Genetic diversity and its relationship to hybrid performance in High Erucic Acid Rapeseed

BY

MARC RENE LUC VINCENT

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Plant Science

University of Manitoba

Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

Genetic diversity and its relationship to hybrid performance in High Erucic Acid Rapeseed

BY

Marc Rene Luc Vincent

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

MASTER OF SCIENCE

Acknowledgements

I would like to acknowledge all the help and support that I received from many people throughout my research program:

Dr. Peter B.E. McVetty for both his supervision and support throughout my research program.

Dr.Genyi Li for both his guidance and help in the conductance of my molecular genomics work throughout the duration of my research program and also for serving on my advisory comitee.

Dr. Gary H. Crow for serving on my advisory committee and for his help with my statistical analysis.

Dr. Brian Fristensky for all his help with my data analysis in Unix.

Sandra Fuller for her guidance and knowledge for the care of my seedlings in the greenhouse.

Greenhouse staff: Cathy Bay, Linda Pearn and Ian Brown for taking care of my plants in the greenhouses.

Genomics Lab: Yu Chen, Rice, Arvind, and Ravneet for their assistance in my genomics work.

Also to my fellow graduate students for all their help and support.

My wife Kathleen, my two daughters Mackenzie and Chloe, my Mom and Dad, my in-laws Sharon and Jack Johnston for their encouragement and support throughout the duration of my research program.

Thanks for everything.

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

Vincent, Marc Rene Luc. March 2008. Genetic diversity and its relationship to hybrid performance in High Erucic Acid Rapeseed. Major Professor: Dr. Peter B.E. McVetty, Department of Plant Science

Determination of genetic diversity and distance within oilseed rape and correlations between genetic distance and hybrid performance would cluster genetically similar inbreds, cultivars or lines together, create genetically distinct groups and predict hybrid performance. A DNA marker technique known as sequence related amplified polymorphism (SRAP) was used to quantify genetic diversity among parental lines of 45 derived hybrids in order to predict heterosis. The major assumption behind marker based heterosis prediction is a strong linear correlation between marker heterozygosity and hybrid performance. This study evaluated this correlation by evaluating molecular marker heterozygosity and hybrid performance of 45 hybrids derived from crosses of twelve High Erucic Acid Rapeseed (HEAR) cultivars / lines. These cultivars / lines had been selected based upon their diverse pedigree, favorable agronomic performance and seed quality. The cultivars and their 45 hybrids had previously been evaluated in field trials grown across six environments over two years (2004-2005) in replicated field trials and accessed for agronomic performance and seed quality traits by Cuthbert (2006). A total of 102 SRAP primer combinations used in this study produced 885 polymorphic loci. The assignment of HEAR cultivars to genetically distinct groups was in excellent agreement with available pedigree and geographic origin information. Two major genetically distinct groups based on cluster analysis were determined to be in agreement with hybrid performance data collected by Cuthbert (2006). Significant correlations between parental

genetic distance and hybrid seed yield, flowering time, days to maturity, plant height, protein content, oil content and GCA were observed ($p < 0.01$). This suggests that some of the SRAP molecular markers developed in this study may be linked to quantitative trait loci for the above traits. By selecting the top fifteen most genetically divergent hybrids it was possible to correctly predict high seed yield 13 out of 15 times and oil concentration 13 out of 15 times correctly. Genetic distance between parental combinations appears to be a good predictor of hybrid performance / heterosis. It was predicted that hybrids derived from EU HEAR #1 and EU HEAR #3 lines crossed with selected UM HEAR cultivars would result in maximum heterosis, and this was consistent with both the hybrid agronomic and seed quality data collected by Cuthbert (2006). Based upon cluster analysis and genetic distance correlations for individual traits, sufficient marker density for accurate heterosis prediction was determined to be 200 polymorphic markers. SRAP molecular markers along with the use of a ABI DNA analyzer appears to be a cost effective means of potentially assigning inbreds, cultivars or lines to different genetically distinct groups and accurately predicting hybrid performance / heterosis.

Introduction

Oilseed rape (*Brassica napus* L.) is an economically valuable oil seed crop, grown primarily for its oil and meal. Its production has greatly increased globally over the past 30 years. Oilseed rape is cultivated in Asia and Europe as winter habit whereas in Canada, Australia and Northern Europe it is spring habit. Oilseed rape is very well adapted to the temperate climates of the Canadian Prairies and for these reasons it occupied nearly 6 million hectares in 2007 in Western Canada (Statistics Canada 2007).

Hybrid oilseed rape cultivars have an advantage over open-pollinated population cultivars, since they frequently display hybrid vigor and have superior agronomic performance (Poehlman and Sleper 2006). Hybrids have shown to out perform open pollinated populations (OPP) varieties with seed yield increases of 30 to 60% over high parent values (Serynk and Stefansson 1983; Brandle and McVetty 1989). Currently, over 50% of the acreage grown to oilseed rape is devoted to hybrid cultivars (Statistics Canada 2007) and this is expected to increase over time based upon increases in both seed yield and agronomic performance. Hybrid vigor or heterosis is defined as an increase in size, productivity or vigor over the high parent value. It is expected that crosses between individuals with genetically divergent genotypes will result in higher heterosis when dominant gene action is occurring (Falconer 1981). There are two main theories for the occurrence of heterosis; the Dominance and the Overdominace hypotheses. The dominance hypothesis suggests that the genes responsible for hybrid vigor are dominant and recessive genes are unfavorable and thus are detrimental to yield (Crow 1948). The overdominance hypothesis suggests that hybrid vigor is the result of heterozygous loci

contributing more to productivity than homozygous loci, therefore hybrid vigor increases as the number of heterozygous loci increase (Shull 1908; East 1908).

Currently, Canada is the world's largest producer of spring habit high erucic acid, low glucosinolate rapeseed (HEAR) cultivars. The current HEAR cultivars grown in Canada are the open pollinated population cultivars MilleniumUM 03, Red River 1826 and Red River 1852. Several HEAR cultivars at the University of Manitoba have arisen from a relatively narrow genetic background and they likely belong in one heterotic pool. In-house research (Cuthbert 2006) has illustrated that hybrid HEAR lines generated from crosses between University of Manitoba and European HEAR cultivars / lines resulted in increased performance over open pollinated population HEAR cultivars / lines. Superior performing HEAR hybrids displayed high-parent heterosis estimates for seed yield of up to 155% (Cuthbert 2006).

The evaluation of inbred lines suitable for the production of superior hybrid cultivars is extremely costly since they are evaluated over multiple years and locations for superior agronomic and seed quality characteristics. Large amounts of effort and time is devoted to hand crossing of inbred lines in single crosses for field evaluation. Screening hybrid combinations for superior performance and heterosis is likely the most time consuming and costly step in hybrid cultivar development. Therefore, the development of a simple, efficient, reliable and inexpensive technique to: (1) identify new sources of populations for inbreds that will predictably produce superior hybrids and (2) predict hybrid performance without previously generating and evaluating single-cross hybrids in expensive replicated field trials. This would eliminate much of the work

associated with making crosses and evaluating the crosses in field trials and would greatly accelerate the development of new elite commercial hybrids.

The level of genetic diversity / genetic distance between parental lines has been proposed as a potential predictor of hybrid performance and heterosis (Teklewold and Becker 2005) since crosses between geographically divergent materials normally perform in a superior fashion (Brandle and McVetty 1989). With molecular markers it is possible to intensively analyze the amount of genetic diversity present within a species and to evaluate distance/similarity between individuals and populations (Charcosset and Moreau 2004). Molecular markers can also be used to determine the genetic relationships between the genotype of the parent and hybrid vigor (Joshi et al. 2000) and can be used to predict crosses that might produce new, superior, and desirable gene combinations (Jain et al. 2002). The major assumption behind marker based heterosis prediction is that there is a strong linear correlation between marker heterozygosity and hybrid performance. Therefore, molecular markers could be used to quickly separate inbreds, cultivars or lines into different genetically distinct groups. Choosing individuals from each group with the most genetic distance could result in maximum heterosis being achieved. By assessing genetic diversity or genetic distance with molecular markers, it is possible to overcome some of the drawbacks (i.e. cost, labor and time) and increase the probability of predicting heterotic performance (Riaz et al. 2001). This could allow for the selection of potentially superior inbred parental lines for the development of new hybrid breeding populations. DNA-based markers such as Random Fragment Length Polymorphism, Random Amplified Polymorphic DNAs, Simple Sequence Repeats, Amplified Fragment Length Polymorphism and Sequence Related Amplified Polymorphism have been used to

determine genetic diversity and degree of relatedness in several crop species including *Brassica* (Diers and Osborn 1996; Hallden et al. 1994; Throman et al. 1994; Lombard et al. 2000; Burton et al. 2004, Hasan et al. 2005), rice (Giarroco et al. 2007), maize (Boppenmaier et al. 1993; Benchimol et al. 2000; Lubberstedt et al. 2000; Reif et al. 2004) , sorghum (Perumal et al. 2007), sugarcane (Alwala et al. 2006). There has been varying success with the use of genetic distance in order to predict heterosis in other crop species including maize (Lanza et al. 1997; Xu et al. 2004; Bruel et al. 2006;), rice (Xiao and Wu, 1998; Zhao et al. 1999; Benchimol et al. 2000; Joshi et al. 2001), sorghum (Jordan et al. 2003), wheat (Liu et al. 1999), cotton (Zhang et al. 2007). However, Diers et al. (1996) used RFLP markers and Riaz et al. (2001) used sequence related amplified polymorphisms (SRAP) in order to study the relationship between genetic distance and heterosis. These authors found that genetic distance and yield were moderately correlated to each other; Diers et al. (1996) $r=0.59$ ($p< 0.05$) and Riaz et al. (2001) $r= 0.64$ ($p < 0.05$).

The objectives of this study were to:

1) Determine the amount of genetic diversity present and assign HEAR cultivars to genetically distinct groups
2) Predict hybrid performance for hybrid HEAR development
3) Determine the marker density required for accurate hybrid performance prediction using molecular markers

Literature Review

2.1 *Origin and History of Rapeseed*

Rapeseed (*Brassica napus L.*; genome AACC, 2n =38) is a member of the crucifer family (Brassicaceae). The species is divided into two subspecies, swedes (*B. napus ssp. napobrassica*) and *B. napus ssp.* which include spring and winter oilseeds, fodder and vegetable rape (Snowdon et al. 2007). *B. napus* originated from interspecific hybridization between cabbage (*Brassica oleracea L.*; genome CC, 2n = 18) and turnip rape (*Brassica rapa L.*; genome AA, 2n =20) resulting in a amphidiploid genome with a genome size of 1200 cM. Since there are no wild relatives of *B. napus* known, it can be assumed that it has arisen recently and it is suggested that this may have occurred in the Mediterranean region where both parental species are present (Snowdon et al. 2007). Other crosses between the Brasicaceae family members have occurred in nature and have resulted in the creation of Indian or brown mustard (*Brassica juncea*; genome AABB, 2n = 36) and Abyssinian or Ethopian mustard (*B. carinata*; genome BBCC, 2n=34) which arose from crossing of black mustard (*Brassica nigra*; genome BB, 2n=16) with either *B. rapa* or *B. oleracea.* These interspecific hybridizations can be observed in the triangle of U (Figure 2.1) (U 1935).

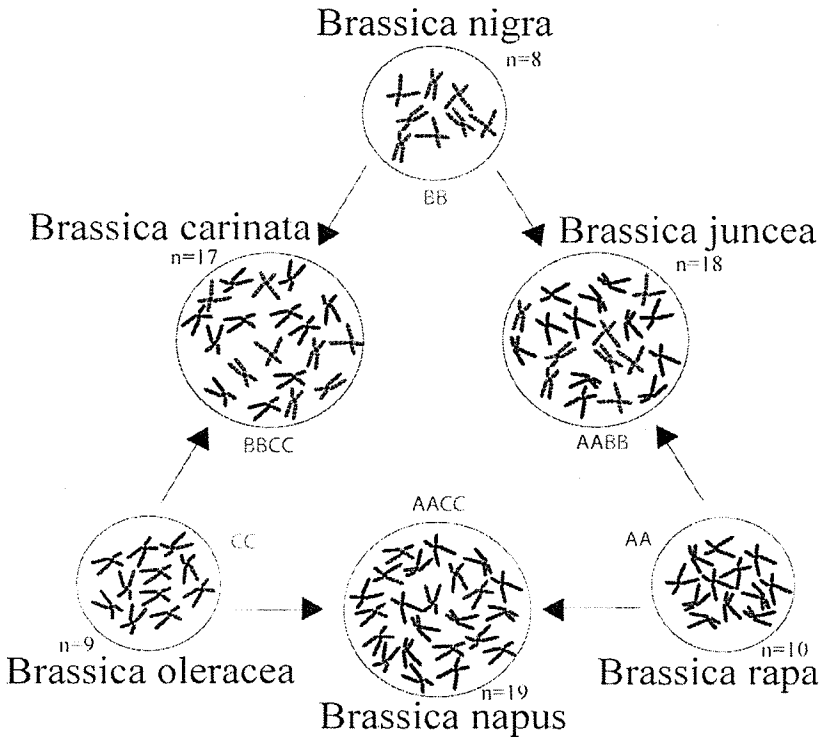

Fig 2.1: Triangle of U – Interspecific Hybridization of *Brassica* species (http://en.wikipedia.org/wiki/Triangle_of_U)

Brassica vegetables and oilseeds were among the first crops grown by mankind. Rapeseed cultivation based upon archeological discoveries dates back to 5000 B.C. in China (Yan 1990). It has been recorded that rapeseed was grown in India about 2000 B.C. and was then introduced to Japan from either China or the Korean Peninsula about 2000 years ago (Bell 1982). Rapeseed oil was used as a fuel for oil lamps since when it burned it was smokeless (Prakash 1980). Rapeseed oil use was recorded to have begun in the 13th century and by the 16th century was the major source of lamp oil in Europe (Snowdon et al. 2007). The use of rapeseed oil greatly increased when it was later on discovered in Europe that it could be used as a high quality lubricant for steamships. Since rapeseed oil would cling to steam washed metal surfaces better than other lubricants. By the 19th century rapeseed oil for lamp fuel was superseded by petroleum as

the major source of lamp fuel and the use of rapeseed as a high quality lubricant for industrial uses continued production throughout the 20th century (Snowdon et al. 2007). Rapeseed acreage quickly expanded in Canada because of a shortage of rapeseed oil during World War II in the early 1940's and *Brassica campestris* (now known as *Brassica rapa)* species commonly known as Polish rapeseed was grown throughout the Prairie region. The *Brassica napus* species obtained from the United States originated from Argentina (Argentine rapeseed) was also grown to meet rapeseed oil demands. After the War rapeseed oil production in Canada fell. The focus of rapeseed oil production became the Asian export market for rapeseed oil as edible oil.

Early rapeseed varieties produced oil that was excellent for industrial lubricant applications since they contained high quantities of erucic acid. Rapeseed oil containing high erucic acid in the oil was shown to have detrimental health effects in humans (Hulan et al. 1975). High levels of erucic acid had been shown to cause cardiac problems (Charlton et al. 1975) and gave a bitter taste to the oil (Snowdon et al. 2007). The seed meal was relatively high in protein, which would be excellent for livestock but elevated glucosinolate levels in the meal had adverse side effects upon livestock. Elevated glucosinolate levels in the meal produced toxic byproducts when the seed was crushed. Monogastric animals displayed liver, kidney and lymph dysfunction (Snowdon et al. 2007) when they consumed rapeseed meal. The first low erucic acid variety Oro (1968) was derived from the identification of a low erucic acid mutant from the German spring forage cultivar Liho and was released in Canada in the early 1970's (Snowdon et al. 2007). In 1969, the rapeseed variety Bronowski from Poland was observed to have low glucosinolate content and was used in order to backcross into high yielding erucic acid

free material (Snowdon et al. 2007). In 1974 the first low erucic acid, low glucosinolate content *B.napus* cultivar Tower was developed by Dr. Baldur Stefansson at the University of Manitoba (Stefansson and Kondra 1975). Cultivars that were low in erucic acid and low in glucosinolate content were termed double low rapeseed and were later named as "Canola" by the Rapeseed Crushers of Western Canada in order to clearly distinguish them from rapeseed (Serynk 1982).

Stefansson at the same time realized the importance of high erucic acid cultivars as sources of industrial lubricants and therefore began to breed cultivars that had a high erucic acid content in the oil (>50%) with a low glucosinolate content in the meal. This allowed the meal to be used as a high protein meal for livestock. These lines consisting of high erucic acid and low glucosinolate content were named High Erucic Acid Low Glucosinolate Rapeseed (HEAR). Advantages associated with HEAR include high smoke and flash points, stability at high temperatures, durability and the ability to remain fluid at low temperatures (Snowdon et al. 2007). HEAR oil derivatives include erucamide, brassilic acid and pelargonic acid, which have various industrial purposes. HEAR oil is mainly used to produce erucamide, which is a slip additive in polyethylene and polypropylene manufacture in order to prevent adhesion of film surfaces. Erucamide is too expensive to be chemically synthesized. HEAR oil is also used in printing inks and many other types of lubricants (Snowdon et al. 2007). Canada is the world's largest producers of spring habit HEAR. Over the past 25 years the University of Manitoba has released many open pollinated population HEAR cultivars which include: Reston (1982), Hero (1988), Mercury (1990), Neptune (1992), Venus (1993), Castor (1994), MillenniUM 01 (1998), MillenniUM 02 (1999), MillenniUM 03 (2000) and the Round-

up Ready cultivars Red River 1826 (2006) and Red River 1852 (2006). In this short period yield increases of nearly 30% from Reston to the MillenniUM cultivars has been observed (McVetty pers. comm. 2007). The formation of hybrid HEAR cultivars could potentially significantly increase seed yield in the future and in a study conducted by Cuthbert (2006) it was reported that HEAR hybrids displayed high-parent heterosis estimates for seed yield of up to 155%.

