucic acid rapeseed cultivars provide crop diversity and financial sustainability for Manitoba farmers because of the diverse market opportunities for either foreign or domestic use of HEAR oil. The physical and chemical properties of industrial rapeseed oil have allowed further diversification into new markets. Table 2.3 shows the unique qualities associated with this oil (Van Dyne et al., 1990).

Table 2.3: Physical and Chemical properties of industrial rapeseed oil (Van Dyne et al., 1990)

Properties	Industrial Rapeseed (HEAR Oil)
Specific gravity (g/cc)	0.91
Specific heat 20°C	0.488
Pour point (°C)	-12
Viscosity	260
Refractive index	1.472 (25°C)
Flash point (°C)	282
Smoke point (°C)	218
Dielectric constant (cgs units)	3.06
Saponification number	170 to 180

When analyzing the diversified markets associated with HEAR oil, it is best to divide them into three basic groups:

1. Oil.
2. Erucic acid and derivatives.
3. Cleavage products of erucic acid.

2.5.2.1 Oil

Triglyceride containing oils with high levels of erucic acid are remarkably stable at high temperatures due to the long chain hydrocarbon (22C) and an isolated double bond. These oils have a high flash (282°C) and smoke points (218°C), enabling them to withstand high temperatures. This allows lubricants using erucic acid to tolerate high temperatures, but also remain fluid at low temperatures (Kramer and Sauer, 1983).

HEAR oils have a high degree of lubricity, and adding high erucic triglycerides to mineral oil increases the oiliness and improves oil durability under high speed and high-pressure operations. Therefore, these improved oils have functioned exceptionally well either directly as a lubricant or in lubrication formulations (Van Dyne et al., 1990). The excellent lubricity is illustrated by their use in textile, steel and shipping industries as spinning lubricants; as metal forming, rolling, fabricating and drilling oils; and as marine lubricants (Nieschlag and Wolff, 1971).

Hydrogenated HEAR oils make hard, glossy waxes (Miwa and Wolff, 1963), while sulfurized oils can be used in rubber compounding as a lubricant. There are also many functions as dielectric fluids (Van Dyne et al., 1990).

2.5.2.2 Erucic acid and derivatives

This category includes erucic acid and its hydrogenated derivatives, behenic acid (docosanoic acid) and erucyl (13-docosen-1-ol) and behenyl (docosan-1-ol) alcohols and other derivatives such as esters, amides, amines and metal salts.

Erucamide is one of the most well known premium products made from erucic acid (Molnar, 1974). This amide and its N-substituted derivatives have served as processing aids, mold release and slip promotion, anti-block agent for plastic films of polyethylene, polypropylene, polyesters, polystyrene and various copolymers. Figure 2.8 shows the chemical alteration path erucic acid would follow to produce erucamide and derivatives.

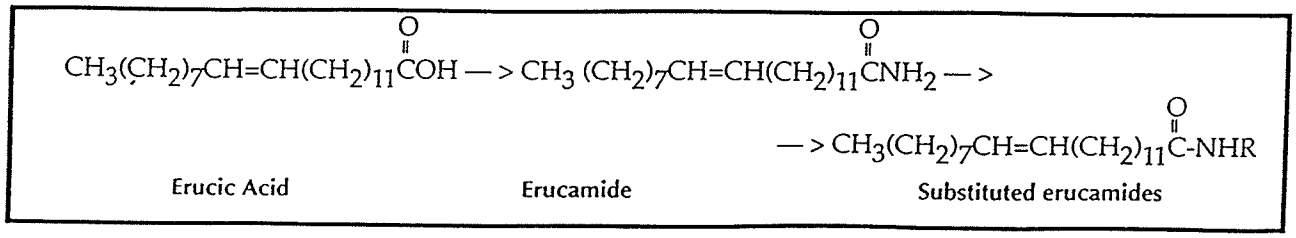

Figure 2.8: Erucic acid and erucamide derivatives (Van Dyne et al., 1990)

Much of the exported erucamide to the United States has been for the plastic industries needs. It is added to plastics at low concentrations to function as a lubricant to speed and ease production of plastic parts and to provide a thin layer on the plastic surface to prevent film sheets from adhering to one another (Van Dyne et al., 1990).

Di-substituted amides have a potential use as plasticizers for polyvinylchloride (PVC) plastics, resulting in the products having good tensile strength and elongation, low brittle points, low volatility losses, and low extraction loss and compatibility (Mod et al., 1969).

There are also many formulations that include behenic acid, a product of hydrogenation of erucic acid. These include use for coatings for carbon black used in rubber formulations, coatings for paper and plastic films used in photothermography, and in the production of audio and optical tapes.

Behenic acid, its esters and derivatives also enhance performance in pharmaceuticals, cosmetics, fabric softeners, and hair conditioners and rinses. These large hydrocarbon-like molecules serve as flow improvers, and pour point depressants for distillate fuel oils. They can also serve as pumping aids for offshore crude oils. In textile companies, these materials are used as antifriction coatings. Release agents for injection

moldings of thermoplastic polymers as well as increased drug absorption in pharmaceutical suppositories result from the use of this acid.

Aluminum complexes made from behenic acid improve properties of lubricant oils and grease formulations and marine coatings.

Fatty amines based on erucic or behenic acid can take the form of primary, secondary, tertiary or quaternary amines depending upon the level of substitution on the N atom. These will serve as the precursors for, or act as antistatic agents, emulsifier and solubilizers, conditioners, wetting agents, surfactants, dispersants, and corrosion inhibitors. There is the possibility that these compounds can be used as flotation agents for recovery of metals and minerals in the mining industry.

Hydrogenation of high erucic acid oils can alter the point of unsaturation and/or the carboxyl group, depending on the catalyst and conditions used, thus producing the saturated and unsaturated alcohols – erucyl [$CH_3(CH_2)_7CH{=}CH\text{-}(CH_2)_{11}CH_2\text{-}OH$] and behenyl [$CH_3(CH_2)_7CH_2CH_2(CH_2)_{11}CH_2\text{-}OH$] alcohols (Van Dyne et al., 1990). These are used in cosmetics, and in other personal care products, in pharmaceuticals, thermographic printing inks, and in antifriction coatings for magnetic tapes.

2.5.2.3 Cleavage products of erucic acid

New markets are being developed for HEAR oils for products derived from brassylic acid, a 13 C dicarboxylic acid obtained by cleavage of erucic acid at its double bond (Nieschlag et al., 1967b; Carlson et al., 1977). From the cleavage reaction there is

also a 9C by-product, pelargonic acid, which also has potential for further use. Figure 2.9 shows the cleavage of the erucic acid into its two derivatives

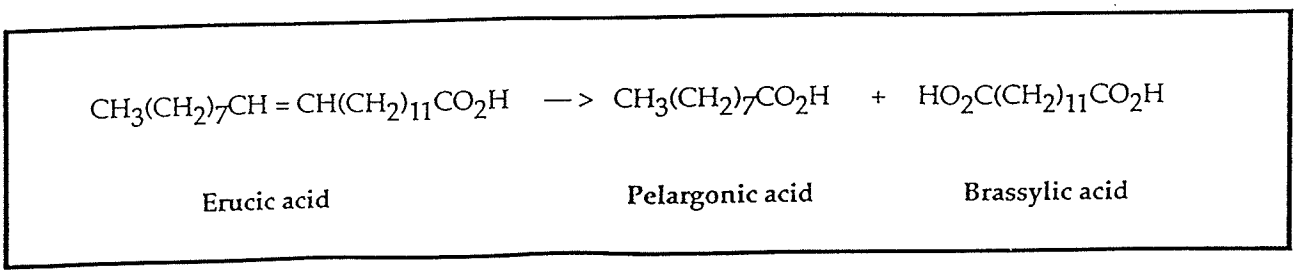

Figure 2.9: Erucic acid and its derivatives as a result of a cleavage reaction (Van Dyne et al., 1990)

Many items are made from bassylic acid such as alkyl, allyl and vinyl ester. The first type has been used for PVC plastics and synthetic lubricants (Nieschlag et al., 1964, Nieschlag et al., 1967a; Nieschlag et al., 1969, Chang et al., 1975), while the other two form polymers and copolymers, used in molding compounds, reinforced plastics, laminates, sealants and coatings (Chang et al., 1967; Chang et al., 1969; Chang et al., 1974).

Brassylic acid has great potential in the growing plastics industries, resin and nylon industries and this suggests that the greatest quantities of these polymers will be used in the automotive industry. Long chain nylons, such an 11 & 12, have found important niches in the automotive parts and product lines because their properties match specifications where performance and weight restrictions are critical. Different nylon products such as 13, 613 and 1313 are prepared from cleavage products of erucic acid, such as brassylic acid (Kestler, 1968; Perkins et al., 1969; Greene et al., 1967; Greene et

al., 1969; Nieschlag et al., 1977). All though these markets are fairly new, further development may lead to a commercially viable nylon market.

Pelargonic acid (9C) is used as an active ingredient in two unique pesticide products. First, it is used as the active ingredient for a non-specific broad spectrum herbicide that was registered in 1994, and has a commercial name of Scyth. The second use of pelargonic acid is as a fruit blossom thinner that promotes returning blooms and increases fruit size and quality in apples and pears.

2.5.2.4 Meal

Meal is the by-product after the seed has been crushed. The meal has a market as a protein supplement in livestock rations. The ability to reduce the levels of glucosinolates to non-toxic levels has increased markets not only in the beef industry but also in the hog and poultry industries as well.

The bulk of Canada's low glucosinolate meal supply is exported to the United States feed ingredient market, which imports over a million tones annually. This represents eighty percent or more of the Canadian total meal exports. Feed deficient Asian countries such as Japan, Taiwan and South Korea are also a consistent market for Canadian low glucosinolate meal (Canola Council of Canada, 2000).

2.5.3 Identity Preserved Crop Production System in Canada

The distribution and marketing of high erucic acid rapeseed cultivars to farmers is on a contract based, closed loop, identity preserved system. This identity preserved system is regulated by the Federal Government of Canada. This system requires the segregation of high erucic acid rapeseed, from the time of initial seeding through to seed crushing, from other rapeseed or canola varieties of any kind. The production and crushing of high erucic acid rapeseed cultivars and derived seed products is done in Canada by CanAmera Foods. CanAmera Foods control the seed distribution of HEAR cultivars, the contacts for production growth, and seed crushing plants in Altona and Russell in Manitoba and at Nipawin in Saskatchewan. The by-product meal, due to its low levels of glucosinolates, enters the low glucosinolate Brassica animal feed markets after crushing is completed. The primary determinant on the number of hectares that are contracted for production in Canada annually is set by market requirements for high erucic acid rapeseed oil. Premiums are paid to growers. This premium is dependent, in part, on the relative performance of current high erucic acid rapeseed cultivars and current canola cultivars. Growers need a financial incentive to grow high erucic acid rapeseed cultivars instead of canola cultivars because there are additional management costs, which would ultimately result as a loss to the farmer, if not compensated by a premium.

2.5.4 Cost Factors

Assessing two very distinct breeding methods to develop new varieties can present many challenges, especially when comparisons are to be made. To alleviate these challenges, both breeding methods were assessed using marginal benefits and cost concepts. Costs were separated into two categories, fixed and variable. Fixed costs were those costs that did not fluctuate with the volume, where variable costs were those costs that changed directly with volume. Graphically, with respect to costs and volume, fixed costs appeared horizontal, while variable costs appeared to be sloping upwards, showing the normal relationship of increased costs with increased volume. In costs associated with economics of scale, the cost per unit falls with increasing volume of production.

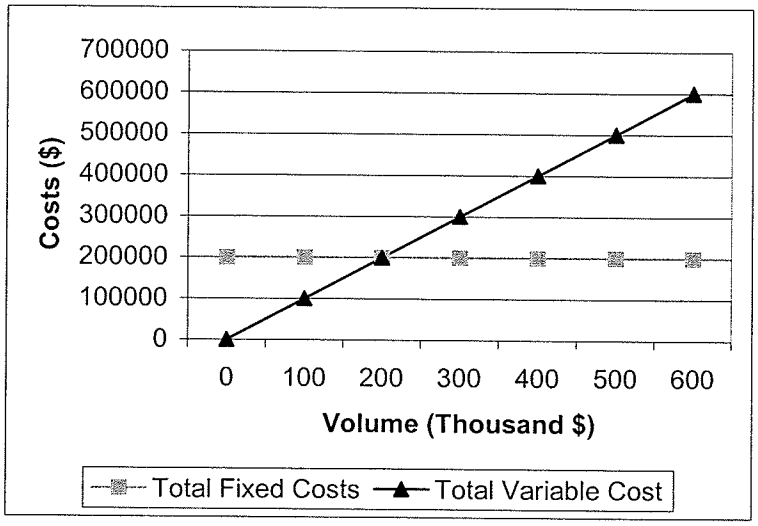

Figure 2.10: Fixed and variable costs as compared to volume

3.0 Materials and Method

3.1 DEVELOPMENT OF PEDIGREE SELECTION FAMILIES

Three separate and distinct crosses were used to generate the F4 families used in this study. The crosses were as follows:

Cross I: 03801BX/Neptune*3//Neptune

Cross II: 03801BX/Neptune*3//(Castor/Quantum) F_4

Cross III: 03801BX/Neptune*3//(Mercury/Cyclone) F_4

Cross I has the narrowest genetic diversity between parents, while cross II has the largest genetic diversity between the parents. Cross III is an intermediate in genetic diversity as compared to cross I and II.

3.1.1 Commonalities among generation development

The generations involved in development F4 families using the pedigree selection breeding method have many overlapping methodology, therefore these will be discussed first, followed by a description of the differences in associated with each generation. All seeds of the parental through F3 generation were planted into a soil-less mixture, metro-mix, into flats at a depth of six millimeters. The flats were lightly water and placed into a growth room. The growthroom used was an Enconaire, model GR-192-5 set to a 17 hour, 21°C day and 7 hour, 16°C night. Growth in the growthroom occurred for approximately twelve days, where water was applied every

second day. When the first true leaf was present, the flats were sprayed with bromoxynil herbicide at 560 g a.i. ha^{-1} to identify plants with bromoxynil resistance. The best developed plants were transferred from the flats into 15 cm plastic pots. The pots contained a soil mixture of black soil, sand, and peat moss in 2:2:1 ratio respectively. The pots were placed into the greenhouse and water applied every second day. Temik, a systemic insecticide used to control aphids, along with NPK 20-20-0 fertilizer at 8 ml l^{-1}, were applied to the plants just before bolting. Plant watering was increased to a daily regime. Selfing bags (clear plastic) were placed over the plant at the beginning of flowering, to ensure that the pollen was transferred to the style of the same plant, guaranteeing "selfing". Visually, plants showing signs of weakness or mutation were discarded. This was the primary selection applied to the pedigree selection breeding method. After seed set had occurred, watering of the plants was gradually reduced, to promote seed maturation. Seeds harvested from each mature plant were packaged in individual envelopes.

3.1.2 Development of F1 seed

All of the parental lines used in the crosses were planted in the beginning part of the month October, in 1998. Twelve seeds were randomly selected for each female line and planted, while thirty-six seeds were randomly selected for each male line and planted 12 at a time in weekly intervals, starting 1 week before the female line was planted. The female line plants were sprayed at the one true leaf stage with bromoxynil herbicide to identify parental plants that were resistant to bromoxynil

herbicide. The male parent plants were not sprayed with the bromoxynil herbicide. Visual selections of the eight best bromoxynil resistant female parent plants were transferred from the flats into fifteen centimeter plastic pots, while sixteen of the best male parent plants based on visual selection were transferred, two to a pot.

Transfer of pollen from the male parent plants to the female parent plants required hand emasculation techniques on the female, where the anthers are removed, and then direct hand transfer of the pollen from the male plants to the appropriate female plants. The female plants that were successfully pollinated were tagged and allowed to mature and set seed. After seed set had occurred, watering of the plants was gradually reduced. Seeds were harvested from each of the eight female plants per cross and packaged in individual envelopes. Harvesting of the F1 seeds for all three crosses was completed in by the end of January 1999.