2.2 *Origin of Hybrid Breeding*

Reports about advantages associated with plant hybrids date back to Koelreuter (1763) who observed this in tobacco, other reports of hybrid advantages included Knight (1799), Gartner (1849) and Darwin (1877) concluded that inbreeding or self-fertilization was detrimental in plants and cross-pollination in plants was beneficial (East and Hayes 1912). Also Beal (1880) observed that hybrid corn out-performed open-pollinated corn varieties (Poehlman and Sleper 2006).

Modern hybrid breeding first began in maize in the early 1900's Shull (1909). Shull devised a method of selecting inbred lines (pure lines) that would be used to develop single cross hybrid maize cultivars. He developed pure lines by repeatedly self fertilizing plants for years in order to attain a near homozygous state and then all possible crosses between these pure lines were made. The hybrid plants from these crosses were grown in ear-to-the-row tests, where each row would be the product of a different cross of pure lines. The hybrid plants from each cross were evaluated for yield and the possession of favorable characteristics. Upon selection of the most desirable crosses for both yield and quality, two isolated plots were grown where plot I consisted of only the mother strain and plot II consisted of both mother and father lines planted in alternate

rows. The mother strain rows in plot II were detasseled and the seed collected from these rows was hybrid seed. Seed in the father line rows was similarly collected and reused as pure father line seed. This method, known as the pure-line hybrid breeding method totally revolutionized corn breeding and led to the development of hybrid cultivars in many crop species. However, it was observed that the production of hybrid seed from inbred corn lines was limited.

Jones (1922) came up with a different method of producing corn hybrids. He inbred the parental lines for a minimum of five years and then he crossed parental strains to make hybrid seed. He then crossed two hybrids to create the next generation, which he denoted as the double cross hybrid generation. This was done to overcome low parental seed production in inbred lines and subsequent low hybrid seed production. Jones (1922) reported that double crossed hybrids displayed yield increases as high as 35% over the top yielding open-pollinated population (OPP) varieties of the day and that the double cross hybrids frequently outperformed single cross hybrids. It was also reported that hybrids from the double cross technique showed superior quality as compared to currently available OPP varieties. The double cross method made hybrid production in corn economically feasible since hybrid seed was produced in large quantities from the intercrossing of hybrid plants instead of from intercrossing inbred plants.

2.3 *Heterosis*

Heterosis was first defined by Shull as the increase in size, yield, and vigor of a hybrid (Shull 1948). It is now more commonly defined as the increase in size, vigor, or productivity of a hybrid plant over the mid parent (Poehlman and Sleper 2006). Even though the exact mechanism responsible for heterosis is not fully understood, there have

been two main theories proposed to explain the genetic basis that underlies hybrid vigor or heterosis. These were the dominance (Davenport 1908; Bruce 1910; and Keeble and Pellew 1910) and overdominance hypothesis (Shull 1908; East 1908).

2.3.1 *Dominance Hypothesis*

The dominance hypothesis was first stated by Davenport (1908), Bruce (1910) and Keeble and Pellew (1910). It assumes that hybrid vigor is the result of combining favorable dominant genes. It suggests that the genes responsible for vigor are dominant and that recessive genes are unfavorable and thus are detrimental to yield (Crow 1948; Jain et al. 2002). In cross pollinating populations, recessive alleles are hidden in heterozygotes by dominant genes. In self pollinating populations, one half of heterozygous loci will become homozygous recessive genes and will contribute to the decline in vigor (Poehlman and Sleper 2006). Therefore, the dominance hypothesis suggests that it should be possible to overcome inbreeding depression by creating inbred lines containing entirely dominant alleles (homozygous dominant) or entirely recessive (homozygous recessive) (Keeble and Pellew 1910; Jones 1917). It would therefore be observed that the completely recessive strain would be less vigorous than the parents and the hybrid and completely dominant strain would be more vigorous than the parents (Jones 1917).

There are two objections to the dominance hypothesis. (1) if heterosis was a result of dominance, then the distribution of the F_2 progeny would be unsymmetrical in comparison to characteristics observed in the F_1 as heterosis (Emerson and East 1913) (Jones 1917). This follows Mendelian expectations where dominance and many other factors are concerned (Jones 1917). (2) It should be possible to obtain strains that contain

all dominant allele loci and one with all recessive allele loci. In this case, the all recessive allele loci individual would be less productive and the completely dominant allele loci individual would be more productive. Both of these objections are considered null if a large number of loci are involved or if linkage is occurring (Bernardo 2002).

2.3.2 *Overdominance Hypothesis*

The overdominance hypothesis, first suggested by Shull (1908) and East (1908) suggests that heterozygotes per se are superior to homozygotes (Crow 1948). Hybrid vigor is the result of heterozygous loci contributing more to productivity than homozygous loci, therefore hybrid vigor increases as the number of heterozygous loci increase (Poehlman and Sleper 2006). Therefore, each allele produces a contrasting combined effect that is more favorable to the plant than what the individual alleles could contribute alone.

2.3.3 Mid-Parent and High-Parent Heterosis

Determination of heterosis is a very important in the evaluation of hybrids. If the hybrid performs better than the mean performance of the parents this is known as mid-parent heterosis.

$$\% \text{ Mid-parent Heterosis} = \frac{\overline{F1} - \overline{MP}}{\overline{MP}} \times 100$$

Where:

$\overline{F1}$ = the mean of the hybrid combination

$\overline{MP}$ = the mean performance of the two parental lines

If the hybrid out-performs the best parent used in the cross to make the hybrid, this is known as high-parent heterosis.

$$\% \text{ High-parent Heterosis} = \frac{\overline{F1} - \overline{HP}}{\overline{HP}} \times 100$$

Where:

$\overline{F1}$ = the mean of the hybrid combination

$\overline{HP}$ = the mean performance of better performing parental line (the high-performing parent)

2.3.4 Commercial Heterosis

When developing hybrid cultivars breeders aim to develop hybrids that out perform the best currently available commercial cultivars of the day. This is known as commercial heterosis.

$$\% \text{ Commercial Heterosis} = \frac{\overline{F1} - \overline{\text{Best cv}} \times 100}{\overline{\text{Best cv}}}$$

Where:

$\overline{F1}$ = the mean of the hybrid combination

$\overline{\text{Best cv}}$ = mean performance of the best commercial cultivar currently in the market

2.4 *Breeding*

Techniques for breeding crops differ if the crop species is self- or cross-fertilizing. *B. napus* is a facultative out-crossing species with a high degree of self pollination. In Western Canada *B.napus* is approximately 97% self pollinating and 3%

cross pollinating (Cuthbert and McVetty 2001). Cross pollination rates are dependent upon insect activity and wind. *B. napus* is generally bred as a self-fertilizing species.

2.4.1 *Hybrid Breeding*

Hybrid breeding is the crossing of homozygous parent genotypes that combine favorably to produce superior hybrids which will be grown by the producer. Sufficient levels of heterosis must be achieved in order to justify the increased cost of production. Hybrid breeding methods were first proposed by Shull (1909) in order to exploit potential heterosis in maize. Heterosis potential is maximized by crossing genetically divergent inbreds since it has been observed that crossing individuals with similar pedigree or geographic origin results in equal or poorer performance than that of the parents (Brandle and McVetty 1989). The development of hybrid cultivars has greatly improved over time especially from 1909 (Shull) through use of pollination control systems such as male sterility in hybrid breeding. Hybrid breeding consists of three main steps:

1) Inbred Line Development
2) Testing for Combining Ability
3) Production of Hybrid Seed

2.4.2 Inbred line Development

The first step in hybrid breeding is the development of inbred lines, since they will produce reproducible hybrids. Inbred lines could be developed from a variety of populations including open pollinated population varieties, single, three-way or four-way hybrids, or an improved population. They will then be self pollinated for several generations in order to achieve near homozygosity, usually for a minimum of three or four generations (S_3-S_4). A selection procedure such as pedigree selection in self-

pollinating crops could also be used. Similarly, one could develop double haploid (DH) populations for inbred use. This would allow for complete fixation of alleles at all loci, thus eliminating heterozygous loci. The DH lines could be evaluated in replicated field trials grown in several environments to select superior DH parental lines.

2.4.3 Combining Ability

The evaluation of combining ability requires consideration of a set of crosses among several parents to determine if the observed variations between crosses are due to statistical additive effects of the parents.

$$X_{AB} = X + G_A + G_B + S_{AB}$$

where X is the general mean, G_A and G_B are the general combining abilities of the parents and S_{AB} is the specific combining ability (Simmonds 1981). Combining abilities are estimated from either diallel or M x N crossing scheme (Fig 2.4.1.2).

General combining ability (GCA) "is the average contribution that the inbred line makes to hybrid performance in a series of hybrid combinations in comparison to the contribution of other inbred lines to hybrid performance in the same series of hybrid combinations" (Poehlman and Sleper 2006). Specific combining ability (SCA) "is the contribution of an inbred line to hybrid performance in a cross with a specific inbred line, in relation to its contributions in crosses with an array of specified inbred lines" (Poehlman and Sleper 2006). GCA evaluates the additive portion of the genetic effect, while SCA evaluates non-additive genetic effect (Poehlman and Sleper 2006). GCA is evaluated by crossing inbred lines in every possible combination (diallel mating) and then evaluating the hybrids in replicated field trials for varying agronomic and seed quality characteristics. When evaluating large numbers of inbred lines, it may not always be

feasible to evaluate all potential hybrid combinations (full diallel) in performance trials. To calculate all potential single cross combinations excluding reciprocals of *n* inbred lines (half diallel), the formula is: *[n(n-1)]/2*. Therefore with 12 inbreds the possible single cross combination would be 66; with 100 inbreds it would be 4950. A testcross which is a cross between a hybrid with one of its parents can similarly be used to screen the inbred population for GCA and will reduce the amount of crosses and only individuals with a high GCA will be further tested in a single cross.

Once we have identified the superior performing inbred lines (those with high GCA) we can evaluate SCA by crossing the superior performing inbred lines in a diallel crossing scheme and evaluate them in replicated field trials for specific agronomic and seed quality characteristics. It has been observed that individuals that are distantly related will frequently combine to produce better progeny than closely related individuals (Poehlman and Sleper 2006).

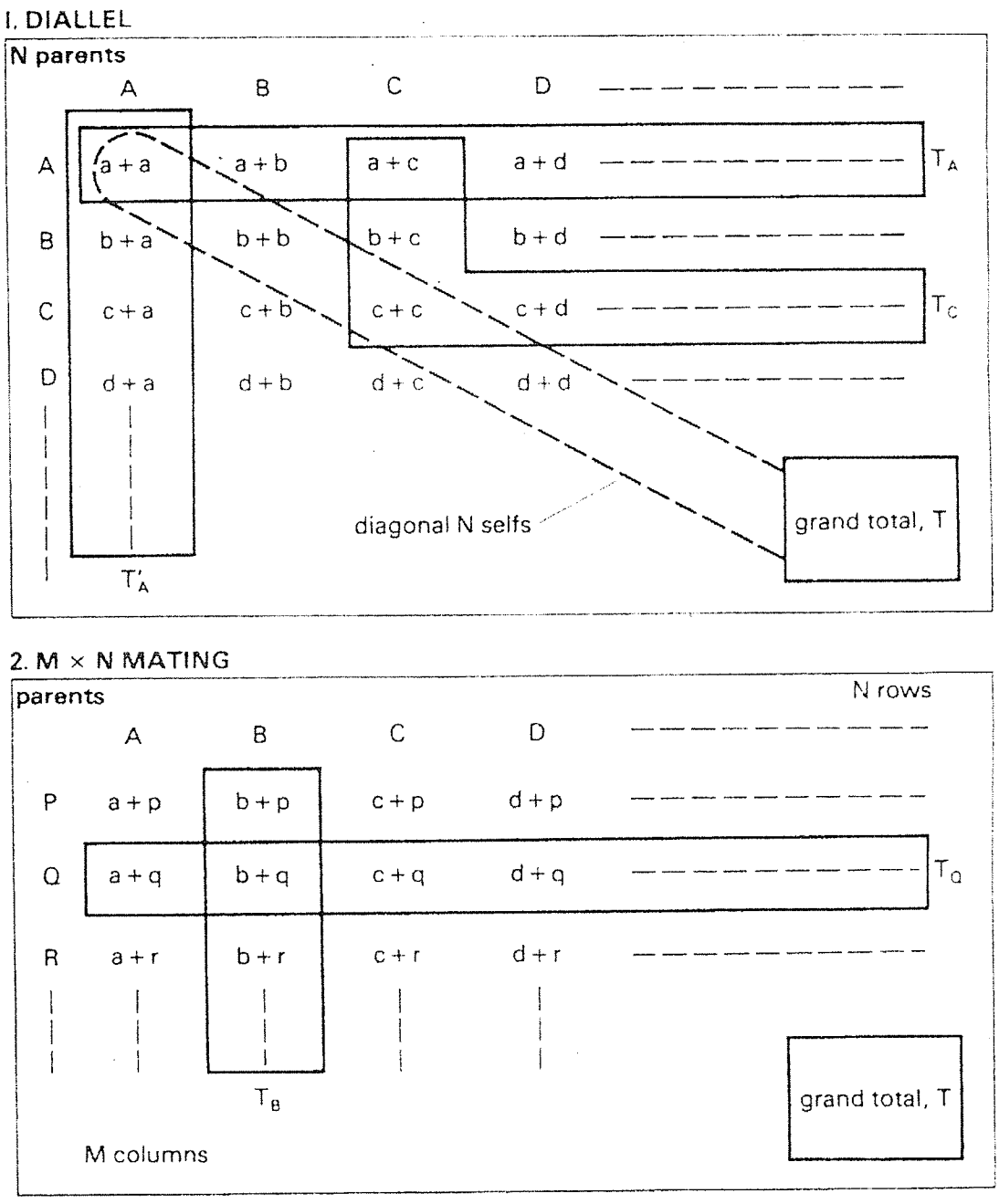

Fig 2.4.1.2: Mating patterns for estimation of combining abilities (Simmonds 1981)

2.4.4 Production of Hybrid Seed

After superior inbred parental line combinations have been identified, commercial production of hybrid seed from genetically pure inbred parental lines begins. Originally, hand emasculation was used in the majority of crops, but this technique was very laborious and time consuming so hybrid seed is now obtained using some form a male sterility i.e. cytoplasmic male sterility (CMS) (McVetty 1997), self incompatibility (SI) (McCubbin and Dickinson 1997), chemically induced male sterility (Cross and Schultz 1997), genetic male sterility (GMS) (Sawhney 1997), or genetically engineered male sterility (Williams et al. 1997). Male sterility systems are employed in order to inhibit sib- or self- pollination of the female plants. The majority of hybrid oilseed rape crops are now produced using either cytoplasmic male sterility or genetically engineered male sterility systems in order to produce large quantities of hybrid seed (McVetty pers. comm. 2008).

Cytoplasmic male sterility (CMS)

CMS was first used commercially in the 1940's for commercial production in onions and was later used in corn and sorghum. CMS systems generally result in the malformation of the tapetum and anthers (McVetty 1997). The genetic determinants of CMS are located within the mitochondria and nuclear genes control the expression of CMS (McVetty 1997). In CMS systems a source of sterile cytoplasm is needed and a reliable fertility restorer gene or genes in the nucleus is (are) also required. Cytoplasmic sterile lines called A-lines (female line) have a sterile cytoplasm (S) and are homozygous recessive at the nuclear restorer locus (rfrf); male-fertile maintainer lines called B-lines have a fertile cytoplasm (N) and are homozygous recessive at the nuclear restorer locus

(rfrf); while male fertile, fertility restoring lines called R-lines have a fertile cytoplasm (N) or a sterile cytoplasm (S) and are homozygous dominant at the nuclear restorer locus (RfRf). A- lines are developed by backcrossing B-lines into a CMS A-line for four to six generations in order to development a new A-line and B-line pair (McVetty 1997). R-lines are developed by using a CMS R-line as a female for the original cross and then a new line as the recurrent backcross parent for a subsequent four to six generations (McVetty 1997). A-, B- and R-lines are used in order to either maintain male sterility or to produce male fertile restored hybrid seed which is sold to producers (Figure 2.4.1.3). Restorer genes will temporarily suppress male sterility even in the presence of a sterile cytoplasm. Hybrid seed is produced by using the block method of hybrid seed production where alternate rows or strips of female and male plants are planted.

In rapeseed the major CMS systems are *nap* (Fan 1985), Ogura (*ogu*) (Heyn 1978), and Polima (*pol*) (Fang and McVetty 1988, 1989). Some CMS systems, however, have limitations that make them unreliable for the use as pollination control systems in oilseed rape. The *nap* CMS system in oilseed rape is unstable at moderate to high temperatures (McVetty 1997). The *ogu* CMS system fertility restorer gene (Rf) has adverse affectes upon female fertility and in general at low temperatures oilseed rape demonstrates poor growth and chlorosis symptoms (McVetty 1997). The *pol* CMS system in oilseed rape is unstable at temperatures above 30°C and will result in partial male fertility (McVetty 1997). Also *pol* CMS system hybrids in oilseed rape have lower seed yield, oil content, harvest index, and total dry matter than hybrids with normal *nap* cytoplasm (McVetty 1997).

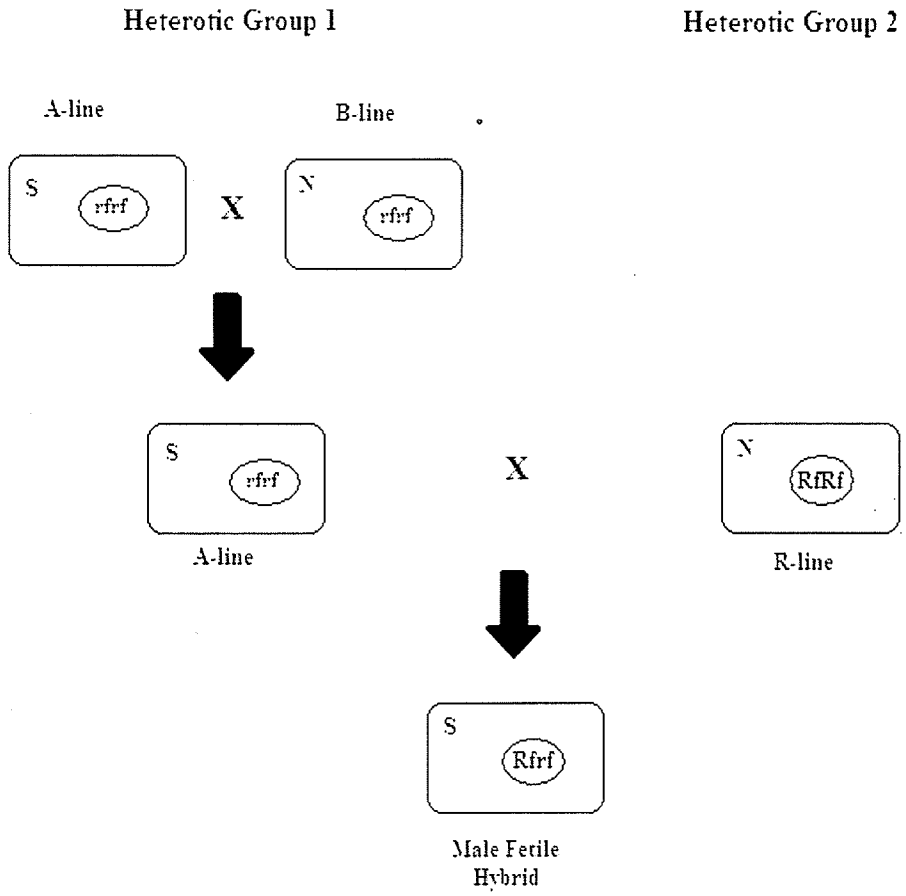

Fig 2.4.1.3: Hybrid seed production with CMS system (Bernardo 2002)

Genetic male sterility (GMS)

Genetic male sterility (GMS) is commonly occurring within angiosperms and is a result of a mutation within any one of the genes controlling pollen and/or stamen development (Sawhney 1997). GMS can be controlled by a single dominant gene for fertility (MsMs or Msms). The major drawback to this system is that it is extremely hard to develop a completely homozygous male sterile population (msms) unless one uses rogueing. This can be achieved by manually rogueing individuals but will need a tightly linked marker to the male sterility gene to effectively rogue prior to flowering.

Genetically engineered male sterility has been created by chimeric ribonuclease genes that cause malformation of the tapetum, which resulted in the formation of male sterile plants (Williams et al. 1997). This genetically engineered male sterility system also has a herbicide tolerance gene linked to the male sterility system gene to simplify rogueing. It is used in the SeedLink InVigor System by Bayer Crop Science (Williams et al. 1997).

2.5 *Markers*

There are three kinds of markers, morphological (plant traits), biochemical (proteins and isozymes) and molecular (DNA) markers (Jain et al. 2002). Molecular genetic markers are heritable entities that can be associated with economically important traits that can be used by plant breeders as selection tools (Jain et al. 2002). Molecular markers are heritable entities that are discreet, nondeleterious, not effected by environmental factors and which are transmitted by the simple laws of inheritance and are therefore superior to morphological markers (Jain et al. 2002). They may be located near or within genes. Since DNA within cells of an organism is constant throughout the organisms life cycle, and it can therefore be tested using molecular markers at any developmental stage. For these reasons, molecular markers are superior to biochemical markers where sampling at differing developmental stages can result in different results and is superior to morphological markers that are highly influenced by environment.

2.5.1 *DNA molecular markers*

There is a great diversity of DNA molecular markers available. Such markers have been used for crop improvement via QTL mapping, marker assisted selection (MAS), and genetic diversity studies (Jain et al. 2002). The marker systems can be differentiated based upon their technical requirements, cost, labor, relative difficulty or

number of polymorphic markers. Every marker system possesses both advantages and disadvantages, therefore certain marker systems are more suitable for certain applications than others. The type of marker system used is dependent upon species, cost, application and convenience. DNA molecular markers are classed into two separate groups: (1) hybridization-based molecular markers and (2) PCR-based molecular markers (Jain et al. 2002).

2.5.2 Hybridization-based molecular markers

RFLP

Restriction Fragment Length Polymorphism (RFLP) was the first DNA molecular marker system developed. It has been used in genome mapping, chromosome tagging, phylogenetic studies, DNA fingerprinting and cultivar identification. RFLP marker analysis involves the digestion of genomic DNA with restriction endonucleases (enzymes which cut DNA at specific nucleotide sequence sites) in order to create copious amounts of fragments (Poehlman and Sleper 2006). Upon digestion, the fragments are separated using gel electrophoresis and the fragments of interest are detected using specific labeled DNA probes. Advantages are that RFLP's are a codominant marker system that allows one to distinguish between homozygous and heterozygous individuals and they are highly reproducible. Disadvantages associated with this method are that it is time consuming, labor intensive, relatively expensive compared to other marker methods and not suitable for evaluation of large segregating populations in a breeding program (Jain et al. 2002). There is also often only one polymorphism per probe.

2.5.3 PCR-based molecular markers

RAPD

Random Amplified Polymorphic DNA (RAPD) is a PCR-based technique that utilizes short 10 base long oligonucleotide random sequences as primers in order to amplify genomic DNA under low annealing temperatures (Williams et al. 1990). The primer hybridizes at multiple locations throughout the genome and when two hybridization events occur in close proximity to each other, the DNA segment will be amplified. Amplified products are then separated on an agarose gel and stained with ethidium bromide. Advantages to the RAPD method are that it is inexpensive and easy method that requires relatively small amounts of DNA. RAPD bands can be cloned and sequenced to make SCAR (sequence-characterized amplified region) markers that are highly reproducible. Disadvantages are that it is a dominant marker system, meaning that it cannot distinguish between heterozygotes and homozygotes. This is limiting to the amount of polymorphic information provided for a given primer combination. RAPD's are sensitive to laboratory conditions and therefore difficult to reproduce.

AFLP

Amplified Fragment Length Polymorphism (AFLP) is a PCR-based molecular marker system that was developed in the mid 1990's by Vos et al. (1995). AFLP is a DNA fingerprinting technique that generates DNA fragments from restriction enzyme digestion amplified with PCR. Upon digestion the end-specific oligonucleotide adapters are ligated to the fragments. The adapters and the restriction site sequence are used as primer sites for non-specific PCR amplification. Then sequence specific primers containing one to three arbitrary nucleotides are used to selectively amplify the DNA.

Amplified fragments are then separated by gel electrophoresis and viewed using either radioactive or fluorescent labeling (Vos et al. 1995). Advantages of AFLP are that no sequence information is required, large numbers of polymorphisms are created and it is a highly reproducible technique. Disadvantages are that AFLP is a dominant marker system, is relatively time consuming and is a proprietary technology.

SSR

Simple sequence repeats (SSR) or microsatellites are regions within the locus that consisting of two or more base repeats that are tandemly repeated a number of times. The unique sequence flanking the repeated regions are used as forward and reverse primers. PCR is used in order to amplify microsatellites which are then separated by gel electrophoresis. Advantages of SSRs are that it is a codominant marker system that produces large amounts of polymorphism that is highly reproducible. Disadvantages of SSRs are that the initial identification of SSRs can be extremely time consuming, relatively expensive and DNA sequences are required.

SNP

Single nucleotide polymorphisms or SNP's are variations within the DNA sequence that occur when a single nucleotide (A, T, C or G) is altered in the genome. They can be found in a variety of regions including coding gene sequences, non-coding regions or intergenic regions. The SNPs within coding regions may or may not have an effect upon protein development. Advantages of SNPs are that they are co-dominant, and highly reproducible. Disadvantages are that SNP's are extremely time consuming to develop, expensive, produce small amounts of polymorphic loci and require sequence information.

SRAP

Sequence related amplified polymorphism (SRAP) is a simple PCR-based marker system with two-primers of about 17 or 18 nucleotides long (Li and Quiros 2001). The primers, starting at the 5' end, consist of a filler sequence of 10 to 11 bases, followed by CCGG sequence in the forward primer or AATT sequence in the reverse primer and three selective nucleotides at the 3' end. It is believed that these primers will target coding regions, rather than inter genic regions (Li and Quiros 2001). SRAP amplifies moderate quality genomic DNA with either a labeled or unlabeled forward primer and an unlabeled reverse primer. The annealing temperature for the first five cycles is set at 35 ^{o}C in order to ensure maximum primer binding to target DNA. Annealing temperature is then raised to 50 ^{o}C for 35 cycles, this will allow the amplified DNA from the first five cycles to produce consistent banding for all remaining cycles. Upon amplification products are separated by gel electrophoresis. Forward labeled primer products can be separated on an ABI Gene Sequencer. This greatly increases the number of samples that can be run at a given time as compared to agarose gel electrophoresis which is extremely laborious and time consuming. Advantages of SRAP's are that they produce very large numbers of polymorphic fragments in each reaction, are inexpensive and easy to do, are a dominant / codominant marker system and are very highly reproducible. Also SRAP was originally designed for Brassica crop species. This allows SRAP to be an ideal method to study genetic diversity in *B.napus*.

Table 2.5: Summary of molecular marker techniques

	RFLP	RAPD	AFLP	SSR	SNP	SRAP
Principle	Endonuclease restriction, Southern blot hybridization	DNA amplification with random primers	Endonuclease restriction use of adaptors and selective primers	Amplification of simple sequence repeat using specific primers	Sequence analysis	DNA amplification with random primers
Reproducibility	High	Low	High	High	High	High
Inheritance	Co-dominant	Dominant	Dominant	Co-dominant	Co-dominant	Both Dominant and Co-dominant
Technical Difficulty	Medium-High	Low	High	Low	Medium-High	Low
Automation possible	No	No	Yes	Yes	Yes	Yes
Cost	Medium	Low	High	High	High	Low
# of loci detected	1-5	1-10	>70	1-3	1	5-50
Sequence information needed	No	No	No	Yes	Yes	No

Adapted from Jain et al. 2002

2.6 Genetic Diversity Studies in B. napus

The gene pool of oilseed rape material has been considerably reduced in recent decades by breeding for specific quality traits (Snowdon and Friedt 2004). Breeding for double low seed quality in *B. napus* in the 1970's and 1980's has resulted in the formation of a genetic bottleneck since only two sources of these quality traits, Liho and Bronowski have been used by all breeding programs globally to create double low quality rapeseed (canola) cultivars. This has limited the genetic variability in characters of

importance in breeding. In order to increase genetic diversity the development of resynthesised rapeseed by crossing to ancestral Brassicaceae members such as *B. oleracea* and *B. rapa* has been suggested. This would potentially increase genetic variability for hybrid breeding and would be a source genetic variability for disease and pest resistance (Snowdon and Friedt 2007).

Studies to determine the amount of genetic variability within the species have been conducted with molecular markers. Diers and Osborn (1994) explored the amount of genetic diversity in *B. napus* germplasm with RFLP markers and observed that it was possible to separate spring from winter type accessions. This was similarly observed by Plieske and Struss (2001) with SSR molecular markers. Hallden et al. (1994) studied genetic diversity in *B. napus* using RFLP and RAPD molecular markers and found them to agree with pedigree information. Thorman et al. (1994) determined genetic distance between cruciferous species with RFLP and RAPD markers. Becker et al. (1995) examined the diversity between cultivars and resynthesised rapeseed lines with allozymes and RFLP markers and determined that resynthesised rapeseed lines would be a good source of genetic diversity. Similarly, Seyis et al. 2003 studied the genetic diversity between resynthesised rapeseed lines and spring rapeseed cultivars using AFLP markers. Lombard et al. (2000) used AFLP markers to genotype winter rapeseed cultivars in order to estimate genetic similarity.

2.7 Prediction of Hybrid Performance

Bernardo (1992) stated that in order to accurately predict heterosis the following criteria would have to be met: (1) dominance effects are strong; (2) trait heritability is high; (3) at least 30 to 50% of the quantitative trait loci (QTL) are linked to molecular

markers; (4) not more than 20 to 30% of the molecular markers are randomly dispersed or unlinked to QTL; (5) allele frequencies at individual loci in the parental inbreds are negatively correlated; (6) average parental allele frequencies vary only within a narrow range. The correlation between genetic distance and heterosis is expected to decrease if (1) QTL influencing heterosis is not closely linked to markers used for calculation of genetic distance estimates; (2) markers used for calculating genetic distance are not linked to QTLs (Melchiniger 1999). Therefore uniform marker genome coverage is not necessarily important, but rather the associating of the marker with the specific trait / gene is more important in order to accurately predict hybrid performance.

The relationship between genetic distance and heterosis in oilseed rape has been explored by Diers et al. (1996) with RFLP markers, Riaz et al. (2001) with SRAP and Yu et al. (2005) with morphological characters, isozymes, proteins and RAPD molecular markers. The ability to accurately predict heterosis based upon genetic distance has however, had varying success. This could be due to any of the above reasons or this could be due to the utilization of non-optimum marker systems that randomly distribute markers throughout the genome and are not associated with the trait itself.

Materials and Methods

3.1 *Plant material*

Parental material was selected based upon its diverse pedigree and favorable agronomic and seed quality (Table 3.1). All plant material for the 12 HEAR cultivars / lines were grown in standard potting soil (2 soil: 1 sand: 1 peat mix) in the University of Manitoba Department of Plant Science greenhouses. Six to twelve individuals of each line were grown and tissue harvested at the five leaf stage to determine degree of similarity between individuals from parental lines. All parental material grown was then selfed and the seed was collected and stored in cold storage. In this study 12 HEAR *Brassica napus* cultivars / lines were used (Table 3.1). Of these lines 7 were HEAR quality rapeseed material selected from Manitoba, Canada and 5 were HEAR quality rapeseed European lines. Canola quality lines and rapeseed lines from China (Table 3.1.1) were also included in this study in order to determine the ability of the SRAP molecular markers to differentiate the lines of distinctly different genetic backgrounds into genetically distinct groups.

Table 3.1: Parental HEAR cultivars / lines

HEAR Cultivars / Lines	Habit	Breeding Origin
Castor	Spring	Canada
MillenniUM 03	Spring	Canada
MillenniUM 01	Spring	Canada
HR 102	Spring	Canada
HR199	Spring	Canada
HR200	Spring	Canada
RRHR102	Spring	Canada
EU HEAR 1	Spring	Europe
EU HEAR 2	Spring	Europe
EU HEAR 3	Spring	Europe
EU HEAR 4	Spring	Europe
EU HEAR 5	Spring	Europe

Table 3.1.1: Canola / rapeseed quality cultivars

Canola Cultivars	Habit	Breeding Origin
Sentry	Spring	Canada
Westar	Spring	Canada
Surpass 400	Spring	Australia
Quinta	Winter	Europe
Glacier	Winter	Europe
Zhong You #9	Semi-winter	China
Zhong You #821	Semi-winter	China
Huashaung #3	Semi-winter	China

3.2 *DNA extraction*

The DNA was extracted from all plants using a modified version of the 2x CTAB method previously described (Li and Quiros 2001). DNA was extracted from six plants per cultivar / line. Two grams of tissue from each plant was collected in a sample envelope and flash frozen with liquid nitrogen. One and half grams of crushed tissue were then transferred to a 15 mL centrifuge tube, and then 10% CTAB buffer was added to it. The samples were then incubated at 65 °C for 90 minutes. Proteins were removed with a chloroform extraction and centrifuged at 4600 rpm for ten minutes. DNA was

precipitated by adding 0.55 v/v isopropanol and centrifuged at 4600 rpm for three minutes. The pellet was washed with 70% ethanol and air dried for two hours. DNA was then resuspended in three milliliters of distilled water. DNA samples were then frozen at -20 ^{o}C until use.

3.3 *Quantitation and electrophoresis*

DNA products were mixed together with bromophenol blue (BPB) and sterile distilled water and loaded into a 1% agarose gel stained with ethidium bromide in TBE buffer. A DNA sized marker of 1Kb was used in order to estimate quantity and quality of DNA. The gel was run at 150 volts for 30 minutes or until bands were within 1 cm from the bottom of the gel. Gels were then viewed using a UV transilluminator. DNA was also quantified using a spectrophotometer and readings were taken at 260 nm and 280 nm. The reading at 260 nm reading allows for the calculation of concentration of nucleic acids and the 280nm reading gives the amount of protein in the sample. Ratios of OD260 / OD280 were taken in order to determine the quality of DNA present for each DNA extraction (Saunders and Parkes 1999). An OD260 / OD 280 of 1.8 to 2.0 signify pure DNA. Quantity of DNA in the sample was determined by diluting the samples to 1/10 and 1/100 and taking optical density (OD) readings at 260 nm, where 1 OD at 260 nm for dsDNA is equivalent too 50 ng/uL. DNA concentration can be calculated using the following formula:

DNA Concentration = OD260 * 50 ng/uL * dilution factor

Once DNA quantity was determined, each sample was standardized to make a 10 ng/uL DNA sample. DNA samples were pooled together from within the same cultivar / line in order to reduce variability within the same cultivar / line. This is since the parental

material used in this experiment were not inbred lines, therefore variation between individuals within the same cultivars / lines were expected. Six DNA samples of the same cultivar / line were then pooled together to make 1.5 mL of 10 ng/uL DNA template for each cultivar / line.