3.1.3 Development of F2 seed

The F1 seeds were planted February 4, 1999. Twenty-four seeds were randomly selected from each of the eight packages of F1seeds and planted. When the first true leaf was present, the flats were sprayed with bromoxynil herbicide. This occurred on February 16, 1999. The plants were scored on February 18, 1999 to verify bromoxynil herbicide resistance. All F1 plants were resistant to bromoxynil herbicide. Visual selection of the best twelve F1 plants were transferred to 15 cm plastic pots. Harvesting of the F2 seeds for all three crosses was completed by the end of May 1999.

3.1.4 Development of F3 seed

The F2 seeds were planted May 13, 1999. Sixteen seeds were randomly selected from the F2 seeds packages for each cross and planted. The F2 plants were sprayed with bromoxynil herbicide at the first true leaf stage. As expected, 75% of the F2 plants were resistant to bromoxynil herbicide, while 25% of the F2 plants were susceptible to bromoxynil herbicide and died within three days of herbicide application. Table 3.1 shows the phenotypic segregation results for bromoxynil resistance/susceptibility for all F_2 plants in three crosses. The results confirm the 3:1 phenotypic segregation ratio expectations for the F_2 generation.

Table 3.1: Observed phenotypic segregation ratios for resistance (R) and susceptibility (S) to bromoxynil herbicide for the F2 generation of three crosses

Cross	Observed Segregation Ratio R:S	Expected Segregation Ratio R:S	X^2	P-value
Cross I	154:58	3:1	0.6289	0.5 - 0.1
Cross II	169:48	3:1	0.9601	0.5 - 0.1
Cross III	155:48	3:1	0.1823	0.5 - 0.1

Of the resistant F2 plants identified, 48 per cross were selfed. Harvesting of the F3 seeds of all three crosses was completed by June 10, 1999.

3.1.5 Development of F4 seed

F3 family seed was planted September 3 to 10, 1999. There were twenty-four seeds randomly selected from each F3 family seed package. The F3 families were sprayed with bromoxynil herbicide at the first true leaf stage. The F3 families segregated into a ratio of 1 pure breeding bromoxynil resistant family to 2 F3 families segregating for bromoxynil resistance/susceptibility. Table 3.2 shows the phenotypic segregation results for the F3 families for all three crosses confirming the 1:2 F3 family phenotypic segregation ratio expectations.

Table 3.2: Observed phenotypic segregation ratios for resistance (R) and susceptibility (S) to bromoxynil herbicide for F3 generation families of three crosses

Cross	Observed Segregation Ratio R:S	Expected Segregation Ratio R:S	X^2	P-value
Cross I	50:96	1:2	0.0548	0.5 - 0.1
Cross II	53:94	1:2	0.4898	0.5 - 0.1
Cross III	49:94	1:2	0.0559	0.5 - 0.1

Visual selection of the best six F3 plants in each identified pure breeding bromoxynil resistant F3 family of each cross were grown to maturity. Harvesting of the F4 family seed occurred from December 12 to 20, 1999.

3.2 DEVELOPMENT OF DOUBLED HAPLOID LINES

The identical three crosses used for development of pedigree selection F4 families were used to produce F1 seed for doubled haploid (DH) line development. The crosses were:

Cross I: 03801BX/Neptune*3//Neptune

Cross II: 03801BX/Neptune*3//(Castor/Quantum) F_4

Cross III: 03801BX/Neptune*3//(Mercury/Cyclone) F_4

3.2.1 Commonalities among generation development

The generations involved in development of DH lines have overlapping methodologies, therefore these will be discussed first, followed by a description of the differences in methodologies associated with each generation. All seeds of the parental, DH0 and DH1 generations were planted into a soil-less mixture, metro-mix, into flats at a depth of six millimeters. The flats were lightly water and placed into a growth room. The growthroom used was an Enconaire, model GR-192-5 set to a 17 hour, 21°C day and 7 hour, 16°C night. Growth in the growthroom occurred for approximately twelve days, where water was applied every second day. When the first true leaf was present, the flats were sprayed with bromoxynil herbicide at 560 g a.i. ha^{-1} to identify plants with bromoxynil resistance. The best developed plants were transferred from the flats into 15 cm plastic pots. The pots contained a soil mixture of black soil, sand, and peat moss in 2:2:1 ratio respectively. The pots were placed into

the greenhouse and water applied every second day. Temik, a systemic insecticide for aphids, along with NPK 20-20-0 fertilizer at 8 ml l^{-1}, were applied to the plants just before bolting. Plant watering was increased to a daily regime. Selfing bags (clear plastic) were placed over the plant at the beginning of flowering, to ensure that the pollen was transferred to the style of the same plant, guaranteeing "selfing". After seed set had occurred, watering of the plants was gradually reduced, to promote seed maturation. Seeds harvested from each mature plant were packaged in individual envelopes.

3.2.2 Development of F1 seed

All of the parental lines used in the above crosses were planted in October, 1998. All subsequent parental line plant selection, growth, crossing and harvesting procedures were the same as for the pedigree selection breeding method described in section 3.1.2.

3.2.3 Development of DH1 seed

The DH lines developed from F1 plants of these three crosses were started in February, 1999, produced at the Saskatchewan Wheat Pool Research Labs at Saskatoon, Saskatchewan during the spring and summer of 1999. The F1 seeds from these crosses were grown to the bud stage and microspore culture was performed to produce up to 100 homozygous DH lines per cross. The microspore culture protocol

for oilseed rape (Ferrie et al., 1995) was followed to produce DH1 seeds in June to October 1999. The DH0 plants were sprayed with bromoxynil herbicide at the one true leaf stage DH0 plants segregate in a 1:1 ratio for bromoxynil resistance and as a result were sprayed to identify bromoxynil resistant DH1 plants.

3.2.4 Development of DH2 seed

Because the DH lines are homozygous at all loci at their origin, there is no genetic advance provided by producing additional selfed generations of DH lines. Selfing of DH1 seed to DH2 seed is frequently done, however, to increase seed amount of each DH line prior to planting in the field. The DH1 seeds were planted October 17, 1999. All DH lines of subsequent DH generations were sprayed with bromoxynil herbicide to ensure that they were bromoxynil resistant. Eight seeds were randomly selected from each package of bromoxynil resistant DH1 seeds and planted. DH2 Seeds harvested from each mature DH1 plant were packaged in individual envelopes for all three crosses and was completed by February 2000.

3.3 FIELD TRIALS

The pedigree selection breeding method and the doubled haploid line development breeding method field trial comparisons were performed at the

University of Manitoba Research Farm at Carman and on the University of Manitoba campus in Winnipeg, in the year 2000.

Sixty F4 families and sixty random DH2 lines were grown from each cross, therefore there was 120 families or lines associated with each cross, plus 12 Neptune BX that were used as checks were planted in field nurseries in three-meter rows with 40 cm spacing between rows. Random numbers were assigned to the sixty F4 families and DH2 lines in each cross and pairing occurred so that each pedigree selection family was paired with the corresponding numbered DH2 line. Each pair of a pedigree selection F4 family and DH2 line was planted side by side in the field to minimize the effects of soil and environmental heterogeneity on the mean performance of all pedigree selection F4 families and DH2 lines. Two randomized replications of each pair of pedigree selection family and DH2 line per cross were planted at each location.

The Carman trial was seeded on May 15, 2000 into a moderately fine/very fine clay/sandy loam (Agriculture and Agri-Food Canada, 1998), while the Winnipeg trial was seeded on May 17, 2000 into a Riverdale silty loam (Agriculture and Agri-Food Canada, 1998). Each F4 family or DH2 line was planted in a three-meter row using half a gram of seed, with 40.64 cm row spacing. An insecticide, Counter 5G, was incorporated with the seed at a rate of 55.6 g a.i. ha^{-1} of terbufos, to control flea beetles (*Phyllotreta crucifera Goeze* and *P. striolata F.*). A Hege belt cone seeder was used to plant the seeds at 3 cm depth.

Fertilizer, 20-0-0-14, NPKS, was applied to the Winnipeg trial on May 30, 2000, at a rate of 111 kg ha^{-1}. The Winnipeg and Carman trials were sprayed with

bromoxynil herbicide, at a rate of 560 g a.i. ha^{-1}, on June 5, 2000 and June 6, 2000 respectively.

Hand weeding was done as needed during the growing season. Rainfall for the growing season in both Winnipeg and Carman were above the long term normal average, especially in June. This excessive rain slowed plant growth in June, but the plants recovered in July and August. Lontrel, for thistle control, with the active ingredient clopyralid, was applied at a normal rate of 231 g a. i. ha^{-1} to the Carman trial only.

Flowering occurred the first week of July at both locations. Deceis, with the active ingredient deltamethrin, was applied at a normal rate of 6 g a. i. ha^{-1}on the Winnipeg trial only, to control diamond back moths, in late July.

The Winnipeg trial was harvested on August 24, 2000 while the Carman trial was harvested on August 16, 2000. The plants in each rows were cut by hand, tied together, and placed in stooks on the field to dry. The Winnipeg trial was threshed September 01, 2000 while the Carman trial was threshed September 5, 2000, using a Kincaid stationary thresher. The harvested seed was placed into labeled brown paper bags. The seeds were placed in a dryer room for up to four weeks to dry, and then cleaned, and weighted. These post harvest operations were completed by mid October of 2000.

3.3.1 Agronomic Traits

Selected agronomic traits were measured on all F4 families and DH2 lines at both locations. These agronomic traits included: number of days to flower, number of days to maturity, plant height and seed yield.

Number of days to flower was measured as the number of days from planting to first open flower on at least 50% of the plants in a plot.

Number of days to maturity was measured as the number of days from planting to physiological maturation of the plant in each plot. For a plant to be physiologically mature, the seeds in the middle pods on the main stem should have a 30 to 40% color change.

Height was measured when the plants finished flowering as the distance from the ground to the tip of the plant for five random plants per plot.

Yield was initially measured in g $plot^{-1}$ and then converted to kg ha^{-1}.

3.3.2 Quality Traits

Selected seed quality traits were also measured on all F4 families and DH2 lines at both locations. These quality traits included; seed oil concentration, seed protein concentration, sum of seed oil concentration and seed protein concentration, erucic acid concentration and glucosinolate concentration.

Seed oil concentration was measured using near-infrared reflectance (NIR) on a Foss 6500 system (Daun et al., 1994). Seed oil concentration values were adjusted to 0% moisture.

Seed protein concentration was also measure using near-infrared reflectance (NIR) on a Foss 6500 system (Daun et al., 1994). Again seed protein concentration values were adjusted to 0% moisture.

The sum of seed oil and protein concentration was calculated by summing the NIR results for seed oil concentration and seed protein concentration adjusted to 0% moisture.

Erucic acid concentration was determined by gas chromatography. Gas chromatography protocols developed for oilseed rape, (Hougen and Bodo, 1973) were used.

Glucosinolate concentration was measured using near-infrared reflectance (NIR) on a Foss 6500 system (Daun et al., 1994).

3.3.3 Selection Protocols used to Determine Acceptable Families or Lines

High erucic acid rapeseed candidate cultivars must meet minimum agronomic performance and quality standards to be eligible for entry into official registration tests, the HEAR Contract Registration Tests. The minimum standards were determined using a Neptune BX check. Number of days to first flower, height, days to maturity, seed yield, oil concentration, protein concentration, sum of oil and protein concentration erucic acid levels and glucosinolate concentration were compared to the in-house check, "Neptune BX", the first BX HEAR line in the University of Manitoba HT HEAR breeding program. Neptune BX check comparison

was used to determine the number of acceptable families or lines (AFL) for official registration tests.

The selection criteria used for determining AFL's for this study, was based on the Neptune BX agronomic and quality trait performance, and the registration criteria for new HEAR cultivars in Canada, as set by the SCRC of the WCC/RRC Inc.

1. Number of days to flower was the days to flower of Neptune BX plus or minus one day with preference to earlier maturing.
2. Height was the height of Neptune BX plus or minus 5cm, with preference to shorter.
3. Number of days to maturity was the days to maturity of Neptune BX check plus not more than three days.
4. Yield was equal to the mean yield of Neptune BX as a minimum, plus two more stringent yield criteria of Neptune BX plus 10% and 20%.
5. Oil concentration and protein concentration selection criteria was equal to the oil concentration and protein concentration of Neptune BX as a minimum.
6. Erucic acid concentration was not less than 2% below the mean erucic acid concentration of Neptune BX.
7. Glucosinolate concentration was equal to the Neptune BX mean glucosinolate concentration.

3.4 STATISTICAL ANALYSIS

All agronomic traits and seed quality traits for all entries in all trials at all locations were analyzed using a statistical analysis service (SAS) version 6.12 for Windows (SAS Institute Inc. Cary, North Carolina, USA). All traits were analyzed to determine their distribution and correlation. This was achieved using SAS Proc Corr and Proc Univariate. Means, standard error, maximum and minimum values were calculated using appropriate SAS commands.

Comparison of breeding method means was done using analysis of variance (ANOVA). Due to the presence of missing data, results of the ANOVA were taken from the type III sums of squares.

To determine the significance of mean differences within families and DH lines and over breeding methods, days to flower, days to maturity, height, yield, oil concentration, protein concentration, sum of oil and protein concentration, and glucosinolate concentration were analyzed using a split-split plot design with a randomized complete block design in the main plot, thus a split-RCBD design. The following model was used:

$$Y_{ijkl} = \mu + Location_i + Line_j + (Location*Line)_{ij} + Rep_k + Treatment_l + e_{ijkl}$$

Where:

Y_{ijkl} = trait of interest

μ = mean

$Location_i$ = locations with ith equal either Winnipeg or Carman

$Line_k$ = there are 60 lines used

$(Location * Line)_{ij}$ = this is error A and will be used as a residual term to compare location and line

Rep_k = replications in the model, with the kth term representing two replications

$Treatment_l$ = there are two treatments either pedigree selection breeding method or doubled haploid line breeding method

e_{ijkl} = residual values

To determine significance differences between breeding method means over all crosses for the same traits mentioned above, the model included cross, making it a split-split-split plot analysis.

Erucic acid concentration was analyzed using a simple split plot analysis with a complete randomized design in the main plot due to the fact that the data was only collected at Carman and for only one replication. The model used for this analysis is as follows:

$$Y_{ijkl} = \mu + Cross_i + Line_j + (Cross*Line)_{ij} + Treatment_k + e_{ijk}$$

Where:

Y_{ijkl} = trait of interest

μ = mean

$Cross_i$ = there are three crosses of interest

$Line_j$ = there are 60 lines used per cross

(Cross *Line)$_{ij}$ = this is error A and will be used as a residual term to compare cross and line

Treatment$_k$ = there are two treatments either pedigree selection breeding method or doubled haploid line breeding method

e_{ijk} = residual values

Seed yield was collected in g 0.6096m^{-2} plot size and conversion was done to express seed yield in kg ha^{-1}.

Data analysis used for determining phenotypic segregation ratios in the development of pedigree selection lines were analyzed using Chi-square goodness of fit tests. Chi-square goodness of fit tests measures the size discrepancy between the observed and expected results. The significance level for testing hypotheses for Chi-square was 0.05 and the degree of freedom was equal to one for all the Chi-square tests conducted in this study.

3.5 COST ANALYSIS

The two breeding methods compared in this study were distinctly different, thus to aid in the efficiency of estimating the allocations of costs, time and space, each breeding method was divided into seven stages. Figures 3.1 and 3.2 provide an outline of each breeding method and the seven allocated stages within each breeding method. Stage 4 in the doubled haploid line development breeding method is an optional section and will be treated as such in any further calculations.