3.4 *DNA amplification*

DNA was amplified using sequence related amplified polymorphism (SRAP). The reaction constituents were: 10X PCR buffer (500 mM KCl, 100 mM Tris, 1% Triton, 1.5mM $MgCl_2$, pH 9.3); dNTP 25 mmol/L; forward labeled primer 10 mmol/L; reverse unlabeled primer 10 mmol/L; *Thermus aquaticus* (Taq) DNA polymerase; genomic DNA 10 ng/uL. Distilled water brought the total reaction volume to 10 uL. The PCR reaction was carried out in the following fashion. The cycling profile included: (1) five cycles at 94°C for one minute, 35°C for one minute and 72°C for one minute, for denaturation, annealing and extension; (2) then the annealing temperature was raised to 50°C for the remaining 27 cycles; (3) 4°C soak for 30 minutes. After amplification, SRAP plates were stored in the refrigerator at 4°C until analysis on an ABI 3100 DNA Analyzer. This was repeated for the four colors of labeled primer (Table 2). As it is observed in (Table 2) the florescent dye FAM is blue, NED is yellow, PET is red and VIC is green. A total of 102 primer combinations were run for each parental line resulting in 885 polymorphic markers being scored.

Table 3.4: Labeled primers information

Primer Name	Fluorescent Dye	Primer Sequence
ME2	FAM	TGAGTCCAAACCGGAGC
ODD3	FAM	CCAAAACCTAAAACCAGGA
FC1	FAM	TCAAGGGCAGGTAAGAACAA
EM2	NED	GACTGCGTACGAATTCTGC
SA12	NED	TTCTAGGTAATCCAACAACA
BG23	NED	ATTCAAGGAGAGTGCGTGG
DC1	PET	TAAACAATGGCTACTCAAG
ODD20	PET	TCGTTGTTATGGCTGGAGA
PM88	PET	CGAAACCTCACCTCTCTCA
EM1	VIC	GACTGCGGTACGAATTCAAT
GA3	VIC	TCATCTCAAACCATCTACAC
SA7	VIC	CGCAAGACCCACCACAA

3.5 *Marker detection*

All markers were assessed using an ABI 3100 DNA analyzer (Applied Biosystems Institute, California). For every sample, 2 uL of each color was pooled together and then 2.5 uL of the four color solution was added to the corresponding well on 384 well plates. Then 5 uL of formamide with LIZ –labeled (orange) GeneScan 500 (ABI) as a standard was added to each well. All pooling and the addition formamide with standard were accomplished with the use of a robotics machine (Evo150 Tecan, Toronto). The plate was heated to 94°C for three minutes and then chilled for ten minutes on ice. The sample plate was then loaded into the ABI 3100 DNA analyzer and the SRAP products were separated using 36 cm long 16 channel arrays, run at 15.0 kV for 45 minutes.

3.6 *Data Analysis*

Data was analyzed using GenScan software (ABI) and was viewed using Genographer. Data exported into Genographer created a gel picture which enabled for the identification and scoring of polymorphic bands. All bands that showed polymorphism were scored as a dominant polymorphic marker. Presence of a band was scored as 1 and

absence was scored as 0. Scored polymorphic markers were then copied into a Microsoft Excel sheet. Data was then transferred into Phylip software (Felsenstein 2007) and analyzed by mGDE in order to calculate genetic distance and to perform the cluster algorithm by Dollop parsimony. Marker densities for 200, 400, 600 and 786 polymorphic markers were selected at random for both the cluster analysis and genetic distance calculations.

There are three potential major methods for cluster analysis; (1) distance methods, (2) parsimony and (3) maximum likelihood. Distance methods such as UPGMA, Fitch and Margoliash or Neighbor-joining use genetic similarity matrix data in order to build their trees. Distance methods are quick and require little computer power to analyze. However, UPGMA and Neighbor-joining distance methods test only a single tree, do not consider intermediate ancestors and miss homoplasies (Fristensky 2007). Whereas Fitch and Margoliash does consider multiple trees but does not consider intermediate ancestors and misses homoplasies (Fristensky 2007). The parsimony methods such as Dollo parsimony ($1\rightarrow0 >> 0\rightarrow1$) and Polymorphism parsimony ($1\rightarrow0 >> 0\rightarrow1$) choose the most parsimonious tree as the tree which minimizes the total number of steps (in this case, gain or loss of a site) for the entire tree from an evolutionary point of view (Fristensky 2007). Parsimony uses raw polymorphic marker data to make the most parsimonious tree and therefore the contributions of rare alleles are not lost as they are in the distance methods. Parsimony makes a new tree with every replication in order to find the most likely tree. Parsimony method can, however, be computer intensive and the time for analysis drastically increases with the number of lines added. Maximum likelihood is very similar to parsimony but calculates probability of change from one character to

another. Maximum likelihood is very computer intensive and time for analysis increases exponentially with the addition of lines (Fristensky 2007).

Nei and Li's index of similarity was used in order to calculate genetic distance, using the following formula:

$$\text{Similarity} = 2\ N_{ab} / N_a + N_b$$

where N_{ab} is the number of bands shared by a and b, N_a is the bands amplified in fragment a and N_b is the bands amplified in b (Nei and Li 1979). Genetic distance was calculated using the following formula:

$$\text{Genetic Distance} = 1 - \text{Similarity}$$

Genetic distances were then used to calculate Pearson's Product moment correlation coefficient (SAS 2001) to mean hybrid performance, mid- and high-parent heterosis for seed yield, oil, protein, glucosinolate, and erucic acid content, plant height, days to maturity and flowering time of all hybrid combinations and for GCA and SCA .

Determination of significant differences between correlations and molecular marker density for sets of 200, 400, 600, 786 molecular markers was explored with a Z-test.

$$\text{Z-test} = (\text{Z-score}_1 - \text{Z-score}_2) / \text{SE}$$

The conversion of the correlation coefficient (r) to the Z-score is as follows:

$$\text{Z-score} = 0.5\ln [(1+r) / (1-r)]$$

where r is the correlation coefficient and ln is the natural logarithm

$$\text{SE} = \text{SQRT} [(1/n_1-3) + (1/n_2-3)]$$

where n_1 and n_2 are the sample sizes of the two independent samples and SQRT is the square root.

Results and Discussion

4.1 Cluster Analysis of Twenty Cultivars / Lines

Parsimony cluster analysis of the 12 HEAR cultivars / lines along with eight canola or rapeseed quality cultivars based upon 885 polymorphic markers, clustered the cultivars into six major groups (Fig 4.1.1). The cluster analysis was able to accurately distinguish between canola, rapeseed and HEAR quality cultivars / lines, and also between spring and winter type canola cultivars. There was clear separation between cultivars of different origin which was consistent with both pedigree and geographic origin information.

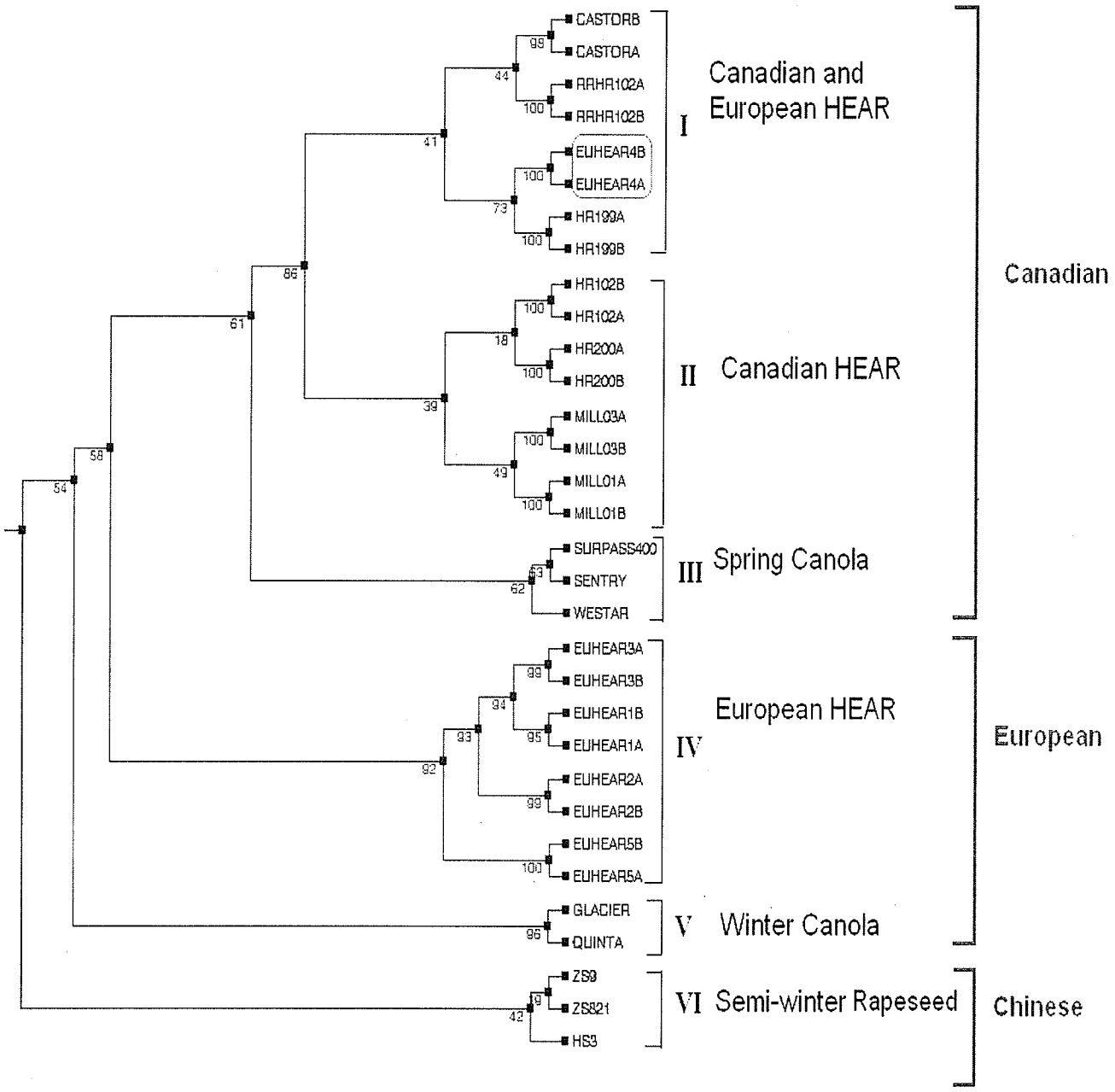

Fig 4.1.1: Phenogram of the 20 cultivars / lines revealed by parsimony cluster analysis from 885 SRAP polymorphic markers.

4.2 DNA polymorphism and Cluster Analysis of Twelve HEAR Cultivars / Lines

Sufficient polymorphism was obtained in order to calculate genetic distance between the 12 HEAR cultivars / lines. Molecular marker polymorphisms not including the 12 HEAR cultivars / lines and monomorphic markers just including the 12 HEAR cultivars / lines were removed for the analysis of only the HEAR material, which reduced the number of polymorphic loci from 885 to 786. A total of 786 polymorphic markers were generated from 102 primer combinations with an average of 7.7 polymorphic markers per primer combination. The average genome coverage was a marker every 1.5 cM when all 786 polymorphic markers were used.

Parsimony cluster analysis based upon 200 markers separated the 12 HEAR cultivars / lines into five groups (Figure 4.2). With 400 markers the cluster analysis separated the cultivars / lines into six groups (Figure 4.2.1) and with 600 markers the cluster analysis separated the cultivars / lines into five groups (Figure 4.2.2). Cluster analysis based upon all 786 polymorphic markers grouped all 12 HEAR cultivars / lines into five groups, which was consistent with available pedigree and geographic origin information. (Fig 4.2.3) Grouping of individuals within groups with parsimony cluster analysis is fairly consistent from 200 markers to 786 markers. There were some minor discrepancies with cultivar / line placement within the University of Manitoba material which consisted of two groups. There were two major genetically distinct groups and one minor group (Figure 4.2.3). The assignment of individuals to distinct genetic groups was found to in excellent agreement with available agronomic and seed quality data by Cuthbert (2006). The use of molecular markers for genetic diversity studies and group assignment has been used in other species such as rice (Giarroco et al. 2007), maize

(Boppenmaier et al. 1993; Benchimol et al. 2000; Lubberstedt et al 2000; Reif et al. 2004) , sorghum (Perumal et al. 2007), and sugarcane (Alwala et al. 2006). Therefore, the use of SRAP molecular markers for the determination of genetic diversity and possible assignment of cultivars to genetically distinct groups appears to be appropriate.

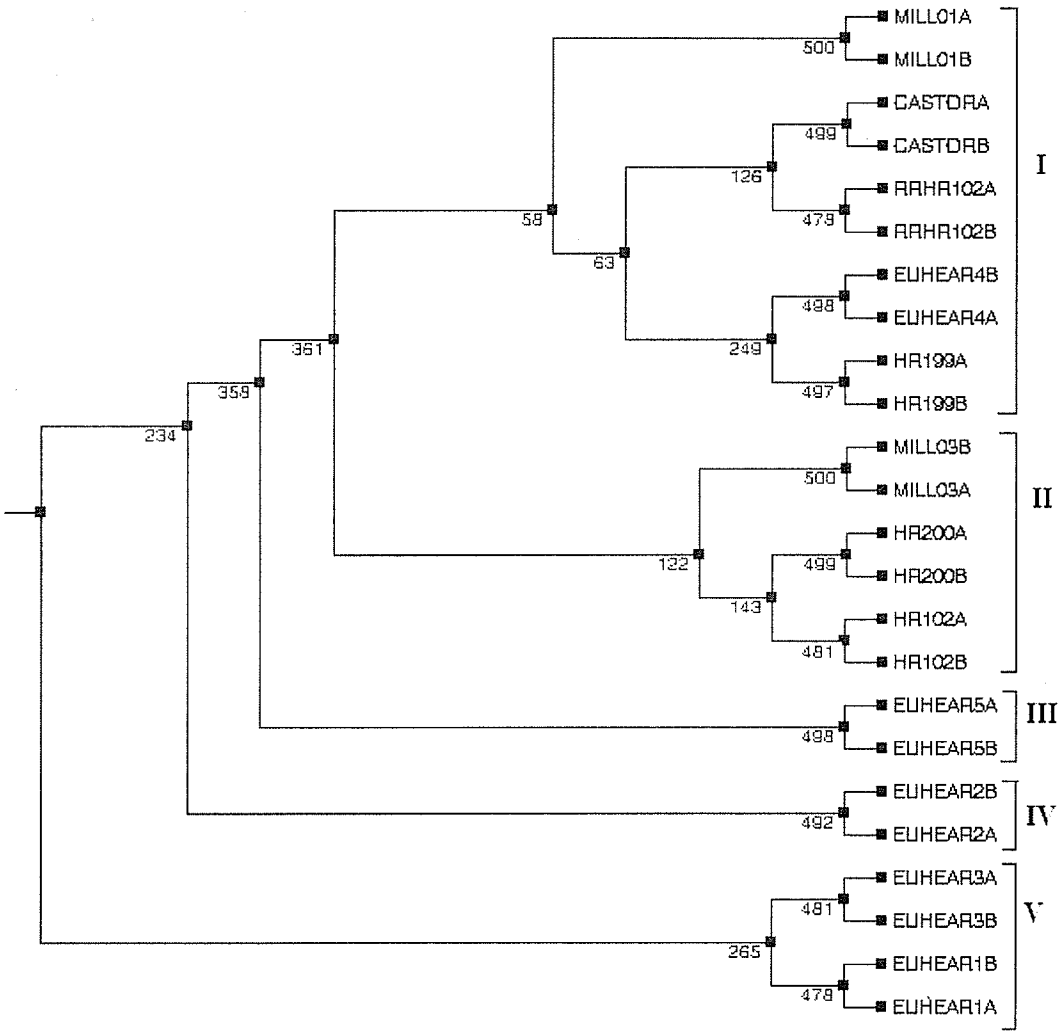

Fig 4.2: Phenogram of 12 HEAR cultivars / lines revealed by parsimony cluster analysis from 200 SRAP molecular polymorphic markers.

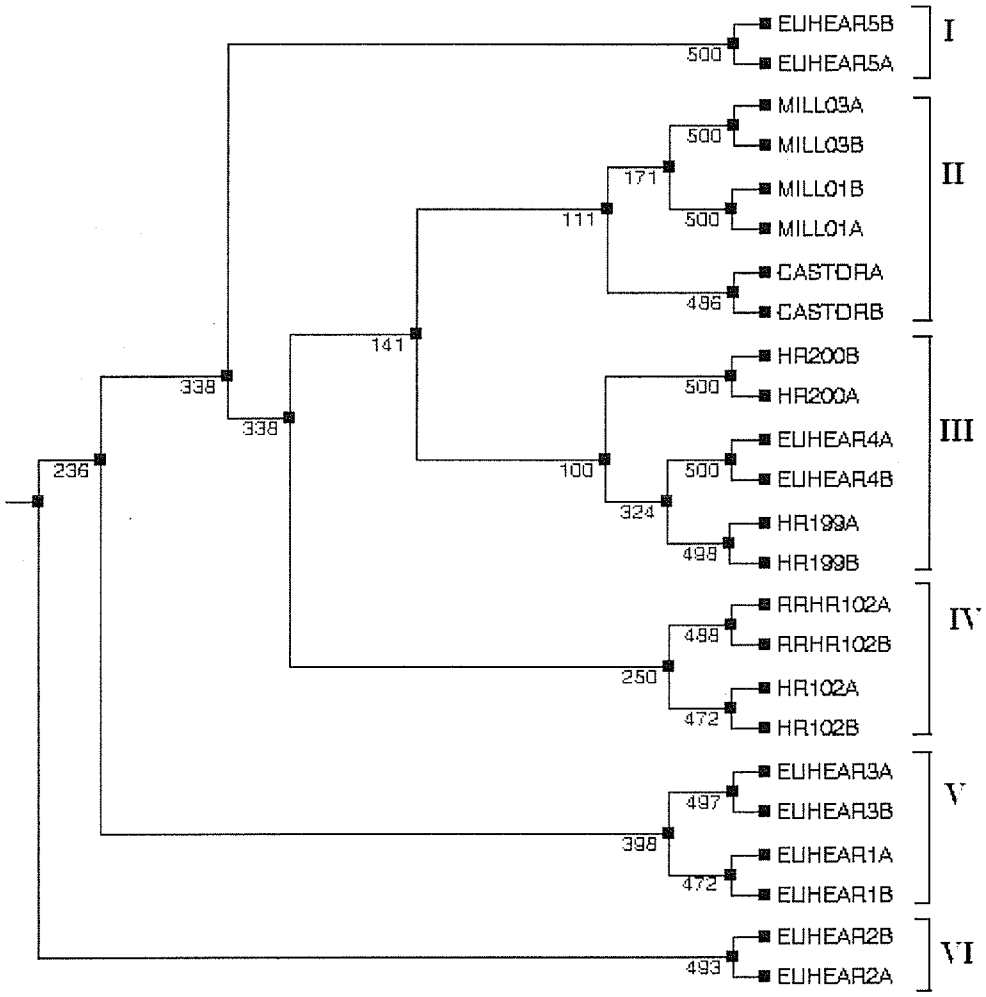

Fig 4.2.1: Phenogram of 12 HEAR cultivars / lines revealed by parsimony cluster analysis from 400 SRAP polymorphic markers.

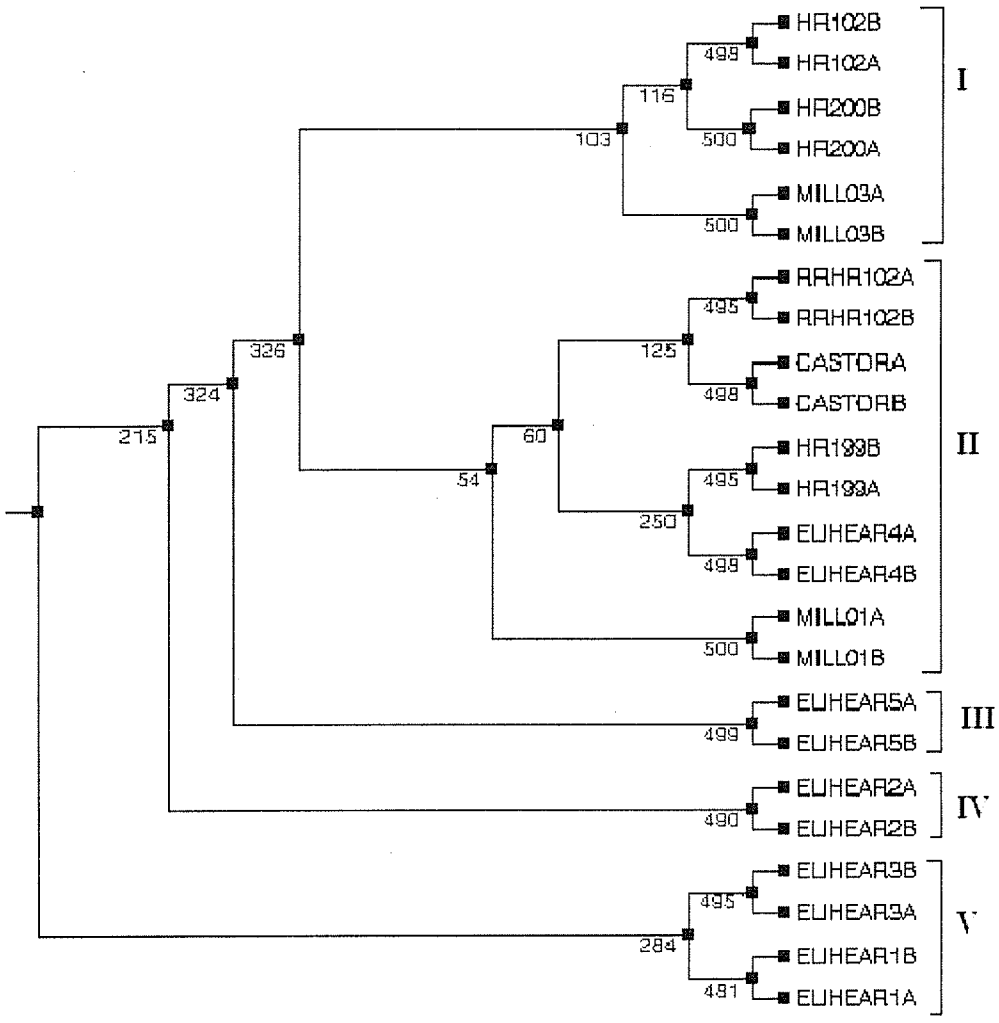

Fig 4.2.2: Phenogram of 12 HEAR cultivars / lines revealed by parsimony cluster analysis distances from 600 SRAP polymorphic markers.

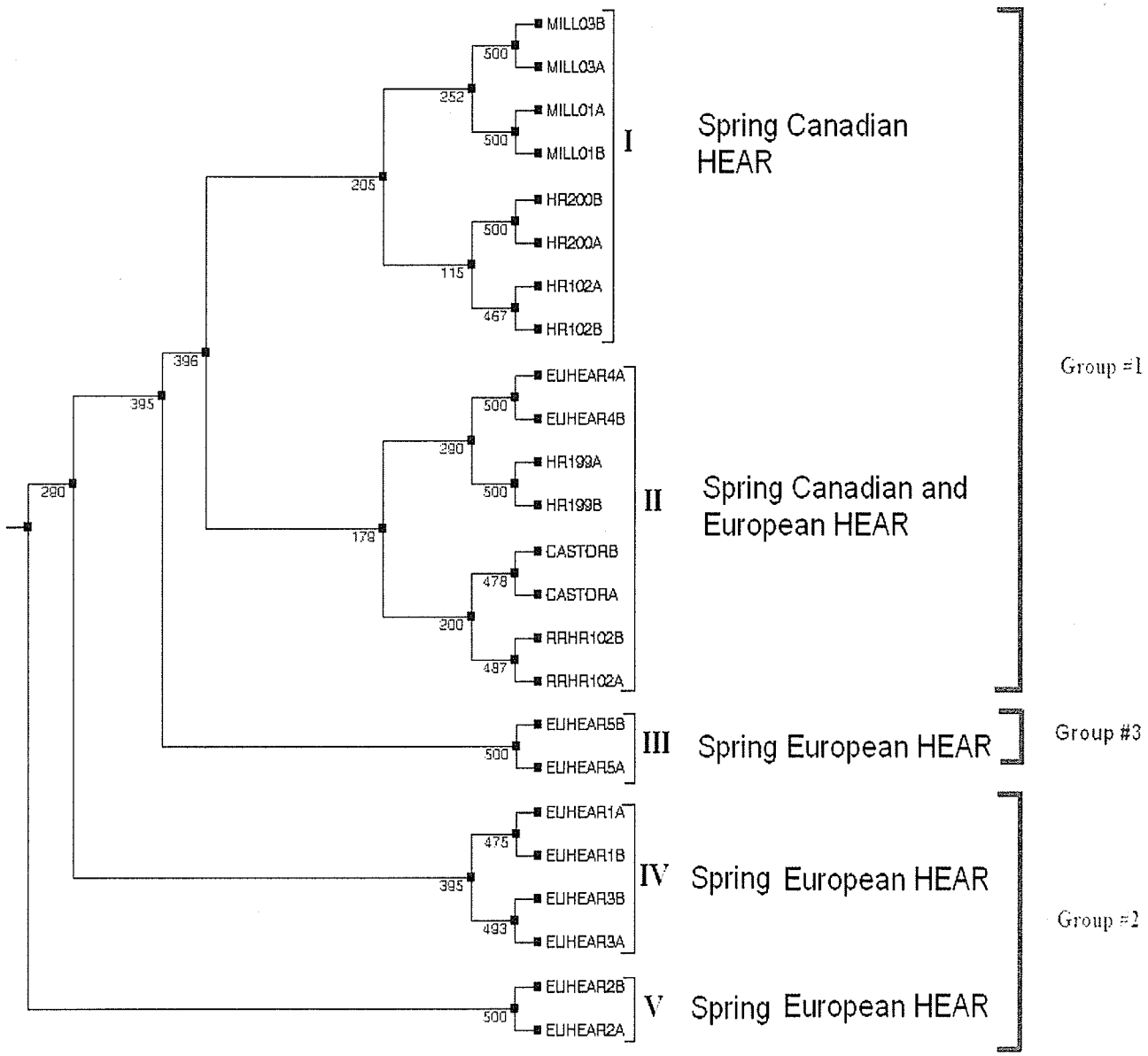

Fig 4.2.3: Phenogram of 12 HEAR cultivars / lines revealed by parsimony cluster analysis from 786 SRAP polymorphic markers.

4.3 Genetic Distance and Hybrid Performance

Genetic distances were calculated for sets of 200, 400, 600 and 786 molecular markers in order to determine what marker density would be necessary in order to accurately predict heterosis (i.e. every 6, 3, 2 or 1.5 cM) (Table 4.3). It should be noted that crosses of the 12 HEAR cultivars / lines were evaluated for agronomic and seed quality characteristics in a topcross design (Appendix A) by Cuthbert (2006). The calculated genetic distance ranged from 0.21 to 0.04 for HEAR cultivars. Genetic distances observed in Table 4.3 were lower than reported by Yu et al. (2005). These authors used RAPD markers and reported that genetic distance in rapeseed lines ranged from 0.309 to 0.553. They are also lower than reported by Riaz et al. (2001) who observed genetic distances of 0.03 to 0.54 among spring type rapeseed lines using SRAP.

Table 4.3: Nei's Genetic Distance for 200, 400, 600 and 786 polymorphic markers

UM x EU Hybrids	**GD 200**	**GD 400**	**GD 600**	**GD 786**
Castor x EU HEAR 1	0.18	0.20	0.19	0.20
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15
Castor x EU HEAR 2	0.16	0.17	0.16	0.15
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14
Castor x EU HEAR 3	0.18	0.20	0.19	0.20
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04
Castor x EU HEAR 5	0.08	0.09	0.10	0.10
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09
UM x UM / EU x EU Hybrids				
Castor x HR200	0.05	0.06	0.06	0.06
Castor x HR102	0.10	0.09	0.08	0.08
MillenniUM01 x HR102	0.08	0.08	0.07	0.06
MillenniUM03 x HR200	0.03	0.04	0.04	0.04
MillenniUM03 x HR102	0.06	0.06	0.06	0.05
HR200 x HR102	0.07	0.07	0.06	0.06
HR200 x RRHR102	0.06	0.06	0.05	0.05
HR200 x HR199	0.05	0.05	0.05	0.04
HR102 x RRHR102	0.09	0.07	0.06	0.06
HR102 x HR199	0.11	0.09	0.08	0.08
RRHR102 x HR199	0.06	0.06	0.06	0.06
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10

Table 4.3.0: Parental agronomic and seed quality data (Cuthbert 2006)

Parents	Flower (d)	Maturity (d)	Height (cm)	Yield (kg ha^{-1})	Oil Conc. (g kg^{-1})	Pro Conc. (g kg^{-1})	Gluc (umol g^{-1} seed)	Erucic Acid (%)
EU HEAR 1	61.3	124.7	143.8	1128.2	462.5	247.5	18.8	45.1
EU HEAR 2	45.1	108.6	114.6	1897.8	515.8	228.3	21.9	51.9
EU HEAR 3	53.2	123.4	141.3	1005.5	467.5	245.0	17.9	47.1
EU HEAR 4	43.2	99.6	95.0	1161.5	452.5	263.3	19.6	44.8
EU HEAR 5	42.1	97.4	100.4	1300.3	476.7	231.7	16.3	56.5
Castor	43.2	101.0	100.6	1640.3	462.5	256.7	26.9	49.1
MilleniUM01	42.8	100.2	62.3	1166.9	457.9	269.2	24.0	54.0
MilleniUM03	42.9	102.0	94.4	1527.2	466.7	265.8	17.7	56.3
HR200	43.8	100.8	90.6	1391.0	458.8	257.5	16.2	55.8
HR102	42.6	101.7	96.0	1774.5	490.9	242.1	17.7	55.1
RRHR102	46.2	102.7	112.1	1865.1	475.4	253.0	18.8	51.7
HR199	43.9	107.5	107.5	1789.1	471.7	252.5	20.0	54.7

4.3.1 Genetic Distance and Seed Yield

As expected inter-cluster crosses were in general higher yielding than intra-cluster crosses (Table 4.3.1). Crosses involving UM cultivars / lines to the more genetically divergent EU HEAR #1 and EU HEAR #3 lines were observed to be significantly higher yielding than other inter- and intra-cluster crosses. For example the MillenniUM03 x HR200 intra-cluster cross (1989 kg/ha) was lower yielding than the inter-cluster cross HR102 x EU HEAR #3 (3162 kg/ha) and this paralleled the observed differences in genetic distance. The MillenniUM03 x HR200 intra-cluster cross had a genetic distance of 0.03 to 0.04 whereas the inter-cluster cross HR102 x EU HEAR #3 had a genetic distance of 0.17 to 0.21. It was observed that as the number of polymorphic loci scored increased from 200 to 786, so did the correlations to mean seed yield, mid- and high-parent heterosis (Table 4.3.1). Relatively high correlations suggest that some of the markers may be linked to quantitative trait loci for seed yield.

Correlations to mean seed yield, mid-parent heterosis and high-parent heterosis were higher than previously reported by Diers et al. (1996) who found a significant correlation between genetic distance and mid-parent heterosis (r = 0.58*) and high-parent heterosis (r = 0.58*) and hybrid performance (r = 0.59*). Similarly Riaz et al. (2001) found a significant correlation between genetic distance and mid-parent heterosis (r = 0.63*) and high-parent heterosis (r = 0.66*) and seed yield (r = 0.64*). In contrast, Yu et al. (2005) found no significant correlation between RAPD molecular markers calculated genetic distance and seed yield.

When the top five most genetically divergent parent combinations were selected as predictions for highest seed yield it was observed that it was accurate in predicting

mean seed yield, mid-parent heterosis, and high-parent heterosis 1 of 5 times correctly. When the top ten most genetically divergent parental combinations were selected as predictions for highest seed yield it was accurate in predicting mean seed yield and high-parent heterosis 6 of 10 times correctly and mid-parent heterosis 5 of 10 times correctly. But when the top fifteen most genetically divergent parental combinations were selected as predictions for highest seed yield it was accurate in predicting mean seed yield, mid-parent heterosis and high-parent heterosis 13 of 15 times correctly. Genetic distance estimates could be a good predictor of hybrid performance and heterosis for seed yield.

Table 4.3.1: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for seed yield for 45 hybrids grown at four locations over two years

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Yield (kg/ha)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	2819.9	103.7	71.9
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	2977.0	159.4	155.1
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	2744.6	106.7	79.7
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	3043.9	141.7	118.8
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	2923.4	101.4	64.7
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	2798.8	87.0	50.1
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	2973.8	103.9	66.2
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	2321.3	31.2	22.3
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	2214.8	44.5	16.7
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	2457.1	43.5	29.5
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	2445.9	48.7	28.9
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	2817.6	53.4	48.5
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	2397.9	27.4	26.4
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	2890.7	56.8	52.3
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	2964.0	124.1	80.7
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	2658.0	144.7	127.8
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	2987.2	135.9	95.6
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	2780.1	132	99.9
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	3162.3	127.5	78.2
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	2637.4	83.8	41.4
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	3009.8	115.4	68.2
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	1388.9	19.3	19.0
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	2038.6	51.6	33.5
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	1510.0	18.3	8.6
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	1888.7	28.7	6.4
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	1965.8	29.9	5.4
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	1800.4	22	0.6
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	1918.4	30.5	17.0
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	1846.0	49.6	42.0
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	2210.6	56.4	44.8
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	1821.6	35.4	31.0
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	2169.9	41.1	22.3
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	2063.0	30.3	25.8
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	1388.9	-8.4	-15.3
Castor x HR102	0.10	0.09	0.08	0.08	1843.0	7.9	3.9
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	2100.0	42.8	18.4
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	1989.0	36.3	30.2
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	2180.2	32.1	22.9
HR200 x HR102	0.07	0.07	0.06	0.06	2045.9	29.3	15.3
HR200 x RRHR102	0.06	0.06	0.05	0.05	1807.8	11.0	-3.1
HR200 x HR199	0.05	0.05	0.05	0.04	2136.1	34.3	19.4
HR102 x RRHR102	0.09	0.07	0.06	0.06	2228.7	22.5	19.5
HR102 x HR199	0.11	0.09	0.08	0.08	2089.2	17.3	16.8
RRHR102 x HR199	0.06	0.06	0.06	0.06	1926.6	5.4	3.3
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	2375.2	48.5	25.2
Correlation coefficient							
GD 200					0.87 ***	0.75 ***	0.69 ***
GD 400					0.88 ***	0.75 ***	0.68 ***
GD 600					0.88 ***	0.77 ***	0.71 ***
GD 786					0.88 ***	0.78 ***	0.71 ***

* Signifies statistical significance to 0.05
** Signifies statistical significance to 0.01
*** Signifies statistical significance to < 0.0001

4.3.2 Genetic Distance and Flowering Time

It was observed that crosses between UM cultivars / lines with EU HEAR #1 and EU HEAR #3, which are both late maturing spring type HEAR cultivars, had higher genetic distance values but also had later flowering times than did crosses to EU HEAR #4, EU HEAR #5 and intra-cluster crosses involving UM HEAR x UM HEAR or EU HEAR x EU HEAR cultivars / lines.