Calculations of expenses associated with each breeding method were separated into fixed and variable costs. Fixed costs included salaries and wages, repairs and maintenance, chemicals, and insurance costs were calculated for each pedigree selection breeding method and doubled haploid line development breeding method. Variable costs included supplies used, and rent which incorporates utility costs. Depending on the section that is analyzed for costs, variability exists, i.e. field chemical costs will vary from greenhouse chemical costs.

Space allocation was based on the amount of space required to perform a task, thus, five main areas were looked at: growthroom, greenhouse, biotechnology laboratory, quality laboratory and field space. Each space requirement was estimated in m^2.

The duration of each breeding method was measured in number of days. This was based on 260 physical working days per calendar year. Each working day represents seven hours of work.

Costs were calculated on a per day basis with respect to the single family or DH line developed. This was compared to the calculated costs when the numbers of lines were altered. This was used to determine breeding program efficiency, comparing marginal costs to marginal benefits.

Calculation of the net present value would also be an asset as it represents the value of the benefits minus the costs; therefore, further manipulation of this formula could produce a dollar value for benefits associated with each breeding method. The formula used for the net present value (NPV) is as follows:

$$NPV = \sum_{i\text{-}1}^{n} \frac{values_i}{(1+rate)^i}$$

Where n = the number of cash flows in the list of values

rate = the rate of discount over the length of one period

values = cost values for a specific time period for i^{th} terms

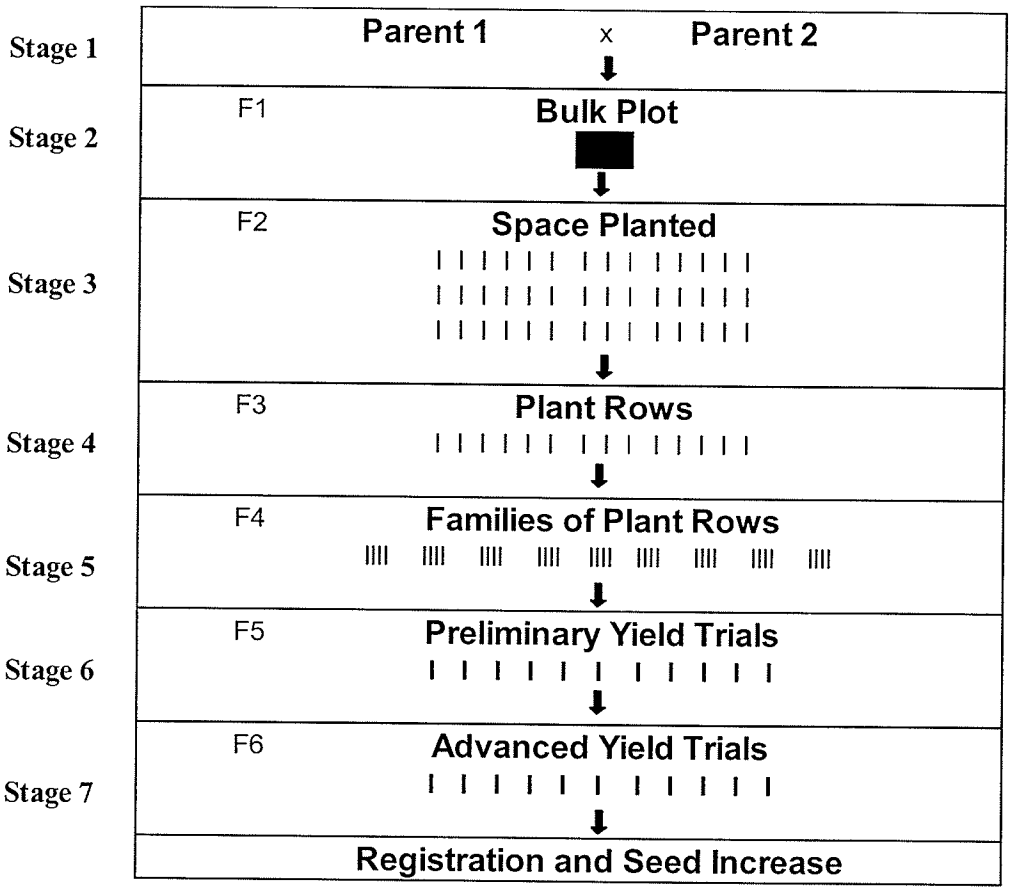

Figure 3.1: Pedigree selection method sections

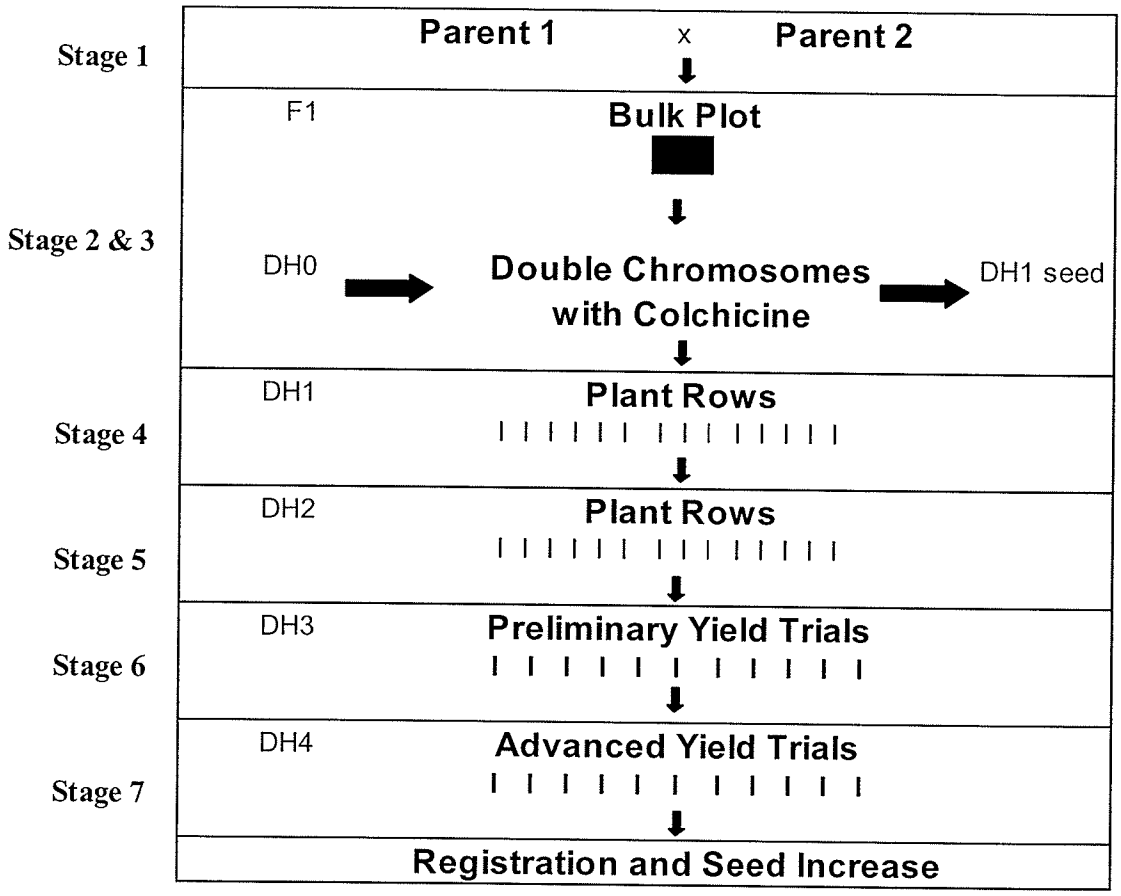

Figure 3.2: Doubled haploid line method sections

4.0 Results and Discussion

4.1 FIELD COMPARISONS

4.1.1 Characterization of agronomic traits for the progeny of three crosses

4.1.1.1 Days to first flower

At Carman, two of three crosses had significantly different mean number of days to first flower for the breeding methods comparison (Cross I and II) (Table 4.1.1) (Appendix Table A1, A2, A3). In both crosses I and II, the doubled haploid lines as a group were earlier than the pedigree selection families as a group by approximately 0.7 and 1.0 day, respectively. The range in number of days to first flower for both breeding methods were similar for all three crosses. The distributions for number of days to first flower as compared to the in-house check Neptune BX for both breeding methods were different for two of the three crosses. Cross I and II showed fewer late flowering doubled haploid lines than pedigree selection families, however, the majority of the families/lines were similar in days to first flower to the Neptune BX check. There were significant differences in mean number of days to flower among the three crosses averaged over breeding methods, with cross II having the earliest mean (Appendix A4). This difference was less than one day, however, so both breeding methods in all three crosses generated progeny acceptable for days to first flower.

At the Winnipeg site, one of three crosses had significantly different mean number of days to flower related to breeding methods (Cross II) (Table 4.1.2) (Appendix A1, A2, A3). As at Carman, the doubled haploid lines in cross II were earlier to first flower than the pedigree selection families, but in this case, only by less than 0.5 of a day. The range in number of days to first flower for both breeding methods, was similar for all three crosses. The distribution for number of days to first flower for both breeding methods as compared to the in-house check Neptune BX was similar for all three crosses, with the majority of the families/lines being similar in days to first flower to the Neptune BX check. There were significant differences in mean number of days to first flower among the three crosses averaged over breeding methods (Appendix A4), with cross III having the earliest mean. This difference was less than 0.5 day, however, so both breeding methods in all three crosses generated progeny acceptable for days to first flower.

There was evidence of a genetic by environment (G x E) interaction (Tables 4.1.1 and 4.1.2), since the mean over families/lines for cross III was latest to first flower at Carman but earliest to first flower in Winnipeg. Thurling and Vijendra Das, in 1979 also found considerable variation in flowering response under different environments. Plant breeders evaluate genetic material in multiple locations per year and occasionally several years, to determine overall mean performance to eliminate G x E interactions.

There are no previous comparisons in the scientific literature of the pedigree selection breeding method and the doubled haploid line breeding method..

A conservative estimate of the narrow sense heritability for days to first flower is low at 14.0% (Singh and Yadav, 1980). This low narrow sense heritability suggests that

little advancement though selection would be achieved for days to first flower. The results for days to first flower observed in this study confirmed this expectation.

Table 4.1.1: Number of days to first flower for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Early[Z]**	**Check[Y]**	**Late[X]**
	(d)		**(d)**	**(d)**			
Cross I							
PS	50.7a	0.10	53.0	49.0	0	44	16
DH	50.0b	0.08	53.0	48.0	0	54	4
all (PS+DH)	50.3B	0.07	53.0	48.0	0	98	20
Cross II							
PS	50.6a	0.11	54.0	48.0	1	42	17
DH	49.5b	0.07	53.0	48.0	4	54	2
all (PS+DH)	50.1C	0.07	54.0	48.0	5	96	19
Cross III							
PS	51.0a	0.08	53.0	49.0	0	40	20
DH	51.0a	0.08	53.0	50.0	0	42	18
all (PS+DH)	51.0A	0.05	53.0	49.0	0	82	38
Neptune BX (Check)	50				<49 days	49 to 51 days	>51 days

[Z] early is more than 1 full day before check mean in flowering.

[Y] +/- one day of check mean in flowering.

[X] late is more than 1 full day after check mean in flowering.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.2: Number of days to first flower for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	Mean	Std. Error	Max	Min	Early[Z]	Check[Y]	Late[X]
	(d)		(d)	(d)			
Cross I							
PS	49.5a	0.08	54.0	48.0	0	56	4
DH	49.4a	0.08	53.0	48.0	0	53	7
all (PS+DH)	49.5A	0.06	54.0	48.0	0	109	11
Cross II							
PS	49.2a	0.07	52.0	48.0	0	59	1
DH	48.9b	0.08	52.0	47.0	2	58	0
all (PS+DH)	49.1B	0.05	52.0	47.0	2	117	1
Cross III							
PS	49.0a	0.07	51.0	46.0	3	57	0
DH	49.0a	0.08	52.0	47.0	3	55	2
all (PS+DH)	49.0C	0.06	52.0	46.0	6	112	2
Neptune BX (check)	49				<48 days	48 to 50 days	>50 days

[Z] early is more than 1 full day before check mean in flowering.

[Y] +/- one day of check mean in flowering.

[X] late is more than 1 full day after check mean in flowering.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.1.2 Height

At Carman, two of three crosses demonstrated significantly different mean height for the breeding methods comparison (Cross I and II) (Table 4.1.3) (Appendix A5, A6, A7). In both crosses I and II, the doubled haploid lines as a group were shorter, upon inspection than the pedigree selection families as a group, by 5 cm and 7 cm respectively. The range in height for both breeding methods, were similar for all three crosses. The distributions of height for crosses I and II, showed large differences, with the doubled

haploid lines having many fewer tall plants as compared to pedigree selection families. These results suggests that the doubled haploid line breeding method may be the breeding method of choice to produce progeny of equal or reduced height compared to an internal check, at least for these crosses grown in these environments. There were significant differences in mean height among the three crosses averaged over breeding methods (Appendix A8), with cross I having the shortest mean height and cross II having the tallest mean height. This difference was less than 4 cm, however, so that both breeding methods in all three crosses generated progeny acceptable for height.

At Winnipeg, two of three crosses demonstrated significantly different mean height for the breeding methods comparison (Cross I and II) (Table 4.1.4) (Appendix A5, A6, A7). In both crosses I and II, the doubled haploid lines as a group were shorter than the pedigree selection families as a group, by approximately 2 cm and 3 cm, respectively. The ranges in height for both breeding methods were similar for all three crosses. As at Carman, cross II displayed many fewer tall doubled haploid lines compared to the pedigree selection families. This again suggests that the doubled haploid line breeding method may be the breeding method of choice to produce progeny of equal or reduced height compared to an internal check, Neptune BX, at least for these crosses grown in these environments. There were significant differences in mean height among the three crosses averaged over breeding methods (Appendix A8), with cross I having the smallest mean height and cross II having the largest mean height. This difference was less than 4 cm, however, so that both breeding methods in all three crosses generated progeny acceptable for height.

Relative cross performance was similar over locations, so that little, if any cross

by environment interaction occurred for height in this study, apparently.

In this study it was found that the doubled haploid breeding method produced shorter plants than the pedigree selection breeding method in two out of the three crosses that showed significant mean differences. Schnell et al., support this result in a similar study in 1980.

In this study, for one of the three crosses, success at decreasing the overall mean height compared to the Neptune BX mean check occurred. Height has both additive and dominant gene action that control this trait (Govil et al, 1984), which accounts for any differences for parental differences. Overall height is also the result of alleles at loci controlling stem termination and maturity (Saindon, et al., 1990), which is also a consideration when significant changes in mean height occur as seen in one of the crosses.

Height is an important agronomic trait that indirectly influences seed yield and thus it is important to make significant improvements. There is evidence that shorter plants improve seed yields since shorter plants are more resistant to lodging (Thompson and Hughes, 1986; Saindon et al., 1990). Lodging decreases seed yield by increasing the occurrence of shattered pods (Thompson and Hughes, 1986). Shorter plants also produced less straw material, which implies that the plant will put more resources into filling the sink, i.e. seed filling, rather than developing the seed, i.e. the vegetative portion of the plant (Thompson and Hughes, 1986). Thus, it is advantageous to develop shorter plants, which have a higher harvest index by default.

Table 4.1.3: Height for pedigree selection (PS) families and doubled haploid (DH) lines for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Short[Z]**	**Check[Y]**	**Tall[X]**
	(cm)		**(cm)**	**(cm)**			
Cross I							
PS	110.4 a	0.73	130.0	90.0	2	33	25
DH	105.9 b	0.94	135.0	80.0	15	31	14
all (PS+DH)	108.1 C	0.62	135.0	90.0	17	64	39
Cross II							
PS	116.1 a	0.89	140.0	100.0	1	16	43
DH	109.8 b	0.75	130.0	90.0	2	35	23
all (PS+DH)	112.8 A	0.61	140.0	90.0	3	51	66
Cross III							
PS	110.4 a	0.75	130.0	90.0	3	28	29
DH	110.0 a	0.76	130.0	90.0	6	26	28
all (PS+DH)	110.2 B	0.54	130.0	90.0	9	54	57
Neptune BX (Check)	106				<101 cm	101 to 111cm	>111 cm

[Z] short is more than 5cm shorter than the check mean for height.