As the number of polymorphic loci increased so did the correlation to mean flowering time (Table 4.3.2). It was observed that there was a negative correlation between mid-parent heterosis flowering time and genetic distance and this correlation generally increased as the amount of polymorphic loci increased (-0.63*** to -0.65***). Similarly, it was observed that the positive correlation increased between genetic distance and mean flowering time (0.62*** to 0.68***) and high-parent heterosis (0.61*** to 0.66***) as the amount of polymorphic markers increased. These relatively high correlations suggests that some of the markers may be linked to quantitative trait loci for flowering time and that genetic distance could be a good predictor of flowering time. In contrast, Yu et al. (2005) found no significant correlation between genetic distance and flowering time.

When the top five most genetically divergent parent combinations were selected as predictions for latest flowering time it was observed that it was accurate in predicting latest mean flowering time 0 of 5 times , mid-parent heterosis 2 of 5 times, and high-parent heterosis 1 of 5 times correctly. When the top ten most genetically divergent parental combinations were selected as predictions for latest flowering time it was accurate in predicting latest mean flowering time 5 of 10 times, mid-parent heterosis 6 of

10 and high-parent heterosis 7 of 10 times correctly. But when the top fifteen most genetically divergent parental combinations were selected as predictions for latest flowering time it was accurate in predicting latest mean flowering time 12 of 15, mid-parent heterosis 14 of 15 and high-parent heterosis 12 of 15 times correctly.

Table 4.3.2: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for flowering time in 45 hybrids grown at four locations over two years.

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Flower (Days)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	46.2	-11.6	7
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	47.3	-9.1	10.5
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	45.2	-13.2	5.4
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	45.7	-13	4.3
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	45.3	-12.9	6.2
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	48.3	-10.2	4.4
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	48.9	-7.0	11.4
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	43	-2.5	-0.4
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	42.9	-2.4	0.2
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	42.7	-3.0	-0.5
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	43.8	-1.4	0.1
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	43	-1.9	0.9
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	44.9	-1.6	-0.4
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	44.3	-0.4	1.0
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	44.1	-8.5	2.1
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	43.5	-9.4	1.6
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	43	-10.5	0.3
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	43.8	-9.6	0.1
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	44.6	-6.9	4.6
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	47.3	-4.9	2.3
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	44.3	-8.8	0.8
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	42.3	-1.7	-1.4
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	41.2	-4.3	-4
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	42.9	-1.3	-0.6
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	41.8	-2.5	-1.9
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	43.9	-1.7	1.7
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	42.1	-3.3	-2.5
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	42.8	0.5	1.8
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	41.9	-1.3	-0.4
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	42.3	-0.3	0.6
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	42.7	-0.6	1.4
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	41.8	-1.4	-0.8
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	42.2	-4.5	-2.3
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	42.7	-1.9	-1.1
Castor x HR102	0.10	0.09	0.08	0.08	41.6	-3.1	-2.5
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	41.5	-2.9	-2.6
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	42	-3.1	-2.0
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	42.1	-1.6	-1.3
HR200 x HR102	0.07	0.07	0.06	0.06	42.4	-1.8	-0.5
HR200 x RRHR102	0.06	0.06	0.05	0.05	43.4	-3.5	-0.8
HR200 x HR199	0.05	0.05	0.05	0.04	42.3	-3.6	-3.5
HR102 x RRHR102	0.09	0.07	0.06	0.06	42.7	-3.9	0.1
HR102 x HR199	0.11	0.09	0.08	0.08	41.9	-3.1	-1.7
RRHR102 x HR199	0.06	0.06	0.06	0.06	43.3	-3.8	-1.3
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	42.8	-1.9	1.6
Correlation Coefficient							
GD 200					0.62 ***	-0.63 **	0.61 ***
GD 400					0.66 ***	-0.63 **	0.63 ***
GD 600					0.66 ***	-0.63 **	0.64 ***
GD 786					0.67 ***	-0.65 **	0.66 ***

* Signifies statistical significance to 0.05
** Signifies statistical significance to 0.01
*** Signifies statistical significance to < 0.0001

4.3.3 Genetic Distance and Days to Physiological Maturity

It was observed that crosses involving UM cultivars / lines to EU HEAR #1 and EU HEAR #3, which were more genetically divergent, were later maturing than crosses involving EU HEAR #2, EU HEAR #4, EU HEAR #5, and intra-cluster crosses that were less genetically divergent. Days to physiological maturity would therefore be expected to increase as genetic distance increases.

A strong positive correlation was observed between genetic distance to mean days to physiological maturity (0.75*** to 0.79***) and to high-parent heterosis (0.65*** to 0.69***); while a negative correlation to mid-parent heterosis (-0.61*** to -0.62***) was observed that increased as the amount of polymorphic loci increased (Table 4.3.3). Days to maturity increased as genetic distance increased since EU HEAR #1, EU HEAR #2 and EU HEAR #3 appear to have remnant winter habit genes in their genetic makeup from crosses to Chinese winter habit rapeseed. These relatively high correlations suggests that some of the markers may be linked to quantitative trait loci for days to maturity and that genetic distance could be a good predictor of maturity. In contrast, Riaz et al. (2001) found no significant correlation between genetic distance and maturity, mid-parent heterosis and high-parent heterosis.

When the top five most genetically divergent parent combinations were selected as predictions for later maturity it was observed that it was accurate in predicting later mean maturity 1 of 5 times , mid-parent heterosis 2 of 5 times, and high-parent heterosis 2 of 5 times correctly. When the top ten most genetically divergent parental combinations were selected as predictions for later maturity it was accurate in predicting later mean maturity 6 of 10 times, mid-parent heterosis 6 of 10 and high-parent heterosis 6 of 10

times correctly. But when the top fifteen most genetically divergent parental combinations were selected as predictions for later maturity it was accurate in predicting later mean maturity 14 of 15, mid- parent heterosis 14 of 15 and high-parent heterosis 12 of 15 times correctly.

Table 4.3.3: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for days to physiological maturity for 45 hybrids grown at four locations over two years

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Maturity (Days)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	107.9	-4.4	6.9
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	108.8	-3.2	8.6
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	106.7	-5.9	4.6
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	106.4	-5.6	5.6
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	107.3	-5.2	5.6
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	108.3	-4.7	5.5
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	110.1	-5.2	2.4
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	102.6	-2.1	1.6
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	102.6	-1.7	2.4
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	103.8	-1.4	1.8
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	102.9	-1.7	2.1
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	105.0	-0.1	3.3
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	105.8	0.2	3.1
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	107.0	-1.0	-0.5
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	103.3	-7.9	2.3
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	103.8	-7.1	3.6
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	103.3	-8.3	1.3
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	103.9	-7.3	3.1
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	105.7	-6.1	4.0
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	107.5	-4.9	4.7
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	108.3	-6.2	0.7
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	101.5	1.6	1.9
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	100.0	-0.8	0.4
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	100.7	0.5	1.1
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	99.6	-1.0	0.0
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	99.8	-1.3	0.2
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	100.7	-2.8	1.1
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	100.5	1.3	3.2
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	98.3	-0.5	0.9
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	99.9	0.2	2.6
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	97.5	-1.6	0.1
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	99.1	-0.4	1.7
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	98.1	-1.9	-2.7
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	99.7	-1.2	-1.1
Castor x HR102	0.10	0.09	0.08	0.08	100.3	-1.0	-0.6
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	100.8	-0.1	0.6
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	99.8	-1.6	-1.0
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	102.2	0.4	0.5
HR200 x HR102	0.07	0.07	0.06	0.06	99.3	-1.9	-1.5
HR200 x RRHR102	0.06	0.06	0.05	0.05	98.9	-2.8	-1.9
HR200 x HR199	0.05	0.05	0.05	0.04	100.0	-4.0	-0.8
HR102 x RRHR102	0.09	0.07	0.06	0.06	99.8	-2.3	-1.8
HR102 x HR199	0.11	0.09	0.08	0.08	102.5	-2.0	0.8
RRHR102 x HR199	0.06	0.06	0.06	0.06	100.7	-4.2	-1.9
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	103.0	0.0	5.7
Correlation Coefficient							
GD 200					0.75 ***	-0.61 ***	0.65***
GD 400					0.78 ***	-0.62 ***	0.66 ***
GD 600					0.78 ***	-0.62***	0.69 ***
GD 786					0.79 ***	-0.62 ***	0.69 ***

* Signifies statistical significance to 0.05

** Signifies statistical significance to 0.01

*** Signifies statistical significance to < 0.0001

4.3.4 Genetic Distance and Plant Height

It was observed that as genetic distance increased so did plant height (Table.4.3.4). Therefore, hybrids involving UM HEAR cultivars / lines crossed to EU HEAR #1, EU HEAR #2 and EU HEAR #3 were taller than hybrids from crosses to EU HEAR #4, EU HEAR #5 or intra-cluster crosses.

A strong positive correlation to mean plant height was found (0.81*** to 0.84***) and no correlation of genetic distance mid-parent heterosis, but a correlation of genetic distance to high-parent heterosis (-0.65*** to -0.67***) was observed (Table 4.3.4). As the number of polymorphic markers increased so did the correlation between genetic distance and plant height. These relatively high correlations suggests that some of the markers may be linked to quantitative trait loci for plant height and that genetic distance could be a good predictor of plant height. Diers et al. (1996) similarly observed a significant correlation between genetic distance and hybrid plant height (r = 0.67*). In contrast, Riaz et al. (2001) observed no significant correlation between genetic distance and plant height, mid-parent heterosis or high-parent heterosis. Similarly, Yu et al. (2005) observed no significant correlation between genetic distance and plant height.

When the top five most genetically divergent parent combinations were selected as predictions for increased height it was observed that it was accurate in predicting increased mean height 1 of 5 times and high-parent heterosis 2 of 5 times correctly. When the top ten most genetically divergent parental combinations were selected as predictions for increased height it was accurate in predicting increased mean height 7 of 10 times, and high-parent heterosis 7 of 10 times correctly. But when the top fifteen most genetically divergent parental combinations were selected as predictions for increased

height it was accurate in predicting increased mean height 13 of 15 and high-parent heterosis 13 of 15 times correctly.

Table 4.3.4: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for plant height for 45 hybrids grown at four locations over two years

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Height (cm)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	120.4	-1.4	-16.2
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	125.0	5.9	-13
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	122.5	2.9	-14.8
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	119.6	2.0	-16.8
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	119.2	-0.6	-17.1
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	132.9	3.9	-7.5
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	129.6	3.1	-9.9
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	111.3	3.4	-2.9
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	112.5	8.8	-1.8
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	110.8	6.1	-3.3
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	108.3	5.6	-5.5
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	114.2	8.4	-0.4
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	117.9	4.1	2.9
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	118.3	6.6	3.3
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	120.4	-0.4	-14.7
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	115.0	-1.5	-18.6
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	115.0	-2.4	-18.6
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	115.0	-0.8	-18.6
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	123.3	3.9	-12.7
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	127.1	0.3	-10
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	128.3	3.2	-9.1
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	94.2	0.6	-0.9
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	100.0	5.6	5.3
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	97.5	5.1	2.6
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	97.5	2.1	1.5
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	106.3	2.6	-5.2
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	101.3	0.0	-5.8
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	104.6	4.0	3.9
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	97.9	1.6	-2.5
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	104.6	7.4	4.1
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	98.3	2.9	-2.1
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	103.8	5.6	3.3
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	108.8	2.4	8.1
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	93.3	-2.4	-7.2
Castor x HR102	0.10	0.09	0.08	0.08	99.6	1.3	-1.0
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	101.7	8.0	5.9
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	102.5	10.8	8.6
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	101.7	6.8	5.9
HR200 x HR102	0.07	0.07	0.06	0.06	101.7	8.9	5.9
HR200 x RRHR102	0.06	0.06	0.05	0.05	103.8	2.4	-7.4
HR200 x HR199	0.05	0.05	0.05	0.04	102.1	3.0	-5.0
HR102 x RRHR102	0.09	0.07	0.06	0.06	107.1	2.9	-4.5
HR102 x HR199	0.11	0.09	0.08	0.08	106.3	4.4	-1.2
RRHR102 x HR199	0.06	0.06	0.06	0.06	113.8	3.6	1.5
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	110.0	2.3	-4.0
Correlation Coefficient							
GD 200					0.81 ***	-0.23	-0.65 ***
GD 400					0.83 ***	-0.23	-0.65 ***
GD 600					0.83 ***	-0.25	-0.65 ***
GD 786					0.84 ***	-0.28	-0.67 ***

* Signifies statistical significance to 0.05

** Signifies statistical significance to 0.01

*** Signifies statistical significance to < 0.0001

4.3.5 Genetic Distance and Oil Concentration

It was observed that crosses involving crosses of UM HEAR cultivars / lines to the more genetically divergent cultivars EU HEAR #1, EU HEAR #2 and EU HEAR #3 produced higher concentrations of seed oil than did other inter- and intra-cluster crosses (Table 4.3.5).

A positive correlation between mean oil concentration and genetic distance was observed (0.81*** to 0.86***) but it was observed to decrease slightly as the number of molecular markers increased. A positive correlation with genetic distance between mid-parent heterosis (0.67*** to 0.68***) and high-parent heterosis (0.53*** to 0.55***) was observed. This relatively high correlation suggests that some of the markers may be linked to quantitative trait loci for oil concentration and that genetic distance could be a good predictor of oil concentration. However, Diers et al. (1996) observed no significant correlation between genetic distance and hybrid seed oil. Similarly, Riaz et al. (2001) observed no significant correlation between genetic distance and hybrid seed oil, mid-parent heterosis and high-parent heterosis.

When the top five most genetically divergent parental combinations were selected as predictions for oil concentration it was observed that it was accurate in predicting the highest mean oil concentration, mid-parent heterosis, and high-parent heterosis 2 of 5 times. When the top ten most genetically divergent parental combinations were selected as predictions for highest oil concentration it was observed that it was accurate in predicting mean oil concentration 8 of 10, mid-parent heterosis 7 of 10, and high-parent heterosis 5 of 10 times correctly. But when the top fifteen most genetically divergent parental combinations were selected as predictions for highest oil concentration it was

observed that it was accurate in predicting mean oil concentration, mid-parent heterosis, and high-parent heterosis 13 of 15 times correctly.

Table 4.3.5: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for oil concentration for 45 hybrids grown at four locations over two years

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Oil Conc. (g/kg)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	502.5	8.6	8.6
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	496.7	7.9	7.4
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	495.0	6.5	6.1
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	503.3	9.3	8.8
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	533.3	11.9	8.6
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	508.3	8.4	6.9
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	492.5	5.4	4.4
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	487.5	-0.3	-5.5
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	502.5	3.2	-2.6
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	488.3	-0.6	-5.3
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	508.3	4.3	-1.5
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	507.5	0.8	-1.6
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	493.3	-0.5	-4.4
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	486.7	-1.4	-5.6
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	489.2	5.2	4.6
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	494.2	6.8	5.7
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	507.5	8.7	8.6
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	501.7	8.3	7.3
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	511.7	6.8	4.2
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	496.7	5.4	4.5
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	494.2	5.2	4.8
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	450.0	-1.1	-1.7
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	460.8	0.3	-1.3
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	461.7	1.3	0.6
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	473.3	0.3	-3.6
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	475.8	2.6	0.1
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	455.0	-1.5	-3.5
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	473.3	0.8	-0.7
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	469.2	0.4	-1.6
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	480.0	1.8	0.7
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	474.2	1.4	-0.5
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	489.2	1.1	-0.3
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	484.2	1.7	4.7
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	455.8	-1.0	-1.4
Castor x HR102	0.10	0.09	0.08	0.08	481.7	1.1	-1.9
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	478.3	0.8	-2.6
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	477.5	3.2	2.3
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	481.7	0.6	-1.9
HR200 x HR102	0.07	0.07	0.06	0.06	483.3	1.8	-1.5
HR200 x RRHR102	0.06	0.06	0.05	0.05	470.0	0.6	-1.1
HR200 x HR199	0.05	0.05	0.05	0.04	475.0	2.1	0.7
HR102 x RRHR102	0.09	0.07	0.06	0.06	486.7	0.7	-0.8
HR102 x HR199	0.11	0.09	0.08	0.08	477.5	-0.8	-2.7
RRHR102 x HR199	0.06	0.06	0.06	0.06	470.8	-0.6	-1.0
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	484.2	-2.4	-6.1
Correlation Coefficient							
GD 200					0.86 ***	0.68 ***	0.53 **
GD 400					0.85 ***	0.57 ***	0.53 **
GD 600					0.82 ***	0.67 ***	0.53 **
GD 786					0.81 ***	0.68 ***	0.55 ***

* Signifies statistical significance to 0.05

** Signifies statistical significance to 0.01

*** Signifies statistical significance to < 0.0001

4.3.6 Genetic Distance and Protein Concentration

In general hybrid crosses of UM HEAR cultivars / lines to EU HEAR #1, EU HEAR #2 and EU HEAR #3 resulted in lower protein concentrations than did other inter- and intra cluster crosses (Table 4.3.6). Crosses to the EU HEAR #1, EU HEAR #2 and EU HEAR #3 had the highest oil content (they were expected to have the lowest protein content) and since oil and protein content are known to be negatively correlated to each other as previously reported by Serynk and Stefansson (1983). It is expected that as oil concentration increases, protein concentration will decrease.

A relatively high negative correlation of genetic distance to mean protein content (-0.82*** to -0.85***), mid-parent heterosis (-0.67*** to -0.69***) and high-parent heterosis (-0.67*** to -0.68***) was observed. The correlation between genetic distance and protein content, mid-parent heterosis and high-parent heterosis decreased as the number of polymorphic markers increased. These relatively high correlations suggest that some of the markers may be linked to quantitative trait loci for protein concentration. In contrast, Diers et al. (1996) observed no significant correlation between genetic distance and hybrid seed protein concentration.

When the top five most genetically divergent parental combinations were selected as predictions for protein concentration it was observed that it was accurate in predicting the lowest mean protein concentration, mid-parent heterosis and high-parent heterosis 3 of 5 times correctly. When the top ten most genetically divergent parental combinations were selected as predictions for protein concentration it was observed that it was accurate in predicting the lowest mean protein concentration 8 of 10, mid-parent heterosis 7 of 10 and high-parent heterosis 6 of 10 times correctly. But when the top fifteen most

genetically divergent parental combinations were selected as predictions for protein concentration it was observed that it was accurate in predicting lowest mean protein concentration 12 of 15, mid-parent heterosis 13 of 15 and high-parent heterosis 11of 15 times correctly.

Table 4.3.6: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for protein concentration for 45 hybrids grown at four locations over two years.

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Protein Conc. (g /kg)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	232.5	-7.8	-9.4
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	240.0	-7.1	-10.8
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	238.3	-7.1	-10.3
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	231.7	-8.2	-10.0
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	220.0	-10.1	-11.1
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	233.3	-6.8	-7.8
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	240.8	-3.7	-4.6
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	241.7	-0.3	-5.8
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	241.7	-2.8	-10.2
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	242.5	-1.8	-8.8
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	238.3	-1.9	-7.5
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	226.7	-3.6	-6.4
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	240.0	-0.3	-5.1
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	246.7	2.6	-2.3
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	238.3	-5.0	-7.1
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	240.8	-6.3	-10.5
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	228.3	-10.6	-14.1
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	229.2	-8.8	-11.0
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	221.7	-9.0	-9.5
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	235.8	-5.3	-6.8
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	239.2	-3.8	-5.3
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	273.3	2.6	1.5
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	263.3	-0.5	-0.9
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	262.5	0.8	-0.3
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	257.5	1.9	-2.2
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	250.8	-2.8	-4.7
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	264.2	2.4	0.3
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	247.5	1.4	-3.6
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	251.7	0.5	-6.5
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	245.0	-1.5	-7.8
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	245.0	0.2	-4.9
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	240.8	1.6	-0.5
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	238.3	-1.7	-7.5
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	264.2	2.8	2.6
Castor x HR102	0.10	0.09	0.08	0.08	246.7	-1.1	-3.9
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	256.7	0.4	-4.6
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	249.2	-4.8	-6.2
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	250.0	-1.6	-5.9
HR200 x HR102	0.07	0.07	0.06	0.06	247.5	-0.9	-3.9
HR200 x RRHR102	0.06	0.06	0.05	0.05	254.2	-0.4	-1.3
HR200 x HR199	0.05	0.05	0.05	0.04	253.3	-0.7	-1.6
HR102 x RRHR102	0.09	0.07	0.06	0.06	242.5	-2.0	-4.1
HR102 x HR199	0.11	0.09	0.08	0.08	251.7	1.8	-0.3
RRHR102 x HR199	0.06	0.06	0.06	0.06	253.3	0.2	0.1
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	238.3	3.6	2.8
Correlation coefficient							
GD 200					-0.85 ***	-0.69 ***	-0.68 ***
GD 400					-0.85 ***	-0.69 ***	-0.68 ***
GD 600					-0.83 ***	-0.67 ***	-0.67 ***
GD 786					-0.82 ***	-0.68 ***	-0.67 ***

* Signifies statistical significance to 0.05
** Signifies statistical significance to 0.01
*** Signifies statistical significance to < 0.0001

4.3.7 Genetic Distance and Glucosinolate Concentration

No correlation between genetic distance and mean glucosinolate concentration, but significant correlation to mid-parent (-0.39** to -0.45**) heterosis and high-parent heterosis (-0.37** to -0.41**) was observed (Table 4.3.7). This suggests that genetic distance may not be accurate predictor of hybrid performance for glucosinolate concentration and therefore screening of parental inbred lines for glucosinolate concentration would be required prior to crossing. Cuthbert (2006) illustrated that intra- and inter-cluster hybrids were not significantly different from each other and therefore geographic origin and genetic diversity did impact glucosinolate concentration. Since glucosinolate concentration is highly heritable and is controlled by three recessive genes, screening parental material for low glucosinolate content would be a good indicator of glucosinolate concentration in hybrids potentially. It is expected that since glucosinolate concentration is controlled by three recessive loci that parents would be recessive at these loci and that only background genetic factors played a role in determining glucosinolate concentration.

When the top five most genetically divergent parental combinations were selected as predictions for glucosinolate concentration it was observed that it was accurate in predicting the lowest mean glucosinolate concentration, mid-parent heterosis and high-parent heterosis 2 of 5 times correctly. When the top ten most genetically divergent parental combinations were selected as predictions for glucosinolate concentration it was observed that it was accurate in predicting the lowest mean glucosinolate concentration 7 of 10, mid-parent heterosis 8 of 10 and high-parent heterosis 8 of 10 times correctly. But when the top fifteen most genetically divergent parental combinations were selected as

predictions for glucosinolate concentration it was observed that it was accurate in predicting lowest mean glucosinolate concentration 10 of 15, mid-parent heterosis 11 of 15 and high-parent heterosis 11of 15 times correctly.

Table 4.3.7: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for glucosinolate concentration for 45 hybrids grown at four locations over two years

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Gluc (umol / g seed)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	14.8	-35.5	-21.7
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	18.8	-12.2	0.0
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	14.8	-18.7	-16.0
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	13.7	-22.0	-15.7
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	14.8	-19.3	-16.7
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	15.3	-18.8	-18.7
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	17.7	-9.0	-6.2
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	22.2	-9.1	1.1
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	21.4	-6.8	-2.3
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	19.8	-0.2	11.8
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	18.7	-2.1	15.2
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	18.0	-9.2	1.6
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	20.6	1.2	9.8
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	21.5	2.6	7.5
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	16.1	-28.2	-10.3
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	17.4	-17.0	-2.8
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	13.2	-26.0	-25.4
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	14.3	-16.0	-11.6
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	15.5	-13.0	-12.5
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	14.8	-19.1	-17.2
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	18.7	-1.5	4.2
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	22.0	0.9	12.4
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	17.3	-6.9	-1.9
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	17.1	-4.6	5.4
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	16.3	-12.4	-7.8
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	17.8	-7.4	-5.3
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	18.5	-6.5	-5.5
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	18.8	-12.8	15.3
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	18.8	-7.1	14.8
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	15.7	-7.8	-4.0
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	15.6	-4.2	-3.9
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	17.1	0.4	4.6
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	17.8	1.2	9.5
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	18.4	-14.5	13.6
Castor x HR102	0.10	0.09	0.08	0.08	16.4	-24.9	-5.4
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	17.8	-15	0.2
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	16.0	-5.5	-1.3
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	16.7	-5.8	-5.6
HR200 x HR102	0.07	0.07	0.06	0.06	14.3	-15.5	-11.6
HR200 x RRHR102	0.06	0.06	0.05	0.05	15.9	-8.9	-1.8
HR200 x HR199	0.05	0.05	0.05	0.04	17.2	-5.2	5.9
HR102 x RRHR102	0.09	0.07	0.06	0.06	15.4	-15.4	-12.9
HR102 x HR199	0.11	0.09	0.08	0.08	18.3	-2.8	3.5
RRHR102 x HR199	0.06	0.06	0.06	0.06	19.0	-1.9	1.3
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	20.1	5.0	23.0
Correlation coefficient							
GD 200					-0.19	-0.39 **	-0.41 **
GD 400					-0.17	-0.42 **	-0.39 **
GD 600					-0.15	-0.43 **	-0.36**
GD 786					-0.16	-0.45 **	-0.37 **

* Signifies statistical significance to 0.05

** Signifies statistical significance to 0.01

*** Signifies statistical significance to < 0.0001

4.3.8 Genetic Distance and Erucic Acid Concentration

A negative correlation between erucic acid concentration (-0.75*** to -0.80***), no correlation to mid- parent heterosis, and a negative correlation to high-parent heterosis (-0.46** to -0.49**) and genetic distance was observed. In general, as the number of polymorphic loci increased the correlation between genetic distance and mid-parent heterosis and high-parent heterosis decreased, while the correlation between genetic distances and mean erucic acid concentration increased (Table 4.3.8).

When the top five most genetically divergent parental combinations were selected as predictions for lowest erucic acid concentration it was observed that it was accurate in predicting the lowest mean erucic acid concentration 2 of 5, mid-parent heterosis 0 of 5 and high-parent heterosis 1 of 5 times correctly. When the top ten most genetically divergent parental combinations were selected as predictions for erucic acid concentration it was observed that it was accurate in predicting the lowest mean erucic acid concentration 5 of 10, mid-parent heterosis 1 of 10 and high-parent heterosis 5 of 10 times correctly. But when the top fifteen most genetically divergent parental combinations were selected as predictions for erucic acid concentration it was observed that it was accurate in predicting lowest mean erucic acid concentration 13 of 15, mid-parent heterosis 2 of 15 and high-parent heterosis 11 of 15 times correctly.

It can be concluded that genetic distance had a moderately strong predictive ability for erucic acid concentration as was observed by the correlation to mean hybrid performance and high-parent heterosis. Erucic acid concentration is controlled by two dominant loci and has a high heritability and all parents had erucic acid levels > 50% and

were therefore dominant at both major loci. Therefore, background genetic factors must be affecting erucic acid concentration.

Table 4.3.8: Correlation coefficient, genetic distance (GD) obtained from genetic distance matrix for 200, 400, 600 and 786 markers and mid- and high-parent heterosis for erucic acid concentration for 45 hybrids grown at four locations over two years

UM x EU Hybrids	GD 200	GD 400	GD 600	GD 786	Erucic Acid (%)	Mid-Parent (%)	High-Parent (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	50.0	6.1	1.8
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	51.5	3.9	-4.6
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	52.3	3.1	-7.2
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	52.2	3.5	-6.4
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	52.5	4.8	-4.7
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	49.1	1.5	-5.1
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	51.2	2.7	-6.3
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	53.7	6.4	3.5
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	54.4	2.7	0.7
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	53.7	-0.7	-4.6
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	54.9	1.9	-1.7
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	53.1	-0.6	-3.5
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	50.9	-1.7	-1.9
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	52.9	-0.6	-3.2
Castor x EU HEAR 3	0.18	0.20	0.19	0.20	50.9	5.8	3.8
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	51.8	2.5	-4.0
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	53.3	3.1	-5.3
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	52.4	1.7	-6.2
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	53.4	4.4	-3.1
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	49.7	0.6	-3.9
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	51.7	1.6	-5.4
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	55.2	11.8	2.3
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	56.5	11.6	0.2
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	56.8	12.9	1.8
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	56.0	12.2	1.8
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	54.8	13.5	5.9
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	55.3	11.1	1.1
Castor x EU HEAR 5	0.08	0.09	0.10	0.10	55.9	6.0	-1.0
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	56.0	1.3	-1.0
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	56.8	0.7	0.6
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	57.0	1.5	0.8
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	56.4	1.1	-0.2
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	55.2	2.0	-1.1
UM x UM / EU x EU Hybrids							
Castor x HR200	0.05	0.06	0.06	0.06	56.0	6.8	0.4
Castor x HR102	0.10	0.09	0.08	0.08	55.3	6.2	0.4
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	54.2	-0.5	-1.5
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	56.6	0.9	0.4
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	55.4	-0.6	-1.7
HR200 x HR102	0.07	0.07	0.06	0.06	54.9	-0.9	-1.6
HR200 x RRHR102	0.06	0.06	0.05	0.05	55.0	2.2	-1.5
HR200 x HR199	0.05	0.05	0.05	0.04	54.8	-0.8	-1.8
HR102 x RRHR102	0.09	0.07	0.06	0.06	54.4	1.9	-1.2
HR102 x HR199	0.11	0.09	0.08	0.08	56.0	2.0	1.6
RRHR102 x HR199	0.06	0.06	0.06	0.06	53.6	0.7	-2.0
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.10	54.4	0.4	-3.7
Correlation coefficient							
GD 200					-0.75 ***	-0.26	-0.49 **
GD 400					-0.79 ***	-0.27	-0.49 **
GD 600					-0.79 ***	-0.26	-0.46 **
GD 786					-0.80 ***	-0.23	-0.46 **

* Signifies statistical significance to 0.05

** Signifies statistical significance to 0.01

*** Signifies statistical significance to < 0.0001

4.3.9 Genetic Distance and General Combining Ability (GCA)

It was observed that there was a significant relation between GCA and genetic distance for all agronomic characteristics and every seed quality characteristic except for glucosinolate concentration. Genetic distances for GCA are average genetic distances for each cultivar / line for every cross that each cultivar / line was involved in. Highly significant correlations were observed to height, seed yield, oil and protein content ($p<0.01$) (Table 4.3.9). It was observed that in general that GD 400 had a higher correlation to GCA then did the other genetic distances. Since GCA is commonly used in order to effectively describe / predict hybrid performance based on phenotype and there is a strong correlation between genetic distance and GCA, genetic distance could potentially be a good predictor of general combining ability for hybrid cultivar development. It was observed when the four largest genetic distances were selected as predictor of GCA that it was accurate 100% of the time for selecting the cultivars / lines with highest GCA for both seed yield and oil concentration. Diers et al. (1996) observed significant correlation between genetic distance and general combining ability for hybrid seed yield ($r = 0.72^*$), plant height ($r = 0.92^*$), oil concentration ($r = 0.86^*$) and protein concentration ($r = 0.91^*$).

Table 4.3.9: Correlation between Genetic Distance and General Combining Ability (GCA)

	GD				GCA							
	GD 200	GD 400	GD 600	GD 786	Flower (Days)	Maturity (Days)	Height (cm)	Yield (kg/ha)	Oil Conc. (g/kg)	Protein Conc. (g /kg)	Gluc (umol / g seed)	Erucic Acid (%)
EU HEAR1	0.14	0.14	0.13	0.13	5.00	7.30	16.30	348.20	13.30	-8.80	-1.30	-3.50
EU HEAR 2	0.13	0.13	0.12	0.12	0.20	2.00	3.20	102.40	12.70	-6.10	3.20	-0.80
EU HEAR 3	0.14	0.14	0.13	0.13	1.90	4.70	12.90	322.60	9.30	-9.50	-1.40	-2.70
EU HEAR 4	0.08	0.08	0.08	0.08	-1.00	-2.50	-11.50	-648.80	-24.70	17.80	1.00	0.20
EU HEAR5	0.09	0.09	0.08	0.08	-1.20	-3.50	-6.80	-364.90	-7.10	-2.00	0.20	2.10
CASTOR	0.09	0.10	0.10	0.10	-0.20	-0.60	-3.20	-200.00	-7.10	2.50	1.80	-1.00
MilleniUM01	0.09	0.08	0.08	0.08	-0.30	-0.50	-4.80	-277.60	-7.60	9.00	2.70	-0.10
MilleniUM03	0.08	0.08	0.08	0.08	-0.90	-0.50	-3.90	-61.20	-3.80	3.50	-1.00	1.20
HR200	0.08	0.09	0.08	0.08	-0.10	-1.60	-6.20	-218.80	-4.20	2.20	-1.50	0.90
HR102	0.12	0.11	0.10	0.10	-0.60	-0.20	-1.90	53.80	10.30	-6.50	-0.70	0.70
RRHR102	0.10	0.10	0.09	0.09	1.60	0.50	6.60	-91.60	0.40	-0.80	0.40	-1.80
HR199	0.08	0.09	0.08	0.08	1.20	4.00	6.70	164.80	-6.00	4.30	1.90	-0.80
Correlation coefficients												
GD 200					0.63 *	0.69 *	0.74 **	0.73 **	0.86 **	-0.81 **	-0.20	-0.66 *
GD 400					0.70 *	0.76 **	0.80 **	0.78 **	0.84 **	-0.84 **	-0.22	-0.73 **
GD 600					0.68 *	0.74 **	0.76 **	0.73 **	0.79 **	-0.80 **	-0.18	-0.76 **
GD 786					0.68 *	0.74 **	0.76 **	0.73 **	0.79 **	-0.80 **	-0.18	-0.76 **

* Signifies statistical significance to 0.05
** Signifies statistical significance to 0.01

GD is average genetic distance of the cultivar / line in all crosses

4.3.10 Genetic Distance and Specific Combining Ability (SCA)

It was observed that there was only a strong significant correlation between genetic distance and SCA for days to maturity, yield, oil concentration and erucic acid concentration ($p<0.01$) (Table 4.3.10). A significant correlation ($p<0.05$) between genetic distance and plant height and protein concentration were also observed. It can therefore be concluded that genetic distance could be a good predictor of days to maturity, yield, oil concentration and erucic acid concentration for specific combining ability.