[Y] +/- 5cm of the check mean for height.

[X] tall is more than 5cm taller than the check mean for height.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.4: Height for pedigree selection (PS) families and doubled haploid (DH) lines for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	Mean	Std. Error	Max	Min	Short[Z]	Check[Y]	Tall[X]
	(cm)		(cm)	(cm)			
Cross I							
PS	81.2 a	0.96	100.0	50.0	24	33	3
DH	79.3 b	1.08	110.0	50.0	37	17	6
all (PS+DH)	80.2 C	0.73	110.0	50.0	61	50	9
Cross II							
PS	90.7 a	0.96	120.0	55.0	6	24	30
DH	87.8 b	1.06	140.0	60.0	11	33	16
all (PS+DH)	89.2 A	0.72	140.0	55.0	17	57	46
Cross III							
PS	88.2 a	1.10	115.0	60.0	11	30	19
DH	87.4 a	1.03	120.0	60.0	7	36	17
all (PS+DH)	87.8 B	0.75	120.0	60.0	18	66	36
Neptune BX (check)	87				<82 cm	82 to 92cm	>92 cm

[Z] short is more than 5cm shorter than the check mean for height.

[Y] +/- 5cm of the check mean for height.

[X] tall is more than 5cm taller than the check mean for height.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.1.3 Days to maturity

At Carman, there was no significant difference in the mean number of days to maturity for any of the three crosses for the breeding methods comparison (Table 4.1.5) (Appendix A9, A10, A11). The range in number of days to maturity for both breeding methods, were similar for all three crosses. The distributions for number of days to maturity for both breeding methods were also very similar for all three crosses, with the majority of the developed families/lines being similar in days to maturity to the Neptune

BX check. There were significant differences in mean number of days to maturity among the three crosses combined over breeding methods (Appendix A12), with cross II having the earliest mean maturity. Cross II was only 0.4 to 0.7 days earlier to maturity than crosses I and III, respectively, however.

At Winnipeg, there were no significant differences in the means for number of days to maturity for any of the crosses for the breeding methods comparison (Table 4.1.6) (Appendix A9, A10, A11). The ranges in number of days to maturity for both breeding methods were similar for all three crosses. The distribution for number of days to maturity for both breeding methods were also very similar for all three crosses, with the majority of the developed families/lines being similar in days to maturity to the Neptune BX check. There were significant differences in mean number of days to maturity among the three crosses combined over breeding methods (Appendix A12), with cross III having the earliest mean maturity. Cross III were 0.4 days earlier than cross II and 1.2 days earlier to maturity than cross I.

There was evidence of a genetic by environment interaction (Tables 4.1.5 and 4.1.6), since cross III had the latest maturity at Carman and the mean values earliest days to maturity in Winnipeg. The genetic by environment interactions for days to maturity parallels those seen for days to first flower in this study. Interactions are the reason plant breeder's use multiple locations per year and occasionally several years to determine overall mean performance of families and lines under evaluation and thus identify strains with wide range adaptability.

There were no significant differences found between the breeding methods compared in this study for reducing the number of days to maturity. This is similar to the

outcomes of a comparison study of agronomic traits using microspore populations and conventional single seed decent populations in *B. napus* (Charne and Beversdorf, 1991).

Days to maturity has a moderate narrow sense heritability (57%) (Ringdahl et al., 1986), which suggest that there is potential for improvement when selection is applied. This was seen in this study, for two out of the three crosses combined over breeding methods and locations, where there was a decrease in the overall mean number of days to maturity as compared to the Neptune BX check.

Table 4.1.5: Number of days to maturity for pedigree selection (PS) families and doubled haploid (DH) lines for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Early[Z]**	**Check[Y]**	**Late[X]**
	(d)		**(d)**	**(d)**			
Cross I							
PS	91.6 a	0.12	94.0	88.0	0	45	15
DH	91.5 a	0.12	94.0	88.0	2	44	14
all (PS+DH)	91.5 B	0.09	94.0	88.0	2	89	29
Cross II							
PS	91.2 a	0.12	94.0	89.0	2	51	7
DH	91.0 a	0.12	94.0	89.0	3	51	6
all (PS+DH)	91.1 C	0.08	94.0	89.0	5	102	13
Cross III							
PS	91.9 a	0.07	93.0	89.0	0	44	16
DH	91.8 a	0.08	93.0	89.0	0	48	12
all (PS+DH)	91.8 A	0.05	93.0	89.0	0	92	28
Neptune BX (Check)	91				<90 days	90 to 92 days	>92 days

[Z] early is more than 1 full day before check mean in maturity.

[Y] +/- one day of check mean in maturity.

[X] late is more than 1 full day after check mean in maturity.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.6: Number of days to maturity for pedigree selection (PS) families and doubled haploid (DH) lines for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	Mean	Std. Error	Max	Min	Early[Z]	Check[Y]	Late[X]
	(d)		(d)	(d)			
Cross I							
PS	96.6 a	0.13	101.0	94.0	0	45	15
DH	96.6 a	0.11	100.0	94.0	0	47	13
all (PS+DH)	96.6 A	0.08	101.0	94.0	0	92	28
Cross II							
PS	96.0 a	0.15	100.0	93.0	1	52	7
DH	95.8 a	0.13	100.0	93.0	0	55	5
all (PS+DH)	95.9 B	0.10	100.0	93.0	1	107	12
Cross III							
PS	95.8 a	0.12	100.0	94.0	1	50	9
DH	95.7 a	0.12	100.0	92.0	1	54	5
all (PS+DH)	95.4 C	0.33	100.0	92.0	2	104	14
Neptune BX (Check)	96				<95 days	95 to 97 days	>97 days

[Z] early is more than 1 full day before check mean in maturity.

[Y] +/- one day of check mean in maturity.

[X] late is more than 1 full day after check mean in maturity.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.1.4 Seed yield

At Carman, two of the three crosses demonstrated significant differences in mean yield between the breeding methods (crosses I and II) (Table 4.1.7) (Appendix A13, A14, A15). In both crosses I and II, the mean yields for the pedigree selection families, as a group were significantly higher than for the doubled haploid lines as a group, by 900 kg ha^{-1} and 500 kg ha^{-1}, respectively. Considering the range of yield for the two breeding

methods among the three crosses, the yields seen for pedigree selection families as a group in crosses I and II, were the highest. The distributions for yield for both breeding methods showed that both cross I and II had substantially more pedigree selection families in the above average yield group than doubled haploid lines. This suggests that the pedigree selection family breeding method may be the breeding method of choice to improve yield for these crosses grown in this environment. There were significant differences for mean yield among the three crosses combined over breeding methods (Appendix A16), with cross I having the highest mean yield.

At Winnipeg, two of the three crosses had significantly different mean seed yield for the two breeding methods (cross I and II) (Table 4.1.8) (Appendix A13, A14, A15). As seen at Carman, the pedigree selection families as a group for crosses I and II were higher yielding than the doubled haploid lines as a group, by 700 kg ha^{-1} and 300 kg ha^{-1}, respectively. For the two breeding methods among the three crosses, the highest seed yields were seen for pedigree selection families. Both crosses I and II had substantially more pedigree selection families in the above average yield group than doubled haploid lines. This suggests that the pedigree selection family breeding method may be the breeding method of choice to increase yield for these crosses grown in this environment. There were significant differences for yield among the three crosses combined over breeding methods (Appendix A16), with cross III having the largest mean yield.

There is evidence of genetic by environment interaction (Tables 4.1.7 and 4.1.8), since cross III had the lowest mean yield in Carman, and the highest mean yield in Winnipeg. Cross I and III showed many families and/or doubled haploid lines, which were above the check mean yield, suggesting that good progress for yield could be made

for these crosses in these environments.

The pedigree selection breeding method produced increases in seed yield, for two out of the three crosses, which is similar to the results seen in a study by Schnell et al., in 1980. The remaining cross showed more improvement in seed yield using the doubled haploid line breeding method than the pedigree selection breeding method. Seed yield has a low narrow sense heritability of 11.1% (Singh and Yadav, 1980), and is controlled by many factors (Thompson and Huges, 1986), which make breeding for yield the breeder's most difficult task. Breeding methods appear to have little direct effect on yield improvement in this study, as expected for a low heritability trait.

Table 4.1.7: Yield for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

Cross/Type	Mean	Std. Error	Range		Number		
			Max	Min	Below[Z]	Equal[Y]	Above[X]
	(kg ha^{-1})		(kg ha^{-1})	(kg ha^{-1})			
Cross I							
PS	4159 a	7.9	7151	889	18	19	23
DH	3215 b	6.9	6266	440	39	13	7
All (PS+DH)	3673 A	5.6	7151	440	57	32	30
Cross II							
PS	3510 a	7.7	7707	387	31	16	13
DH	3089 b	7.1	6475	246	46	8	6
All (PS+DH)	3293 C	5.3	7707	246	77	24	19
Cross III							
PS	3599 a	6.5	5897	392	31	19	10
DH	3496 a	7.9	7741	397	31	14	15
All (PS+DH)	3546 B	5.1	7741	392	62	33	25
Neptune BX (Check)	3581				<3647	3647 to 4467	>4467

[Z] below 820 kg/ha from the check mean for yield.

[Y] +/- 820 kg/ha from the check mean for yield.

[X] above 820 kg/ha from the check mean for yield.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.8: Yield for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

Cross/Type	Mean	Std. Error	Range		Number		
			Max	Min	Below[Z]	Equal[Y]	Above[X]
	(kg ha^{-1})		(kg ha^{-1})	(kg ha^{-1})			
Cross I							
PS	3564 a	8.4	8481	782	32	14	14
DH	2828 b	7.4	7551	335	52	2	6
all (PS+DH)	3194 B	5.8	8481	335	84	16	20
Cross II							
PS	2797 a	9.4	7863	202	45	8	7
DH	2414 b	7.7	7000	202	54	6	0
all (PS+DH)	2606 C	6.1	7863	202	99	14	7
Cross III							
PS	3826 a	8.6	8054	510	29	13	18
DH	4062 a	11.3	8967	441	25	14	21
all (PS+DH)	3944 A	7.1	8967	441	54	27	39
Neptune BX (Check)	4078				<3668	3668 to 4488	>4488

[Z] below 820 kg/ha from the check mean for yield.

[Y] +/- 820 kg/ha from the check mean for yield.

[X] above 820 kg/ha from the check mean for yield.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.2 Characterization of quality traits for the progeny of three crosses

4.1.2.1 Oil concentration

At Carman, all three crosses demonstrated significantly different mean oil concentration for the two breeding methods (Table 4.1.9) (Appendix A17, A18, A19). In cross I and II, the doubled haploid lines as a group had higher oil concentration than the pedigree selection families as a group, by 1 and 3 percent respectively. In cross III, the pedigree selection families as a group had higher mean oil concentration than the doubled haploid lines, by 1 percent. These differences were reflected in the range of oil concentration in the progeny of two breeding methods, since the highest oil concentration progeny were found in the doubled haploid lines of crosses I and II. The distribution of oil concentration for both breeding methods showed that cross II had more than twice the number of doubled haploid lines with above average oil concentrations than pedigree selection families. In cross III, the distribution showed pedigree selection families to have more doubled haploid lines for above average oil concentration. In cross I, the numbers above average oil concentration families and doubled haploid lines were equal. This suggests that either breeding method may be used to increase oil concentration for these crosses grown in this environment. There were significant differences for oil concentration among the three crosses combined over breeding methods (Appendix A20), with cross III having the highest mean oil concentration and the greatest number of above average oil concentration families and lines.

At Winnipeg, all three crosses demonstrated significantly different mean oil

concentration for the two breeding methods (Table 4.1.10) (Appendix A17, A18, A19). In crosses I and II, the doubled haploid lines as a group had higher oil concentration than the pedigree selection families as a group, by 1 and 2 percent, respectively. In cross III, the pedigree selection families as a group had higher oil concentration than the doubled haploid lines as a group, by 0.5%. These differences were reflected in the range of oil concentration in the progeny in the two breeding methods, since the highest oil concentration progeny were found in the doubled haploid lines of cross II, while in crosses I and III, the highest oil concentration progeny were found in pedigree selection families. The distribution of oil concentration for both breeding methods showed that cross II had more than twice the number of doubled haploid lines with above average oil concentration than pedigree selection families. In cross I, the distribution displayed more doubled haploid lines than pedigree selection families with above average oil concentration. These results suggest that the doubled haploid line breeding method may be the breeding method of choice to improve oil concentration for these crosses grown in this environment. There were significant differences for oil concentration among the three crosses combined over breeding methods (Appendix A20), with cross III having the highest mean oil concentration.

There was evidence of genetic by environmental interaction (Tables 4.1.9 and 4.1.10), since crosses I and II, reversed rank, over the two locations. Interactions, even as small as they are in this case, require breeders to use multiple locations per year, and occasionally several years of testing to determine an overall mean performance of families and lines under evaluation.

There were contradictory results for the breeding methods, which showed mean

increase for oil concentration. This increase can be explained by the influenced by many factors that have genetic and environmental contributions (Downey, 1983). Oil concentration also has both additive and overdominance gene action (Govil et al., 1984) and low narrow sense heritability at 0.26 (Grami et al., 1977). This indicates that increases for parents can be difficult and numerous environmental interactions can be a contributor.

Table 4.1.9: Oil concentration at 0% moisture for pedigree selection (PS) families and doubled haploid (DH) lines for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Below[Z]**	**Equal[Y]**	**Above[X]**
	(%)		(%)	(%)			
Cross I							
PS	45.5 B	0.23	50.4	39.4	24	22	14
DH	46.4 A	0.16	52.3	42.0	11	36	13
all (PS+DH)	46.0 C	0.14	52.3	39.4	35	58	27
Cross II							
PS	45.6 B	0.28	53.1	38.9	25	17	18
DH	48.2 A	0.22	54.0	41.4	7	14	39
all (PS+DH)	47.0 B	0.19	54.0	38.9	32	31	57
Cross III							
PS	48.1 A	0.22	53.5	43.1	3	18	39
DH	47.1 B	0.28	52.9	37.7	14	15	31
all (PS+DH)	47.6 A	0.18	53.5	37.7	17	33	70
Neptune BX (Check)	46.4				<45.4%	45.4 to 47.4%	>47.4%

[Z] below one percent from the check mean for percent oil concentration.

[Y] +/- one percent from the check mean for percent oil concentration.

[X] above one percent from the check mean for percent oil concentration.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.10: Oil concentration at 0% moisture for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Below[Z]**	**Equal[Y]**	**Above[X]**
	(%)		(%)	(%)			
Cross I							
PS	43.5 b	0.21	48.7	38.3	26	20	14
DH	44.6 a	0.16	48.3	41.0	6	33	21
all (PS+DH)	44.0 B	0.14	48.7	38.3	32	53	35
Cross II							
PS	42.7 b	0.23	48.3	35.9	30	19	10
DH	44.4 a	0.19	50.2	39.8	13	25	21
all (PS+DH)	43.6 C	0.16	50.2	35.9	43	44	31
Cross III							
PS	45.7 a	0.18	51.0	41.0	3	17	39
DH	45.3 b	0.20	50.0	40.3	8	16	35
all (PS+DH)	45.5 A	0.14	51.0	40.3	11	33	74
Neptune BX (Check)	44.0				<43.0%	43.0 to 45.0%	>45.0%

[Z] below one percent from the check mean for percent oil concentration.

[Y] +/- one percent from the check mean for percent oil concentration.

[X] above one percent from the check mean for percent oil concentration.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.2.2 Protein Concentration

At Carman, two of three crosses demonstrated significantly different mean protein concentration for the two breeding methods (crosses II and III) (Table 4.1.11) (Appendix A21, A22, A23). In cross II, the pedigree selection families as a group were higher for protein concentration than the doubled haploid lines as a group, by 2 percent. In cross III, the doubled haploid lines as a group were higher for protein concentration than the pedigree selection families as a group. Considering the range of protein concentration for

both breeding methods among the three crosses, the highest mean protein concentrations were seen for cross I and II for pedigree selection families and doubled haploid lines for cross III. The distribution of protein concentration for both breeding methods was variable for the three crosses. Cross I showed equal numbers of protein concentration pedigree selection families and doubled haploid lines with above average protein concentration. Cross II had more pedigree selection families than doubled haploid lines while, cross III had more doubled haploid lines then pedigree selection families. Cross I had the highest mean protein concentration among crosses combined over breeding methods (Appendix A24), with 0.4% and 0.9% higher protein concentration than cross II and cross III, respectively.

At Winnipeg, all three crosses demonstrated significantly different means for protein concentration for the two breeding methods (Table 4.1.12) (Appendix A21, A22, A23). In cross II, the pedigree selection families as a group were higher for protein concentration than the doubled haploid lines as a group, by 2 percent. In crosses I and III, the doubled haploid lines as a group were higher for protein concentration than the pedigree selection families as a group. Considering the range of protein concentration for both breeding methods among the three crosses, the highest protein concentrations were seen for cross I and II for doubled haploid lines, and cross III for pedigree selection families. Cross I and II had more pedigree selection families with above average protein concentration than doubled haploid lines while, cross III had more doubled haploid lines than pedigree selection families. There were no significant mean differences for protein concentration over the three crosses combined over breeding methods (Appendix A24).

There is evidence of genetic by environment interaction in the results presented in

tables 4.1.11 and 4.1.12, since the three cross showed significant differences at Carman, and showed non-significant differences at Winnipeg.

It has been estimated that the narrow sense heritability for protein concentration is 0.26 (Grami et al., 1977), indicating that responses to selection will be minimal.

Table 4.1.11: Protein concentration at 0% moisture for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Below[Z]**	**Equal[Y]**	**Above[X]**
	(%)		**(%)**	**(%)**			
Cross I							
PS	27.8 a	0.17	33.3	23.1	8	28	24
DH	27.9 a	0.17	32.4	22.5	7	27	26
all (PS+DH)	27.8 A	0.12	33.3	22.5	15	55	50
Cross II							
PS	28.4 a	0.22	33.0	21.7	6	20	34
DH	26.5 b	0.18	30.2	20.7	24	24	12
all (PS+DH)	27.4 B	0.15	33.0	20.7	30	44	46
Cross III							
PS	26.5 b	0.20	31.5	21.0	27	23	10
DH	27.3 a	0.23	33.7	21.9	20	19	21
all (PS+DH)	26.9 C	0.15	33.7	21.0	47	42	31
Neptune BX (Check)	27.0				<26.0%	26.0 to 28.0%	>28.0%

[Z] below one percent from the check mean for percent protein concentration.

[Y] +/- one percent from the check mean for percent protein concentration.

[X] above one percent from the check mean for percent protein concentration.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.12: Protein concentration at 0% moisture for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	Mean	Std. Error	Max	Min	Below[Z]	Equal[Y]	Above[X]
	(%)		(%)	(%)			
Cross I							
PS	31.5 b	0.17	35.4	26.9	10	30	20
DH	32.0 a	0.15	34.7	25.4	15	37	8
all (PS+DH)	31.3 A	0.11	35.4	25.4	25	67	28
Cross II							
PS	32.1 a	0.16	36.0	27.1	9	20	30
DH	30.4 b	0.14	34.1	25.8	26	31	2
all (PS+DH)	31.3 A	0.12	36.0	25.8	35	51	32
Cross III							
PS	31.1 b	0.17	34.8	24.4	16	31	12
DH	31.5 a	0.19	37.1	26.8	16	22	21
all (PS+DH)	31.3 A	0.13	37.1	24.4	32	53	33
Neptune BX (Check)	31.3				<30.3%	30.3% to 32.3%	>32.3%

[Z] below one percent from the check mean for percent protein concentration.

[Y] +/- one percent from the check mean for percent protein concentration.

[X] above one percent from the check mean for percent protein concentration.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.2.3 Sum of oil and protein concentration

At the Carman site, two of three crosses demonstrated significantly different mean sum of oil and protein concentration for the breeding methods comparison (cross I and II) (Table 4.1.13) (Appendix A25, A26, A27). In both crosses I and II, the mean sum of oil and protein concentration for doubled haploid lines as a group was significantly higher than for the pedigree selection families as a group, by 1% and 0.5%, respectively. Considering the range of sum of oil and protein concentration for both breeding methods

among the three crosses, the highest sum of oil and protein concentration was with the doubled haploid lines for crosses I and III, while pedigree selection families had the highest sum of oil and protein concentration for cross II. Cross I and II had significantly more doubled haploid lines with above average sum of oil and protein concentration than pedigree selection families, while cross III had equal numbers of pedigree selection families and doubled haploid lines. Based on these results the doubled haploid line breeding method would be the breeding method of choice to improve oil and protein concentration for these crosses grown in this single environment. There were significant differences for sum oil and protein concentration among the three crosses combined over breeding methods (Appendix A28), with cross III having the highest mean sum of oil and protein concentration.

At Winnipeg, one of three crosses demonstrated significantly different mean sum of oil and protein concentration in the two breeding methods (cross I) (Table 4.1.14) (Appendix A25, A26, A27). In cross I, doubled haploid lines as a group had a higher sum of oil and protein concentration than the pedigree selection families as a group, by 1%. Considering the range of sum of oil and protein concentration for both breeding methods among the three crosses, the highest sum of oil and protein concentration was seen in doubled haploid lines as a group for crosses I and II and in pedigree selection families as a group for cross III. Cross I had significantly more doubled haploid lines with above average sum of oil and protein concentration than pedigree selection families, while cross II and III had equal numbers of pedigree selection families and doubled haploid lines in the above average group. Based on these results either the pedigree selection breeding method or the doubled haploid line breeding method would be the breeding method of

choice to improve oil and protein concentration for these crosses grown in this environment. There were significant differences for sum of oil and protein concentration among the three crosses combined over breeding methods (Appendix A28), with cross III having the highest mean sum of oil and protein concentration.

Some genetic by environment interactions exist, since cross I and II reversed rank over two locations with respect to the cross means for sum of oil and protein concentration. Interactions are the reason plant breeders use multiple locations per year and occasionally several years to determine overall mean performance of families and lines under evaluation.

The sum of oil and protein concentration has a higher narrow sense heritability value of 0.33 than for either oil concentration or protein concentration alone (Grami et al., 1977). These traits are also highly negativity correlated indicating that it is necessary to select simultaneously for both oil and protein concentration to maximize the intrinsic value of the seed. (Robbelen, 1978).

Table 4.1.13: Sum of oil and protein concentration at 0% moisture for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	Mean	Std. Error	Max	Min	Below[Z]	Equal[Y]	Above[X]
	(%)		(%)	(%)			
Cross I							
PS	73.3 b	0.12	76.1	70.1	15	33	12
DH	74.2 a	0.10	76.5	69.8	1	31	28
all (PS+DH)	73.8 C	0.08	76.5	69.8	16	64	40
Cross II							
PS	74.1 b	0.17	77.2	68.6	10	20	30
DH	74.7 a	0.12	76.9	70.2	1	22	37
all (PS+DH)	74.4 B	0.10	77.2	68.6	11	42	67
Cross III							
PS	74.6 a	0.18	76.8	72.2	1	22	37
DH	74.3 a	0.21	77.1	70.4	5	21	34
all (PS+DH)	74.5 A	0.09	77.3	68.6	6	43	71
Neptune BX (Check)	73.4				<72.4%	72.4 to 74.4%	>74.4%

[Z] below one percent from the check mean for percent oil and protein concentration.

[Y] +/- one percent from the check mean for percent oil and protein concentration.

[X] above one percent from the check mean for percent oil and protein concentration.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.4: Sum of oil and protein concentration at 0% moisture for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	Mean	Std. Error	Max	Min	Below[Z]	Equal[Y]	Above[X]
	(%)		(%)	(%)			
Cross I							
PS	74.9 b	0.11	77.3	71.8	14	42	4
DH	75.6 a	0.12	77.8	69.0	7	36	17
all (PS+DH)	75.3 B	0.08	77.8	69.0	21	78	21
Cross II							
PS	74.8 a	0.13	77.4	70.5	18	37	4
DH	74.9 a	0.11	78.1	71.2	16	40	3
all (PS+DH)	74.8 C	0.88	78.1	70.5	34	77	7
Cross III							
PS	76.8 a	0.11	79.1	71.6	0	17	42
DH	76.8 a	0.11	78.8	71.3	1	17	41
all (PS+DH)	76.8 A	0.08	79.1	71.3	1	34	83
Neptune BX (Check)	75.3				<74.3%	74.3 to 76.3%	>76.3%

[Z] below one percent from the check mean for percent oil and protein concentration.

[Y] +/- one percent from the check mean for percent oil and protein concentration.

[X] above one percent from the check mean for percent oil and protein concentration.

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.2.4 Erucic acid concentration

At Carman, all three crosses demonstrated significantly different mean erucic acid concentration for the two breeding methods (Table 4.1.15) (Appendix A29, A30, A31). The pedigree selection families mean erucic acid concentration was higher than the doubled haploid line mean for crosses II and III. The highest value achieved for erucic acid concentration for both breeding methods, were similar for all three crosses, however, the range from highest percent to lowest in erucic acid concentration for cross I and II

were wider than for crosses III. Cross I had more doubled haploid lines in the 51 to 60% category, while cross II had more pedigree selection families in the 51 to 60% category, and cross III had equal numbers of both pedigree selection families and doubled haploid line in the 51 to 60% category. There is, therefore, no clear indication of which breeding method is best based on the distribution of erucic acid concentration families or doubled haploid lines. There were significant differences for erucic acid concentration among the three crosses combined over breeding methods (Appendix A32), with cross III having the highest mean erucic acid concentration at 52.1%.

All three crosses were significant differences between breeding method in erucic acid concentration mean value. The presence of these differences suggested that there are in-vitro selection pressure and gamete selection during tissue culture stages not present in the pedigree selection breeding method (Brown and Wernsman, 1982; Zivy et al., 1992; Guiderdoni, 1991).

Fatty acid composition of rapeseed oil is largely determined by the genetic make up of the developing embryo, rather than the maternal parent (Downey and Harvey, 1963; Thomas and Kondra, 1973), but the levels of polyunsaturated fatty acids are strongly influenced by the environment during oil deposition and seed maturation (Downey, 1983). In *B. napus*, there are two genes, each with multiple alleles, acting in an additive manner, controlling erucic acid concentration (Grami and Steffansson, 1977; Harvey and Downey, 1964).

Table 4.1.15: Erucic acid concentration for pedigree selection (PS) families and doubled haploid (DH) lines for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

Cross/Type	Mean	Std. Error	Range		Number		
			Max	Min	< 41	41 to 50	51 to 60
	(%)		(%)	(%)			
Cross I							
PS	48.6b	0.65	56.7	39.0	12	3	41
DH	52.9a	0.26	56.5	46.0	0	3	56
all (PS+DH)	50.5B	0.39	56.7	39.0	12	6	97
Cross II							
PS	47.9a	0.48	54.5	22.5	5	25	25
DH	44.6b	0.51	56.0	25.0	16	36	8
all (PS+DH)	46.0C	0.35	56.0	22.5	21	61	33
Cross III							
PS	52.6a	0.24	56.0	48.5	0	5	53
DH	51.7b	0.28	55.0	42.2	0	11	49
all (PS+DH)	52.1A	0.19	56.0	42.2	0	16	102
Neptune BX (Check)	52.3				0	2	27

a,b - means within cross with same letters are not significantly different at $p<0.05$.
A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.2.5 Glucosinolate concentration

At Carman, all three crosses demonstrated significantly different mean total glucosinolate concentration for the two breeding methods (Table 4.1.16) (Appendix A33, A34, A35). In crosses I and II, pedigree selection families as a group had a higher glucosinolate concentration than the doubled haploid lines as a group. This was reversed for cross III. Considering the range of glucosinolate concentration for both breeding methods, the range of glucosinolate concentration for doubled haploid lines as a group was much smaller than for the pedigree selection families as a group, while in crosses II and III the glucosinolate ranges were equal for both breeding methods. The distribution

for total glucosinolate concentration for both breeding methods at the Carman site were similar for all crosses with the majority of developed families/lines below the Neptune BX check, however, crosses I and II had some families/lines with glucosinolate concentrations above the Neptune BX mean. Cross I had more doubled haploids lines below the Neptune BX check mean, while cross II had more pedigree selection families below the Neptune BX check mean. Based on these results the doubled haploid line breeding method would be the breeding method of choice to lower glucosinolate concentration for these crosses grown in this environment. There were significant differences for total glucosinolate concentration among the three crosses combined over breeding methods (Appendix A36), with cross I having the highest mean glucosinolate concentration, 2 umol g seed $^{-1}$ and 7 umol g seed $^{-1}$ above cross II and III, respectively.

At Winnipeg, all three crosses demonstrated significantly different mean total glucosinolate concentration for the breeding methods comparison (Table 4.1.17) (Appendix A33, A34, A35). In cross I and II, the pedigree selection families as a group had higher glucosinolate concentration than the doubled haploid lines as a group. This was the reverse in cross III. Crosses I and II had narrow ranges of glucosinolate concentration for the doubled haploid lines compared to the pedigree selection families, while cross III displayed equal glucosinolate concentrations for both breeding methods. The distribution for total glucosinolate concentration for both breeding methods were similar for all crosses with the majority of developed families/lines below the Neptune BX check, however, crosses I and II had some families/lines with glucosinolate concentration above the Neptune BX mean. Crosses I and II had more doubled haploid line with glucosinolate concentrations below the Neptune BX check mean. These results

suggest that the doubled haploid line breeding method may be the breeding method of choice to lower glucosinolate concentration for these crosses grown in this environment. There were significant differences for total glucosinolate concentration among the three crosses combined over breeding methods (Appendix A36), with cross I having the highest mean glucosinolate concentration, 2 umol g seed $^{-1}$ and 12 umol g seed $^{-1}$ above crosses II and III, respectively.

There was little, if any evidence of genetic by environment interaction since cross rankings for both locations were the same, which is contradictory to reports of large site and seasonal variations within the seed glucosinolates (Zhao, et al., 1994; Mailer and Wratten, 1985). Two out of the three crosses showed that the doubled haploid line breeding method reduced the mean values for glucosinolate concentration more than the pedigree selection breeding method.

Glucosinolate concentration is determined by the maternal genotype rather than the embryonic genotype (Stefansson, 1983), therefore, special care on the female plant to posses low glucosinolate concentration is required.

Table 4.1.16: Total glucosinolate concentration at 8.5% moisture for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Carman 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Below[Z]**	**Equal[Y]**	**Above[X]**
	(umol)		**(umol)**	**(umol)**			
Cross I							
PS	29.9 a	1.67	72.9	10.6	43	0	17
DH	17.6 b	0.41	30.5	5.4	59	0	0
all (PS+DH)	23.7 A	0.94	72.9	5.4	102	0	17
Cross II							
PS	24.3 a	1.36	71.0	10.6	57	2	1
DH	18.9 b	0.62	67.3	9.7	51	0	9
all (PS+DH)	21.5 B	0.75	71.0	9.7	108	2	10
Cross III							
PS	16.1 b	0.40	26.7	7.6	60	0	0
DH	17.5 a	0.47	29.7	8.6	60	0	0
all (PS+DH)	16.8 C	0.31	29.7	7.6	120	0	0
Neptune BX (Check)	32.8				<31.8 umol	31.8 to 33.8 umol	>33.8 umol

[Z] below one umol from the check mean for percent total glucosinolate

[Y] +/- one umol from the check mean for percent total glucosinolate

[X] above one umol from the check mean for percent total glucosinolate

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

Table 4.1.17: Total glucosinolate concentration at 8.5% moisture for pedigree selection (PS) families and doubled haploid (DH) line for progeny of three crosses grown at Winnipeg 2000 and compared to Neptune BX check

			Range		Number		
Cross/Type	**Mean**	**Std. Error**	**Max**	**Min**	**Below[Z]**	**Equal[Y]**	**Above[X]**
	(umol)		**(umol)**	**(umol)**			
Cross I							
PS	28.3 a	1.65	72.4	10.8	44	0	16
DH	16.6 b	0.48	45.5	6.0	59	0	1
all (PS+DH)	22.4 A	0.93	72.4	6.0	103	0	17
Cross II							
PS	22.7 a	1.39	75.1	3.6	52	1	7
DH	19.0 b	0.53	35.6	8.9	56	2	2
all (PS+DH)	20.9 B	0.76	75.1	3.6	108	3	9
Cross III							
PS	12.2 b	0.36	21.7	0.2	60	0	0
DH	12.9 a	0.41	24.7	3.5	60	0	0
all (PS+DH)	12.5 C	0.27	24.7	0.2	120	0	0
Neptune BX (Check)	29.4				<28.4 umol	28.4 to 30.4 umol	>30.4 umol

[Z] below one umol from the check mean for percent total glucosinolate

[Y] +/- one umol from the check mean for percent total glucosinolate

[X] above one umol from the check mean for percent total glucosinolate

a,b - means within cross with same letters are not significantly different at $p<0.05$.

A,B,C - means among cross with same letters are not significantly different at $p<0.05$.

4.1.3 Characterization of all agronomic and quality traits combined over locations

All agronomic and quality traits data combined over locations for breeding methods within cross were combined to produce grand means for each trait (Table 4.1.18 and 4.1.19). The combination for grand means is what plant breeders do. This is the best way to assess wide range adaptability.

For the combined over location data, the following was noted. For number of days to first flower, the pedigree selection families were significantly later than the doubled haploid lines for crosses I and II, but by only 0.5 and 0.3 days respectably. For height, the pedigree selection families were significantly taller than the doubled haploid lines for crosses I and II, paralleling, the number of days to first flower results. For number of days to maturity, there were no significant differences between breeding methods. For seed yield, the pedigree selection families were significantly higher yielding than the doubled haploid line means for crosses I and II, but the reverse was the case for cross III. For oil concentration, the doubled haploid lines showed significantly higher oil concentrations than the pedigree selection families for crosses I and II, but the reverse was observed for cross III. For protein concentration, the pedigree selection families were significantly higher in mean protein concentration than the doubled haploid line means in cross II, but the reverse was observed for cross III. For the sum oil and protein concentration, the pedigree selection families in mean sum oil and protein concentration were significantly higher than the doubled haploid line means for crosses I and II. For erucic acid concentration, the pedigree selection families were significantly higher in the

mean erucic acid concentration than the doubled haploid line means for crosses II and III, but the reverse was observed for cross I.

Either breeding method could be used to efficiently develop new high erucic acid rapeseed varieties, at least for the crosses and environments based on this study. The real test of breeding method success would be the relative performance of the individual progeny from each cross in each breeding method. The question to be addressed is how many families or DH lines are acceptable to enter official registration tests?

Table 4.1.18: Means for agronomic traits for pedigree selection (PS) families and doubled haploid (DH) lines for three cross over two locations in 2000

Cross/Type	Days to flower		Days to maturity		Height		Yield	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	(d)		(d)		(cm)		(kg/ha)	
Cross I								
PS	50.06 a	0.08	94.17 a	0.19	95.43 a	1.14	3853.84 a	5.87
DH	49.71 b	0.06	94.04 a	0.18	92.56 b	1.12	3021.49 b	5.11
all (PS+DH)	49.88 B	0.05	94.10 A	0.13	93.97 C	0.80	3430.61 B	4.05
Cross II								
PS	49.88 a	0.08	93.67 a	0.18	102.95 a	1.06	3141.32 a	6.27
DH	49.23 b	0.06	93.40 a	0.18	98.77 b	0.96	2751.72 b	5.38
all (PS+DH)	49.54 C	0.05	93.53 C	0.13	100.83 A	0.72	2943.22 C	4.15
Cross III								
PS	49.92 a	0.09	93.88 a	0.15	98.94 a	0.99	3716.50 b	5.45
DH	50.03 a	0.09	93.43 a	0.34	98.69 a	0.97	3779.07 a	6.97
all (PS+DH)	49.97 A	0.06	93.65 B	0.19	98.81 B	0.69	3748.20 A	4.44
Neptune BX (Check)	50		94		96.5		4066.6	

Table 4.1.19: Means for quality traits for pedigree selection (PS) families and doubled haploid (DH) lines for three cross over two locations in 2000

Cross/Type	Percent Oil Mean	SE	Percent Protein Mean	SE	Total Oil & Protein Mean	SE	Glucosinolate Mean	SE	Erucic Acid[z] Mean	SE
	(%)		(%)		(%)		(%)		(%)	
Cross I										
PS	44.47 b	0.17	29.68 a	0.17	74.14 a	0.10	29.12 a	1.17	48.00 b	0.65
DH	45.46 a	0.13	29.47 a	0.15	74.93 b	0.09	17.09 b	0.32	52.95 a	0.26
all (PS+DH)	44.97 B	0.11	29.57 A	0.11	74.53 B	0.07	23.05 A	0.66	50.54 B	0.39
Cross II										
PS	44.11 b	0.20	30.35 a	0.18	74.45 a	0.11	23.45 a	0.97	47.92 a	0.48
DH	46.32 a	0.19	28.44 b	0.17	74.77 b	0.08	18.99 b	0.41	44.64 b	0.51
all (PS+DH)	45.24 C	0.15	29.38 B	0.13	74.61 B	0.07	21.18 B	0.53	46.04 C	0.35
Cross III										
PS	46.85 a	0.16	28.87 b	0.20	75.72 a	0.10	14.11 b	0.30	52.56 a	0.24
DH	46.18 b	0.18	29.36 a	0.20	75.53 a	0.12	15.19 a	0.34	51.72 b	0.28
all (PS+DH)	46.51 A	0.12	29.12 C	0.14	75.63 A	0.08	14.65 C	0.23	52.14 A	0.19
Neptune BX (Check)	45.2		29.2		74.3		31.1		52.3	

[z] Erucic acid values are for one location and one replication only.

The objective of a breeding program for Brassicas include the development of superior yielding, high quality varieties that mature within the limited growing season on the prairies (Ringdahl et al., 1986). A plant breeder is generally interested in maximizing the variation within a cross, which involves the recombination of genes dispersed between the parents (Snape, 1976). Thus when focus is placed on the populations and opportunity, the pedigree selection breeding method would appear to be more efficient than the doubled haploid line breeding method because there is the opportunity for recombination over more than one generation (Snape, 1976).

There are contradictory reports on the superiority of one breeding method over another, a situation paralleled in this study. Several reports suggest that genetic segregation of qualitative traits should be similar in both doubled haploid line breeding method and conventional derived populations of *B. napus* (Chen and Beversdorf 1990 and Litcher et al., 1988; Thiagarajah and Stringam, 1993). The differences that were significant in this study are likely due to genetic x environment interactions that determine the performance of varieties in different agro-climatic conditions and can slow down the progress of selection (Lekh et al., 1996). Difference can also be explained by additive epistasis and coupling phase linkage between factors controlling the trait (Riggs and Snape, 1977; Jinks and Pooni 1981)

Jinks et al. suggest an alternative explanation in 1985, where focus is placed on the breeding method itself. There is greater inbreeding depression in doubled haploid line breeding method than pedigree selection breeding method because there is a lack of residual heterozygosity in doubled haploid line than present in conventional produced inbred families. There can be random mutation during anther culture techniques of the doubled haploid line breeding method not found in pedigree selection breeding method. There can be mutagenic effects of colchicine induced chromosome doubling of haploids in doubled haploid line breeding method not found in pedigree selection breeding method. There can be cytoplasmic deficiency due to unconventional sources of the cytoplasm in doubled haploids obtained from anther culture or preferential selection among microspores during anther culture. Finally poor seed quality in the first generation of the doubled haploid line breeding method as a consequence of the colchicine treatments can result in differences from the pedigree selection breeding method.

All of the above except for preferential selection leads to the expectation that the doubled haploid line samples would be less vigorous. In this study, this was not the case, because when improvements in mean values were present the majority were seen for the doubled haploid line breeding method. This could be due to the nature of the genetical control of each character (Jinks and Pooni 1981). The doubled haploid line breeding method is equal to, or superior to the pedigree selection breeding method for all agronomic and quality traits investigated in this study.

4.2 SELECTION OF ACCEPTABLE FAMILIES OR LINES

Overall mean values were calculated for each trait for each family or line in each breeding method for each cross. These overall mean values were then compared to established selection criteria based on the internal Neptune BX check to determine the number of pedigree selection families and doubled haploid lines (AFL's) that were acceptable for entry into official registration tests (Table 4.2.1).

Table 4.2.1: Number of acceptable families/lines based on selection criteria meeting all requirements, for all pedigree selection families and doubled haploid lines for three crosses, data combined over replications and locations, using two breeding methods

Cross/Breeding Method	Number of AFL's [Z] selected	Sample Size	Percent Selected
Cross I			
Pedigree Selection Method	16	60	26.67%
Doubled Haploid Line Method	6	60	10.00%
Cross II			
Pedigree Selection Method	9	60	15.00%
Doubled Haploid Line Method	5	60	8.33%
Cross III			
Pedigree Selection Method	20	60	33.33%
Doubled Haploid Line Method	26	60	43.33%
Total combined over all crosses			
Pedigree Selection Method	45	180	25.00%
Doubled Haploid Line Method	37	180	20.56%

[Z]AFL = acceptable families or lines

The pedigree selection breeding method produced substantially more AFL's than the doubled haploid line breeding method for cross I and nearly twice as many AFL's in cross II. In contrast, in cross III the doubled haploid line breeding method produced substantially more AFL's than the pedigree selection breeding method. Cross III produced the most AFL's (46 out of 82 total) of any of the crosses. It was the best cross from a plant breeder's perspective of the three crosses used in this study. Combined over all crosses, the pedigree selection breeding method produced eight more AFL's than the doubled haploid line breeding method (45 versus 37), i.e. the pedigree selection breeding method produced 21% more AFL's than the doubled haploid line breeding method. This is a large enough relative difference to suggests that the pedigree selection breeding method would be preferable to the doubled haploid line breeding method.

A more stringent comparison of breeding methods, where an increases in yield are desired, is warranted. To this end, a second set of selection criteria, where yield of the families or lines were at least 10% higher yielding than the internal Neptune BX check were used to determine the number of AFL's, combined over replications and locations, for each breeding method in each cross (Table 4.2.2). The pedigree selection breeding method produced substantially more AFL's than the doubled haploid line breeding method for cross I and three times more AFL's than the doubled haploid line breeding method in cross II. In contrast, cross III produced more AFL's using the doubled haploid line breeding method than the pedigree selection breeding method. Cross III again produced the most AFL's (23 out of 40 total) of any of the other crosses. Combined over all crosses, the pedigree selection breeding method and the doubled haploid breeding

method produced equal numbers of AFL's, suggesting that either breeding method could be used to develop new HEAR varieties with equal effectiveness.

Table 4.2.2: Number of acceptable families/lines based on selection criteria meeting all requirements, exceeding combined yield of Neptune BX by 10%, for all pedigree selection families and doubled haploid lines for three crosses, data combined over replications and locations, using two breeding methods

Cross/Breeding Method	Number AFL's[Z] selected	Sample Size	Percent Selected
Cross I			
Pedigree Selection Method	8	60	13.33%
Doubled Haploid Line Method	5	60	8.33%
Cross II			
Pedigree Selection Method	3	60	5.00%
Doubled Haploid Line Method	1	60	1.67%
Cross III			
Pedigree Selection Method	9	60	15.00%
Doubled Haploid Line Method	14	60	23.33%
Total over all crosses			
Pedigree Selection Method	20	180	11.11%
Doubled Haploid Line Method	20	180	11.11%

[Z]AFL = acceptable families or lines

Cross III was the best cross for producing AFL's, regardless of the breeding method used, while cross II was the poorest cross for producing AFL's. The differences in the crosses is very distinct because of the genetic diversity and nicking that exist.

A final comparison of breeding methods, where the selection criteria included an increase in yield to 20% greater than the internal Neptune BX check was done to determine the number of AFL's combined over replications and locations for each breeding method in each cross (Table 4.2.3).

Table 4.2.3: Number of acceptable families/lines based on selection criteria meeting all requirements, exceeding combined yield of Neptune BX by 20%, for all pedigree selection families and doubled haploid lines for three crosses, data combined over replications and locations, using two breeding methods

Cross/Breeding Method	Number AFL's[Z] selected	Sample Size	Percent Selected
Cross 1			
Pedigree Selection Method	3	60	5.00%
Doubled Haploid Line Method	3	60	5.00%
Cross 2			
Pedigree Selection Method	1	60	1.67%
Doubled Haploid Line Method	0	60	0.00%
Cross 3			
Pedigree Selection Method	8	60	13.33%
Doubled Haploid Line Method	11	60	18.33%
Total over all crosses			
Pedigree Selection Method	12	180	6.67%
Doubled Haploid Line Method	14	180	7.78%

[Z]AFL = acceptable families or lines

The pedigree selection breeding method produced nearly equal numbers of AFL's to the doubled haploid line breeding method for all three crosses, at this 20% yield increase level. Cross III produced the most AFL's (19 out of 26 total) of any of the crosses. It was by far the best cross from a plant breeder's perspective of the three crosses used in this study. Combined over all crosses, the pedigree selection breeding method produced almost equal numbers of AFL's to the doubled haploid line breeding method (12 versus 14). Therefore, either breeding method was equally efficient at producing AFL's. There is therefore, clearly no superior breeding method for the production of AFL's for these crosses grown in these environments.

The determination of AFL's for these three crosses in these environments suggests that at a basic selection criteria level, more AFL's were produced by the pedigree selection breeding method, however, when more stringent selection criteria were applied for increases in yield, the differences in the number of AFL's produced between the breeding methods disappeared. This suggests that breeding methods were equally effective at producing AFL's for these crosses grown in these environments.

4.3 ECONOMIC ANALYSIS OF PEDIGREE SELECTION BREEDING METHOD VERSUS DOUBLED HAPLOID LINE BREEDING METHOD

Resource requirements and the relative costs associated with the pedigree selection breeding method and the doubled haploid line breeding method may be different. Relative costs and resources were calculated to include costs, space requirements and time allocations. Additionally, costs were broken down into their fixed and variable components to determine the component contributions of each component to overall costs. Costs as related to time and capacity were calculated to determine the optimum level of efficiency for breeding programs. Finally, the net present value was calculated to determine the benefits associated with each program. This information established the cost benefit ratio.

4.3.1 Relative Costs and Resource Requirements

The expenses that are required for the development of one potentially enterable family or line from the initial cross to entering official registration tests using the doubled haploid line breeding method showed a savings of $18,668 compared to the pedigree selection breeding method (Table 4.3.1) (Appendix C). This is not a significant savings. A significant savings would be at least a 15% difference. More space is required for the doubled haploid line breeding method than the pedigree selection breeding method. The increase space allocation is required for the biotechnology laboratory used for tissue culture work, which is considerably valuable space for the doubled haploid line breeding method. There is a gain of 61 days using the doubled haploid line breeding method versus

the pedigree selection breeding method, however, in the context of a breeding effort that requires 4 ½ years to produce acceptable families or lines from the initial cross, this gain in time is not substantial. This information demonstrates that the doubled haploid line breeding method is faster, but more expense and has larger space requirements as compared to the pedigree selection breeding method. The gains achieved by the doubled haploid line breeding method are quite small, when viewed in the context of the total costs and the total time required to develop potentially enterable families or lines.

Table 4.3.1: Summary of allocations of cost, space, and time, associated with the development of two breeding programs, pedigree selection breeding method and doubled haploid line breeding method at the university of Manitoba HEAR program

	Pedigree Selection Breeding Method							Doubled Haploid Line Breeding Method						
		Space Allocations							Space Allocations					
Section of Program	Total Expenses	GR	GH	Biotech Lab	Field	Quality Lab	Duration	Total Expenses	GR	GH	Biotech Lab	Field	Quality Lab	Duration
Units	$	m^2	m^2	m^2	m^2	m^2	Days	$	m^2	m^2	m^2	m^2	m^2	Days
1	$45,441	0.04	0.64	N/A	N/A	N/A	122	$45,440	0.04	0.64	N/A	N/A	N/A	122
2	$45,267	0.49	0.32	N/A	N/A	N/A	122							
3	$46,487	5.15	3.32	N/A	N/A	N/A	122	$119,520	1.03	0.48	27.86	N/A	N/A	305
4	$46,433	4.94	3.19	N/A	N/A	N/A	122	$45,408	1.03	0.48	N/A	N/A	N/A	122
5	$233,407	N/A	N/A	N/A	2023.43	111.43	365	$233,407	N/A	N/A	N/A	2023.43	111.43	365
6	$224,916	N/A	N/A	N/A	4046.86	111.43	365	$224,916	N/A	N/A	N/A	4046.86	111.43	365
7	$225,189	N/A	N/A	N/A	12140.57	111.43	365	$225,189	N/A	N/A	N/A	12140.57	111.43	365
Total	$867,140	10.62	7.47	0	18210.86	334.29	1583	$848,472	1.07	1.12	27.86	18210.86	334.29	1522

4.3.2 Total Cost Components

Total calculated costs were separated into two categories, either fixed or variable (Appendix B). Variable costs are those that are dependent on the volume present, and are included in two categories, either supplies or rent/lease. Rent/lease includes land, quality analysis, equipment and fuel. Fixed costs are not dependent on volume and included salary and wages, repairs and maintenance, chemicals, and insurance. It is important to break total cost down into its components, either fixed or variable to determine the amount each contributed to the overall cost. Of the total cost incurred by the pedigree selection breeding method, 21.51% is due to variable costs compared to 22.38% for the doubled haploid line breeding method (Table 4.3.2), which means that almost 80% of the costs for both breeding methods are fixed. This is the amount of funds that are required for one cross to be developed. Increasing the number of crosses would reduce the average fixed costs. This incentive would persuade breeders to have programs with numerous crosses being developed simultaneously. It is also interesting that the during the non-field stages of a breeding program, the costs incurred are almost all fixed for both breeding methods, while in the field stages, approximately ¼ of the costs incurred are variable.

Table 4.3.2: Percentage of variable costs associated with two breeding methods, pedigree selection breeding method and doubled haploid line breeding method at the university of Manitoba HEAR program

	Pedigree Selection Method				Doubled Haploid Line Method			
Section of Program	**Total Expenses**	**Fixed Expenses**	**Variable Expenses**	**Percent Variable Expenses of Total**	**Total Expenses**	**Fixed Expenses**	**Variable Expenses**	**Percent Variable Expenses of Total**
1	$45,441	$45,138	$303	0.67%	$45,441	$45,138	$303	0.67%
2	$45,267	$45,138	$130	0.29%				
					$119,520	$113,419	$6,100	5.10%
3	$46,487	$45,138	$1,350	2.90%				
4	$46,433	$45,138	$1,296	2.79%	$45,408	$45,138	$270	0.59%
5	$233,407	$166,598	$66,809	28.62%	$233,407	$166,598	$66,809	28.62%
6	$224,916	$166,639	$58,277	25.91%	$224,916	$166,639	$58,277	25.91%
7	$225,189	$166,802	$58,387	25.93%	$225,189	$166,802	$58,387	25.93%
Total	$867,140	$680,591	$186,552	21.51%	$848,473	$658,596	$189,876	22.38%

4.3.3 Relationship of Cost to Time and Capacity

The cost per family per day for the pedigree selection breeding method is $547.78, while the cost per line per day for the doubled haploid line breeding method is $557.47 (Table 4.3.3). The cost per family or line per day calculation shows a difference of only $10, which is not sufficient to warrant choosing one breeding method over the other.

The total expenses that were calculated for each breeding method are based on the calculated costs to develop a single line, however, this is not an accurate representation of the operations of breeding programs. Most breeding programs increase the number of initial crosses per year to fall within a range of eight to twelve. To increase the initial crosses from one to eight or twelve crosses, only the variable cost is altered. Revised total costs, adjusted for different capacities, in this case, a range from 8 to 12 crosses per year were calculated (Table 4.3.3). Costs per day per capacity, for one cross, for 8 crosses (low capacity), or for 12 crosses (high capacity) were calculated. This showed that there was a decrease in the average costs as the numbers of crosses handled were increased. There were also minimal differences in the calculated values of cost per day per capacity level for either the pedigree selection breeding method or the doubled haploid breeding method, with differences ranging from $8 to $10 per family or line per day for these capacity scenarios. It appears that either breeding method is equally effective, based on this cost relationship.

Table 4.3.3: Calculated costs per breeding method, either pedigree selection or doubled haploid line method based on the number of lines developed

Factors/amounts		Breeding Methods	
		Pedigree Selection	Doubled Haploid Line
Days		1583	1522
Capacity			
	Number of Lines (Low)	8	8
	Number of Lines (High)	12	12
Expenses			
	Single line	$867,140	$848,472
	Low capacity	$2,172,987	$2,177,599
	High capacity	$2,919,185	$2,937,100
Cost/day/line			
	Single line	$548	$557
	Low capacity	$172	$179
	High capacity	$154	$161

4.3.4 Cost Benefit Analysis

Net present value (NPV) calculates the value of an investment by using a discount rate, payments and income. For example, the discount rate is arbitrarily chosen to be 10%, the total costs represent the payment of the NPV calculation and cultivar royalty will represent the income value of the NPV calculation (Table 4.3.3). The NPV value is the current net value of an investment that will yield a specific amount on a given future date. This concept is based on the time-value of money, which is the case when dealing with breeding programs. Using NPV and its components, aid in make important management decisions looking at the worth of an investment, such as a breeding program (Table 4.3.4).

Table 4.3.4: Calculation of net present value (NPV), benefits, and ratio's used for project evaluations for pedigree selection breeding method and doubled haploid line breeding method

Factors/amounts	Breeding Methods	
	Pedigree Selection	Doubled Haploid Line
Components of NPV		
Total Costs	$867,140	$848,472
Days	1522	1583
Rate	0.1	0.1
Breeders Royalty (Low)	$50,000	$50,000
Breeders Royalty (high)	$250,000	$250,000
Calculation outcome		
NPV (low)[z]	($746,987)	($730,016)
NPV (high)[z]	($581,698)	($564,727)

[z] Microsoft Excel, NPV (rate, - total costs, breeders royalty)

The calculated NPV using both low and high estimated cultivar royalty levels, results in a negative dollar value. This negative value indicates that the economics of both breeding methods to develop HEAR varieties are unacceptable (Lusztig et al. 2001). The NPV values calculated represent the outcome of the benefits minus the costs, thus having a negative NPV value signified that the benefits to the variety developer did not supersede the costs, suggesting little monetary gain from HEAR variety development in this study.

Comparison of the marginal costs per family or line, with respect to capacity level, to the marginal benefits received as cultivar royalties, either at a low level of $50,000 or high level of $250,000, showed that there was an optimal capacity level of greater than 10 crosses per year (Figure 4.1). Developing breeding programs where there

are more than 10 crosses developed each year will produce marginal benefits that exceed marginal costs, assuming that the high cultivar royalty is paid annually. This outcome is dependent on the fact that for every cross made every year, there will be a new variety that is accepted for registration.

This leads to the determination of an optimum capacity level for the breeding programs. An optimum program is at the point of greatest difference between marginal benefits (breeder royaltities) and the total average costs (McConnell, 1999). From this analysis, it has been found that an increase in the number of crosses, produces a decrease in the average total costs. The increase in number of crosses also resulted in an increase in the marginal benefits that were received, assuming that all crosses produce registerable cultivars. Therefore, an optimum breeding program will exist when total average costs and marginal costs show the greatest difference. The limiting factor in determining the greatest difference lies with the number of crosses that can be handled by a breeding program, and this is limited by the economy of size. The economy of size or capacity at which a breeding program could operate depends on the availability of space, trained technicians and funding. The maximum number of crosses that a breeding program can handle will be the point of optimization.

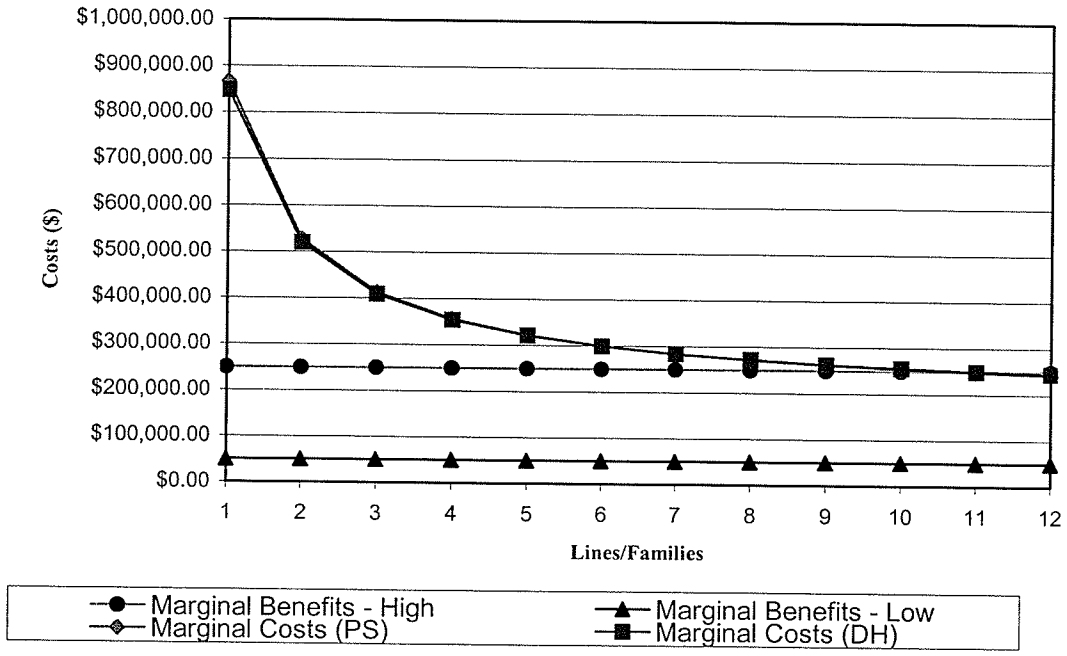

Figure 4.1: Marginal cost and marginal benefits for developed pedigree selection families and doubled haploid lines

The development of new varieties has been carried out in part as a public service to agriculture in the host country. Currently, more variety development is being conducted in the private sector, due to the additional revenue and spin-off benefits generated by commercial production and utilization of new varieties once registered. It is these additional revenues and benefits that justify the variety development effort in many cases.

Variety royaltities were given as a range of values, which suggests that, depending on the agronomic and quality characteristics that a line or family displayed once registered would be reflected in the cultivar royalty. It would then be interesting to see the correlation that exists between variety characteristics and the royalty received.

One could hypothesize that a higher yielding variety should receive a higher royalty because this is a highly desired trait for producers and consumers.

Research is an investment and it has been documented that the research and development in Canola has been profitable in Canada (Nagy and Furtan, 1978), which contrasts with the information reported in this study. HEAR cultivar development is a tiny facet of the entire rapeseed/canola industry in Canada, and it is conceivable to have a portion of this large industry having marginal costs, which exceed marginal benefits.

Gains can be achieved through other levels than mentioned above. For example, Nagy and Furtan, 1978 examine the relationship between producers and consumers when improvement in yield is achieved in a canola cultivar. Higher yielding varieties permit more canola to be grown with the same level of conventional inputs such as land, labor and capital. Obviously the increase in yield will increase the supply. Increased supply brought about by research has an effect on the producers of canola and the consumers. Consumers will see gains because had there not been higher yielding varieties, there would be less quantity offered for sale in the market place. Given that demand is elastic, this would mean lower prices for consumers, thus consumers have gained the difference between the price they would have paid without research and what they actually paid due to the increase in seed yield from research.

What the consumer's gain from the decrease in price the producer loses. Assuming that supply is elastic, producers make up for the loss in price, by the increase in sales, and they are producing at a lower costs because they have obtained higher yields if using the same conventional inputs. Therefore it is a win-win situation for both consumer and producers even with the substantial costs that were incurred from research.

5.0 General Discussion and Conclusion

Choice of a breeding method should be determined by the efficiency with which superior combinations can be assured and thus is dependent on the crop species, breeding objectives and the resources available to the breeder (Poehlman and Sleper 1999). In this study there were no significant differences between using either breeding method, thus providing evidence that for these crosses grown in these environments, it is ultimately the breeder's choice and the decision of which breeding method is superior should be based on trained staff, space and breeder's preference.

The comparison of agronomic characteristics including days to first flower, plant height, days to maturity, and seed yield, and the quality characteristics oil concentration, protein concentration, sum of oil and protein concentration, erucic acid concentration and glucosinolate concentration found that neither breeding method was clearly superior. Of the traits that showed improvements, most came from the doubled haploid line breeding method, conversely, seed yield, one of the most difficult traits to breed for improvement, showed more improvement using the pedigree selection breeding method. It is not unrealistic to see more trait improvement using the doubled haploid line breeding method because trait fixation produced more expression at the extremes.

Using a selection criteria to determine the number of these families or lines that showed trait improvements as compared to an in-house Neptune BX check, this study showed that there were no differences in the number of families or lines that could advance to further trials. This supports the outcome of the trait comparison field trials, in

that there was little difference in the two breeding methods used for these crosses, grown in these environments.

There are no major differences in the costs associated with either breeding methods. There was a savings of approximately $18,000, using the doubled haploid line breeding method compared to the pedigree selection breeding method. The majority of the costs are of a fixed nature, with about 22% being associated with variable costs, supporting increased efficiency with an increased of number of lines. There is a time savings of 61 days using the doubled haploid breeding method, which, is not very significant in a 4½ year total program time period. Comparison of each breeding method to the marginal costs and marginal benefits showed that if the breeder performs over 10 crosses a year, and each one produced a registerable variety, and the maximum variety royalty is paid, tiny marginal benefits will be seen, otherwise variety development costs are substantially higher than the benefits that can be achieved.

Developing new HEAR varieties using the methods compared in this study is a money losing proposition, thus upon reflection, how do the agricultural companies absorb losses of this magnitude? Possibly higher royalty levels? It is known that agricultural companies do make substantial profits, and this is due to vertical integration of the companies into areas of pedigree seed development, seed treatment applications and certified seed sales in addition to plant breeding.

Due to time constraints, only one field season was feasible, thus it would be desirable to repeat the field experiments to look for differences among breeding methods. The calculated costs were specific to the University of Manitoba, a public sector

institution and it would be advantageous to perform the same analysis using a private sector company.

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7.0 Appendix

7.1 Appendix A

Appendix Table A1: General linear model (GLM) analysis for number of days to flower for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	85.17	76.71	***
Lines	59	56.60	0.86	NS
Location*Line[z]	59	65.51	1.31	NS
Replication	1	0.96	1.12	NS
Treatment[y]	1	15.59	18.34	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A2: General linear model (GLM) analysis for number of days to flower for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	122.18	134.23	***
Lines	59	56.70	1.06	NS
Location*Line[z]	59	53.70	1.14	NS
Replication	1	0.34	0.43	NS
Treatment[y]	1	52.74	66.34	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A3: General linear model (GLM) analysis for number of days to flower for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	461.90	531.06	***
Lines	59	52.09	102.00	NS
Location*Line[z]	59	51.32	1.34	NS
Replication	1	6.21	9.55	*
Treatment[y]	1	0.97	1.49	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A4: General linear model (GLM) analysis for number of days to flower for all crosses over field trial locations for 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	47.29	26.17	***

NS non-significant

* ** *** significant at p=0.05, 0.01, 0.001 respectively

Appendix Table A5: General linear model (GLM) analysis for height for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	90627.01	993.87	***
Lines	59	8879.32	1.65	*
Location*Line[z]	59	5380.00	1.11	NS
Replication	1	6334.19	77.29	***
Treatment[y]	1	1176.61	14.36	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A6: General linear model (GLM) analysis for height for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	65938.14	686.96	***
Lines	59	7374.02	1.30	NS
Location*Line[z]	59	5663.17	1.03	NS
Replication	1	1461.94	15.65	***
Treatment[y]	1	2462.27	26.35	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A7: General linear model (GLM) analysis for height for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	59372.50	452.30	***
Lines	59	7107.34	0.92	NS
Location*Line[z]	59	7744.77	1.53	*
Replication	1	2760.94	32.08	***
Treatment[y]	1	58.01	0.67	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A8: General linear model (GLM) analysis for height for all cross, over field trial locations 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	11973.75	59.16	***

NS non-significant

* ** *** significant at p=0.05, 0.01, 0.001 respectively

Appendix Table A9: General linear model (GLM) analysis for number of days to maturity for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	2990.06	2082.26	***
Lines	59	143.75	1.70	*
Location*Line[z]	59	84.72	0.91	NS
Replication	1	9.35	5.95	*
Treatment[y]	1	0.46	0.29	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A10: General linear model (GLM) analysis for number of days to maturity for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	2694.30	1456.57	***
Lines	59	184.04	1.69	*
Location*Line[z]	59	109.14	1.18	NS
Replication	1	79.37	50.49	***
Treatment[y]	1	3.58	2.28	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A11: General linear model (GLM) analysis for number of days to maturity for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	1526.33	126.54	***
Lines	59	635.15	0.89	NS
Location*Line[z]	59	711.63	0.86	NS
Replication	1	8.46	0.60	NS
Treatment[y]	1	17.39	1.24	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A12: General linear model (GLM) analysis for number of days to maturity for all crosses, over field trial locations 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	88.80	7.82	***

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A13: General linear model (GLM) analysis for seed yield for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	108300.99	21.56	***
Lines	59	711964.75	2.40	***
Location*Line[z]	59	296438.01	0.80	NS
Replication	1	32856.90	5.22	NS
Treatment[y]	1	311137.81	49.46	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A14: General linear model (GLM) analysis for seed yield for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	231997.22	52.58	***
Lines	59	542093.02	2.08	***
Location*Line[z]	59	260321.10	0.71	NS
Replication	1	581278.75	93.22	***
Treatment[y]	1	83299.02	13.36	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A15: General linear model (GLM) analysis for seed yield for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	62059.88	7.31	**
Lines	59	781726.98	1.56	*
Location*Line[z]	59	500639.90	1.01	NS
Replication	1	73157.61	8.71	**
Treatment[y]	1	971.46	0.12	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A16: General linear model (GLM) analysis for seed yield for all crosses, among field trial locations 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	579261.95	36.35	***

NS non-significant

* ** *** significant at p=0.05, 0.01, 0.001 respectively

Appendix Table A17: General linear model (GLM) analysis for oil concentration for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	433.36	139.78	***
Lines	59	560.49	3.06	***
Location*Line[z]	59	182.92	0.88	NS
Replication	1	7.17	2.03	NS
Treatment[y]	1	112.23	31.77	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A18: General linear model (GLM) analysis for oil concentration for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	1279.37	383.35	***
Lines	59	1118.70	5.68	***
Location*Line[z]	59	196.91	0.72	NS
Replication	1	0.02	0.00	NS
Treatment[y]	1	539.75	117.01	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A19: General linear model (GLM) analysis for oil concentration for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	517.84	89.03	***
Lines	59	813.60	2.37	***
Location*Line[z]	59	343.16	1.33	NS
Replication	1	41.91	9.59	**
Treatment[y]	1	61.90	14.17	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A20: General linear model (GLM) analysis for oil concentration for all crosses, among field trial locations 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	625.31	56.24	***

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A21: General linear model (GLM) analysis for protein concentration for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	1355.62	402.81	***
Lines	59	363.26	1.83	*
Location*Line[z]	59	198.56	1.28	NS
Replication	1	6.98	2.65	NS
Treatment[y]	1	4.26	1.62	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A22: General linear model (GLM) analysis for protein concentration for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	1663.24	635.83	***
Lines	59	567.26	3.68	***
Location*Line[z]	59	154.34	1.02	NS
Replication	1	82.24	32.17	***
Treatment[y]	1	382.62	149.65	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A23: General linear model (GLM) analysis for protein concentration for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	2229.75	466.72	***
Lines	59	608.06	2.16	**
Location*Line[z]	59	281.87	1.44	*
Replication	1	83.89	25.28	***
Treatment[y]	1	39.18	11.81	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A24: General linear model (GLM) analysis for protein concentration for all crosses, among field trial locations 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	47.03	6.09	***

NS non-significant.
* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A25: General linear model (GLM) analysis for sum of oil and protein concentration for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	255.94	297.54	***
Lines	59	224.56	4.42	***
Location*Line[z]	59	50.75	0.73	NS
Replication	1	0.00	0.00	NS
Treatment[y]	1	72.67	61.88	***

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.
* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A26: General linear model (GLM) analysis for sum of oil and protein concentration for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	25.18	23.89	***
Lines	59	301.31	4.86	***
Location*Line[z]	59	61.97	0.69	NS
Replication	1	79.76	52.41	***
Treatment[y]	1	13.49	8.86	**

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.
* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A27: General linear model (GLM) analysis for sum of oil and protein concentration for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	598.59	484.59	***
Lines	59	146.69	2.01	**
Location*Line[z]	59	72.88	0.88	NS
Replication	1	7.24	5.18	*
Treatment[y]	1	2.60	1.86	NS

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A28: General linear model (GLM) analysis for sum of oil and protein concentration for all crosses, among field trial locations 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	343.31	96.98	***

NS non-significant

* ** *** significant at p=0.05, 0.01, 0.001 respectively

Appendix Table A29: General linear model (GLM) analysis for erucic acid concentration for cross 1, at Carman 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Lines	59	2982.83	1.01	NS
Treatment[Z]	1	459.41	9.16	**

[Z] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A30: General linear model (GLM) analysis for erucic acid concentration for cross 2, at Carman 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Lines	59	2792.21	0.91	NS
Treatment[Z]	1	346.39	6.69	*

[Z] Treatment is either pedigree selection or doubled haploid.
NS non-significant.
* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A31: General linear model (GLM) analysis for erucic acid concentration for cross 3, at Carman 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Lines	59	294.53	1.63	*
Treatment[Z]	1	14.78	4.82	*

[Z] Treatment is either pedigree selection or doubled haploid.
NS non-significant.
* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A32: General linear model (GLM) analysis for erucic acid concentration for all crosses, at Carman 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	2118.27	23.18	***

NS non-significant.
* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A33: General linear model (GLM) analysis for total glucosinolate concentration for cross 1, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	193.36	12.36	***
Lines	59	38882.98	42.14	***
Location*Line[Z]	59	922.76	0.14	NS
Replication	1	8.66	0.08	NS
Treatment[Y]	1	16584.09	149.96	***

[Z] Error a for location and lines.

[Y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A34: General linear model (GLM) analysis for total glucosinolate concentration for cross 2, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	86.80	1.77	NS
Lines	59	24709.17	8.52	***
Location*Line[Z]	59	2899.71	0.54	NS
Replication	1	60.98	0.67	NS
Treatment[Y]	1	2432.71	26.87	***

[Z] Error a for location and lines.

[Y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A35: General linear model (GLM) analysis for total glucosinolate concentration for cross 3, combined over locations in 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Location	1	2144.28	132.98	***
Lines	59	3051.76	3.21	***
Location*Line[z]	59	951.39	1.10	NS
Replication	1	1.78	0.12	NS
Treatment[y]	1	111.26	7.58	**

[z] Error a for location and lines.

[y] Treatment is either pedigree selection or doubled haploid.

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

Appendix Table A36: General linear model (GLM) analysis for total glucosinolate concentration for all crosses, among field trial locations 2000

Factor	Df	Sums of Squares	F-statistic	Significance
Cross	2	18011.16	88.62	***

NS non-significant.

* ** *** significant at p=0.05, 0.01, 0.001 respectively.

7.2 Appendix B

Appendix Table B1: Expense break down for the development of section 1 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$42,964.93
	Repairs and Maintanice[2]	$1,000.00
	Chemicals	$500.00
	Insurance	$672.88
	Total	$45,137.81
Variable Expenses		
	Supplies	$83.04
	Rent[3]	$220.08
	Total	$303.12
	Total Expenses	$45,441

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as fuel, light, power, water, labour etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B2: Expense break down for the development of section 2 for pedigree selection breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$42,964.93
	Repairs and Maintanice[2]	$1,000.00
	Chemicals	$500.00
	Insurance	$672.88
	Total	$45,137.81
Variable Expenses		
	Supplies	$41.52
	Rent[3]	$88.03
	Total	$129.55
	Total Expenses	$45,267

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as fuel, light, power, water, labour etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B3: Expense break down for the development of section 2 and 3 for doubled haploid line breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$107,894.66
	Repairs and Maintanice[2]	$3,342.47
	Chemicals	$500.00
	Insurance	$1,682.20
	Total	$113,419.32
Variable Expenses		
	Supplies	$3,899.47
	Rent[3]	$2,200.82
	Total	$6,100.29
	Total Expenses	$119,520

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as fuel, light, power, water, labour etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B4: Expense break down for the development of section 3 for pedigree selection breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$42,964.93
	Repairs and Maintanice[2]	$1,000.00
	Chemicals	$500.00
	Insurance	$672.88
	Total	$45,137.81
Variable Expenses		
	Supplies	$432.50
	Rent[3]	$917.01
	Total	$1,349.51
	Total Expenses	$46,487

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as fuel, light, power, water, labour etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B5: Expense break down for the development of section 4 optional for doubled haploid line breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$42,964.93
	Repairs and Maintanice[2]	$1,000.00
	Chemicals	$500.00
	Insurance	$672.88
	Total	$45,137.81
Variable Expenses		
	Supplies	$86.50
	Rent[3]	$183.40
	Total	$269.90
	Total Expenses	$45,408

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as fuel, light, power, water, labor etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B6: Expense break down for the development of section 4 for pedigree selection breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$42,964.93
	Repairs and Maintanice[2]	$1,000.00
	Chemicals	$500.00
	Insurance	$672.88
	Total	$45,137.81
Variable Expenses		
	Supplies	$415.20
	Rent[3]	$880.33
	Total	$1,295.53
	Total	$46,433

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as fuel, light, power, water, labour etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B7: Expense break down for the development of section 5 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$150,421.84
	Repairs and Maintanice[2]	$16,000.00
	Chemicals	$57.89
	Insurance	$118.54
	Total	$166,598.27
Variable Expenses		
Rent and Lease[3]		
	Land	$110.00
	Quality	$36,130.25
	Equipment	$27,967.50
	Fuel	$2,601
	Total	$66,808.61
	Total Expenses	$233,407

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as light, power, water, labor (for quality only) etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B8: Expense break down for the development of section 6 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$150,421.84
	Repairs and Maintanice[2]	$16,000.00
	Chemicals	$98.62
	Insurance	$118.54
	Total	$166,639.01
Variable Expenses		
Rent and Lease[3]		
	Land	$220.00
	Quality	$13,138.27
	Equipment	$42,317.50
	Fuel	$2,601
	Total	$58,277
	Total Expenses	$224,916

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as light, power, water, labor (for quality only) etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

Appendix Table B9: Expense break down for the development of section 7 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Fixed Expenses		
	Salary and Wages[1]	$150,421.84
	Repairs and Maintanice[2]	$16,000.00
	Chemicals	$261.56
	Insurance	$118.54
	Total	$166,801.95
Variable Expenses		
Rent and Lease[3]		
	Land	$330.00
	Quality	$13,138.27
	Equipment	$42,317.50
	Fuel	$2,601
	Total	$58,387
	Total Expenses	$225,189

[1] includes benefits

[2] both fixed as it is allocated each year, and variable as repairs are made but this will not be calculated

[3] includes utilities such as light, power, water, labor (for quality only) etc.

Taxes, depreciation, and interest are not incurred by the University of Manitoba

7.3 Appendix C

Appendix Table C1: Total resource requirements for the development of section 1 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Total Expenses		$45,440.93
Space		
	Growthroom	0.0412 m2
	Greenhouse	0.637 m2
	Biotech Lab	N/A
	Field	N/A
	Quality Lab	N/A
Time (Days)		122

Appendix Table C2: Total resource requirements for the development of section 2 for pedigree selection breeding methods at the university of Manitoba HEAR program

Total Expenses		$45,267.36
Space		
	Growthroom	0.4944 m2
	Greenhouse	0.3185 m2
	Biotech Lab	N/A
	Field	N/A
	Quality Lab	N/A
Time (Days)		122

Appendix Table C3: Total resource requirements for the development of section 2 and 3 for doubled haploid line breeding methods at the university of Manitoba HEAR program

Total Expenses		$119,519.61
Space		
	Growthroom	1.03 m2
	Greenhouse	0.4779 m2
	Biotech Lab	27.86 m2
	Field	N/A
	Quality Lab	N/A
Time (Days)		305

Appendix Table C4: Total resource requirements for the development of section 3 for pedigree selection breeding methods at the university of Manitoba HEAR program

Total Expenses		$46,487.31
Space		
	Growthroom	5.15 m2
	Greenhouse	3.318 m2
	Biotech Lab	N/A
	Field	N/A
	Quality Lab	N/A
Time (Days)		122

Appendix Table C5: Total resource requirements for the development of section 4 optional for doubled haploid line breeding methods at the university of Manitoba HEAR program

Total Expenses		$45,407.71
Space		
	Growthroom	1.03 m2
	Greenhouse	0.4779 m2
	Biotech Lab	N/A
	Field	N/A
	Quality Lab	N/A
Time (Days)		122

Appendix Table C6: Total resource requirements for the development of section 4 for pedigree selection breeding methods at the university of Manitoba HEAR program

Total Expenses		$46,433.33
Space		
	Growthroom	4.944 m2
	Greenhouse	3.186 m2
	Biotech Lab	N/A
	Field	N/A
	Quality Lab	N/A
Time (Days)		122

Appendix Table C7: Total resource requirements for the development of section 5 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Total Expenses		$233,406.88
Space		
	Growthroom or Greenhouse	N/A
	Biotech Lab	N/A
	Field	2023.43 m^2
	Quality Lab	111.43 m^2
Time (Days)		365

Appendix Table C8: Total resource requirements for the development of section 6 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Total Expenses		$224,915.64
Space		
	Growthroom or Greenhouse	N/A
	Biotech Lab	N/A
	Field	4046.86 m^2
	Quality Lab	111.43 m^2
Time (Days)		365

Appendix Table C9: Total resource requirements for the development of section 7 for pedigree selection and doubled haploid line breeding methods at the university of Manitoba HEAR program

Total Expenses	$225,188.58
Space	
Greenhouse or Growthroom	N/A
Biotech Lab	N/A
Field	12,140.57 m^2
Quality Lab	111.43 m^2
Time (Days)	365