Table 4.3.10: Correlation between Genetic Distance and Specific Combining Ability (SCA) of Agronomic Characteristics

	GD 200	GD 400	GD 600	GD 786	Flower (Days)	Maturity (Days)	Height (cm)	Yield (kg/ha)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	-0.1	4.5	-0.3	196
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	1.1	5.4	5.7	438.7
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	-0.7	3.3	2.4	-32.5
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	-0.7	3.2	1.6	440.7
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	-0.8	3.8	-2.6	19.5
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	0.7	4.7	3.7	55.3
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	1.6	5.7	0.3	-52.6
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	0.1	0.3	2	-31.4
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	0.1	0.3	4.7	-52.3
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	0.3	1.5	2.2	-48.8
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	0.9	0.8	1.7	113.9
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	0.4	2.6	3.8	184.8
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	0.7	3.3	0.1	-74.4
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	0.5	3.8	0.5	135.5
Castor x EU HEAR 3	0.18	0.20	0.19	0.2	-0.1	0.5	2.7	368.4
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	-0.5	0.9	-1.3	148
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	-0.6	0.5	-2.1	238.4
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	-0.4	1.3	0	205.2
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	0.7	2.8	4.5	286.6
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	1.8	4.4	0.9	-77.8
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	-0.9	4.5	2.1	11.7
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	0.4	-3.4	-0.9	-49.3
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	-0.4	-4.9	4.1	361.5
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	0.9	-4	3.7	6.9
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	0.1	-5.4	-0.1	84.8
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	0.6	-5.3	1.2	322.3
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	-0.9	-5.1	-3.8	-125.9
Castor x EU HEAR 5	0.08	0.09	0.10	0.1	0.9	-0.6	3.9	81.2
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	0.1	-2.8	-1.3	94.4
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	0.9	-1.2	4.6	220.2
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	0.7	-3.4	0.4	5.1
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	0.1	-2.1	2	52.7
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	-1	-3.2	-0.4	106.2
Castor x HR200	0.05	0.06	0.06	0.06	0	-1.8	-7.8	-609.4
Castor x HR102	0.10	0.09	0.08	0.08	-0.8	-1.5	-5.3	-456.1
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	-0.7	-1	-1.8	-112.6
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	-0.2	-1.7	2	-162.5
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	0.2	0.4	-2.6	-272.1
HR200 x HR102	0.07	0.07	0.06	0.06	0	-2.3	-0.5	-232.4
HR200 x RRHR102	0.06	0.06	0.05	0.05	-0.6	-2.8	-5.9	-310.1
HR200 x HR199	0.05	0.05	0.05	0.04	-1.4	-2.5	-7.6	-264.7
HR102 x RRHR102	0.09	0.07	0.06	0.06	-1	-2.2	-6.3	-190
HR102 x HR199	0.11	0.09	0.08	0.08	-1.4	-0.3	-7.2	-612.4
RRHR102 x HR199	0.06	0.06	0.06	0.06	-1.6	-2.2	-7.1	-614.6
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.1	0.6	1.3	3.9	204.3
Correlation Coefficients								
GD 200					0.18	0.79 **	0.37*	0.45 **
GD 400					0.23	0.83 **	0.40 **	0.47 **
GD 600					0.23	0.83 **	0.41 **	0.49 **
GD 786					0.23	0.83 **	0.41**	0.50 **

* Signifies statistical significance to 0.05

** Signifies statistical significance to 0.01

Table 4.3.11: Correlation between Genetic Distance and Specific Combining Ability (SCA) of Seed Quality Characteristics

	GD 200	GD 400	GD 600	GD 786	Oil Conc. (g/kg)	Protein Conc. (g /kg)	Gluc (umol / g seed)	Erucic Acid (%)
Castor x EU HEAR 1	0.18	0.20	0.19	0.20	9.4	-5.6	-2.5	-0.8
MillenniUM01 x EU HEAR 1	0.16	0.15	0.15	0.15	4.2	-4.9	0.8	0
MillenniUM03 x EU HEAR 1	0.16	0.16	0.14	0.14	-1.5	-0.8	0.2	-0.3
HR200 x EU HEAR 1	0.17	0.17	0.15	0.16	7.2	-6.2	-0.5	-0.2
HR102 x EU HEAR 1	0.20	0.19	0.17	0.17	21.9	-8.8	-0.2	0.3
RRHR102 x EU HEAR 1	0.17	0.18	0.16	0.16	7.4	-1.4	-0.6	-0.9
HR199 x EU HEAR 1	0.15	0.16	0.14	0.15	-1.7	0.7	0.3	0.3
Castor x EU HEAR 2	0.16	0.17	0.16	0.15	-5	0.8	0.8	0.6
MillenniUM01 x EU HEAR 2	0.13	0.13	0.12	0.12	10.6	-6.1	-0.8	0.5
MillenniUM03 x EU HEAR 2	0.15	0.15	0.13	0.13	-7.6	0.5	0.9	-1.3
HR200 x EU HEAR 2	0.14	0.15	0.13	0.13	12.7	-2.4	0.3	0.1
HR102 x EU HEAR 2	0.16	0.17	0.14	0.14	-3.4	-5	-1.1	-1.4
RRHR102 x EU HEAR 2	0.15	0.15	0.14	0.14	-7.1	2.5	0.5	-1.5
HR199 x EU HEAR 2	0.15	0.16	0.14	0.14	-7	3.8	0	-0.3
Castor x EU HEAR 3	0.18	0.20	0.19	0.2	0.3	1	-1.1	-0.5
MillenniUM01 x EU HEAR 3	0.16	0.15	0.15	0.15	5.9	-3.4	-0.6	-0.4
MillenniUM03 x EU HEAR 3	0.16	0.17	0.15	0.15	15.2	-10.1	-1.4	0
HR200 x EU HEAR 3	0.19	0.18	0.16	0.16	9.8	-7.9	0.2	-0.7
HR102 x EU HEAR 3	0.21	0.21	0.18	0.17	4.4	-6.4	0.7	0.5
RRHR102 x EU HEAR 3	0.17	0.18	0.16	0.16	-0.1	1.9	-1	-1
HR199 x EU HEAR 3	0.15	0.16	0.15	0.15	4.1	-0.1	1.4	0.1
MillenniUM01 x EU HEAR 4	0.05	0.05	0.04	0.04	-2.3	0.7	1.8	0.4
MillenniUM03 x EU HEAR 4	0.05	0.05	0.05	0.05	4.5	-3.5	0.5	0.6
HR200 x EU HEAR 4	0.05	0.04	0.04	0.04	5.8	-3.1	0.7	1.2
HR102 x EU HEAR 4	0.08	0.07	0.07	0.07	2.1	1	-0.7	0.6
RRHR102 x EU HEAR 4	0.07	0.06	0.05	0.05	15.1	-11.6	-0.3	1.5
HR199 x EU HEAR 4	0.04	0.04	0.04	0.04	1	-3.6	-1	1.1
Castor x EU HEAR 5	0.08	0.09	0.10	0.1	1.9	2.4	0.3	0.3
MillenniUM01 x EU HEAR 5	0.08	0.08	0.08	0.08	-1.7	-0.3	-0.7	-0.4
MillenniUM03 x EU HEAR 5	0.08	0.08	0.08	0.08	5.1	-1.2	-0.3	-0.7
HR200 x EU HEAR 5	0.08	0.08	0.08	0.08	-0.3	0.1	0	-0.3
HR102 x EU HEAR 5	0.13	0.13	0.12	0.11	-0.6	4.9	0.8	-0.6
RRHR102 x EU HEAR 5	0.10	0.10	0.09	0.09	4.9	-3.4	0.5	0.3
Castor x HR200	0.05	0.06	0.06	0.06	-18.8	14.6	1.4	1.4
Castor x HR102	0.10	0.09	0.08	0.08	-8.2	6.1	-1	0.9
MillenniUM01 x HR102	0.08	0.08	0.07	0.06	-11	9.3	-0.9	-0.9
MillenniUM03 x HR200	0.03	0.04	0.04	0.04	-0.5	-1.5	1.5	0.1
MillenniUM03 x HR102	0.06	0.06	0.06	0.05	-11.6	8.4	1.5	-0.9
HR200 x HR102	0.07	0.07	0.06	0.06	-9.6	7.2	-0.4	-1.1
HR200 x RRHR102	0.06	0.06	0.05	0.05	-12.4	8	0.2	1.1
HR200 x HR199	0.05	0.05	0.05	0.04	-0.7	1.7	0	0.1
HR102 x RRHR102	0.09	0.07	0.06	0.06	-11	5.4	-1	0.8
HR102 x HR199	0.11	0.09	0.08	0.08	-13.5	9.2	0.4	1.4
RRHR102 x HR199	0.06	0.06	0.06	0.06	-9.7	4.9	0.1	1.2
EU HEAR 2 x EU HEAR 5	0.09	0.10	0.10	0.1	-8.2	2.1	0.2	-1.4
Correlation Coefficients								
GD 200					0.41 **	-0.41 **	-0.25	-0.40 **
GD 400					0.40 *	-0.41 **	-0.26	-0.44**
GD 600					0.40 **	-0.40 **	-0.26	-0.44 **
GD 786					0.41 **	-0.41 **	-0.27	-0.43 **

* Signifies statistical significance to 0.05

** Signifies statistical significance to 0.01

4.3.11 Heterosis Prediction

The ability to predict hybrid performance for selected parental line combinations is very important in hybrid breeding. Since genetic distances can easily be determined using molecular markers it may be possible to predict hybrid performance or heterosis by determining the degree of relatedness between parents. Correlations between genetic distance and heterosis have been observed using molecular markers in Brassica by Diers et al. (1996) and Riaz et al. (2001) and with morphological and molecular markers by Yu et al. (2005). Similarly, in this study it was observed that correlations were statistically significant between genetic distance and mean seed yield, oil concentration, protein concentration, erucic acid concentration, flowering time, days to maturity, and plant height and for high-parent heterosis ($p<0.01$) for these traits. This suggests that the molecular markers used in this study maybe associated with QTLs for the given traits listed above, since correlations were high between genetic distance and the given agronomic and seed quality characteristic. By selecting the top 15 parental combinations with the greatest genetic distance as predictors of hybrid performance it was possible to accurately predict superior mean seed yield and oil concentrations 13 of 15 times. This suggests that increased genetic distance / genetic diversity would be a good predictor of hybrid performance. The association between parental line combination genetic distance and hybrid performance and high-parent heterosis was observed to decrease as the degree of relatedness between two parental lines increased. It can be concluded that increased genetic diversity / genetic distance would contribute to increased seed yield and oil content, coupled with increased days to first flower, and days to physiological maturity. The hybrids would also be taller than UM HEAR cultivars / lines. With the statistically

significant correlations between genetic distance and GCA it should be possible to select HEAR cultivars / lines which are preferentially good general combiners. Also it should be possible to select specific crosses for yield, oil content and days to maturity based upon the degree of genetic distance between parents since statistically significant correlations ($p = 0.01$) between genetic distance and SCA for these traits was observed.

Therefore, inter-cluster crosses involving UM HEAR cultivars / lines to EU HEAR #1 and EU HEAR #3 would be predicted to be higher yielding, have higher oil concentration and lower protein concentration, have longer days to flowering and physiological maturity and would be taller plants than intra-cluster crosses between UM HEAR x UM HEAR or EU HEAR x EU HEAR material. Since these crosses would involve crosses between different heterotic pools which had the highest amount of genetic diversity / greatest genetic distance, they would be expected to display maximum heterosis.

4.4 Marker Density

One major concern for molecular marker dependent hybrid performance / heterosis prediction is its feasibility. In order for a technique to be considered useful it must first be determined to be simple, efficient, reliable and cost effective. Therefore, the lowest amount of molecular markers required for accurate hybrid performance / heterosis prediction would be favorable. Based upon observation of the cluster analysis and correlations of genetic distance to seed quality and agronomic traits, it is fair to say that a marker density of 200 polymorphic markers or markers every 6 cM could be sufficient for accurate heterosis prediction in HEAR. When selecting top five, ten or fifteen it was similarly observed that there was no increase in the ability to select superior performing

hybrids as marker density increased. Also, since it was determined that there was no significant difference in correlation to hybrid performance / heterosis, GCA, and SCA as marker density increased based upon the Z-test (α= 0.01).

Utilization of SRAP molecular markers along with an ABI 3100 Gene Sequencer allows for molecular work to be completed quickly and efficiently by multiplexing. For a single reaction from DNA extraction to ABI analysis costs approximately two dollars per reaction or 25 cents per polymorphism since a single reaction will yield on average eight polymorphic bands per reaction. For 12 inbreds to be assessed to meet the required 200 molecular markers for hybrid prediction would be a total of 300 reactions and therefore would cost approximately $600. The approximate cost for an analysis of a single 384 well plate is approximately $800 and with the average of 7.7 polymorphic bands per primer combination it would require 6.5 plates (~2500 reactions) to screen a population of 384 inbreds to meet the 200 molecular marker or 6 cM genome coverage target. This would cost approximately $5000. If one was to evaluate all 384 inbred lines for agronomic and seed quality data there would be a total of 73 536 potential hybrid combinations in a diallel, which is too many combinations to evaluate. Therefore screening the population prior to hand crossing material for replicated field trials would greatly reduce the amount of crosses to be evaluated since there are strong statistically significant correlations between genetic distance and hybrid performance / heterosis of agronomic and seed quality characteristics, GCA and certain traits in SCA.

5.0 General Discussion and Conclusions

Predicting hybrid performance has always been a primary goal in hybrid breeding programs (Melchiniger, 1999). Considering the time and expense associated with hand crossing and evaluating hybrids in replicated field trials, it would be best to only cross and send the best combinations to the field in order to reduce cost. In a breeding program, the evaluation of crosses in a diallel crossing scheme can become limiting as the number of hybrids increases exponentially as the number of inbreds included increases where: *[n(n-1)]/2,* such that 12 inbreds would produce 66 hybrid combinations, 100 inbreds would produce 4950 hybrid combinations, and 384 inbreds would produce 73 536 potential hybrid combinations. Creating this many hybrids is also very problematical since adequate greenhouse space is just not available. Therefore, the ability to characterize inbreds with molecular markers and to determine genetic distance between prospective parental lines becomes an attractive option. As this will allow for the screening of relatively large numbers of inbred lines; for example we could screen a population of 384 inbred lines and select the top 25 - 30% most genetically divergent inbred line combinations, since it was observed that it was possible to predict accurately mean seed yield and mean oil concentration 87% of the time (13 of 15) correct with SRAP molecular markers. All screening would be done at the 3 to 5 leaf stage and non-selected inbred line combinations would be removed. The use of molecular markers to assess genetic distance between parental lines would allow a large population of inbreds to be screened and the elimination of unfavorable combinations prior to hand crossing and replicated field trials. This would be beneficial since the *B. napus* gene pool is relatively small and geographic origin and pedigree information may not be able to

accurately depict the true genetic diversity present. This approach would allow for the more efficient production of superior performing hybrids.

Based upon the results of this research it appears that determining genetic diversity / genetic distance in *B. napus* using SRAP molecular markers could be such a pre-screening technique. One major concern with associating molecular marker based genetic distances with heterosis has been that not all polymorphic fragments / markers contribute to heterosis since many of them may be within non-coding regions and therefore have no association with agronomic or seed quality characteristics or QTLs (Yu et al. 2005). Since SRAP molecular marker primers are designed to potentially target coding regions, rather than inter-genic regions it may be a superior marker system for determining genetic diversity / genetic distance. SRAP molecular markers were capable of assigning canola, rapeseed, HEAR and winter and spring habit cultivars to separate genetically distinct groups accurately and consistently with pedigree and geographic origin information. The ability of SRAP molecular markers to separate HEAR cultivars / lines into different genetically distinct groups along with the determination of genetic distance between individual cultivars / lines would allow for maximum heterosis to be achieved. It was also observed that genetic divergence was directly related to hybrid performance and was fairly efficient in predicting it. It was observed that the top fifteen most genetically divergent hybrids were able to accurately predict increased mean hybrid seed yield and mean oil concentration 87 % of the time (13 of 15). Therefore it was predicted that hybrids from crosses with EU HEAR #1 and EU HEAR #3 and UM HEAR parental lines would result in maximum heterosis and this was observed to be consistent with agronomic and seed quality data reported by Cuthbert (2006). Significant

correlations between general combining ability (GCA) and genetic distance for agronomic and seed quality characteristics were observed and predictability of GCA based of genetic distances was 100% for seed yield and oil concentration.. Since GCA is used in hybrid breeding in order to predict the ability of an individual parent to be a good general combiner for hybrid cultivar development, the relationship between genetic distance and GCA signifies that genetic distance between parent lines could be a good predictor of hybrid performance. SRAP molecular markers along with the use of a ABI DNA analyzer appears to be a cost effective means of potentially assigning inbreds, cultivars or lines to genetically distinct groups and accurately predict hybrid performance / heterosis prior to hand crossing and replicated field trials.

Suggestions for further research

Future considerations would be to increase the number of parental inbred lines for evaluation with SRAP molecular markers and reconfirm the ability of genetic distance to be associated with hybrid performance/ heterosis. The top 25 - 30% most genetically divergent parental line combinations would be selected, and include checks such as 2 parental combinations of average genetic divergence and 2 parental combinations that are predicted to perform poorly and evaluate them in replicated field trials. Also evaluation should include other types of *B. napus* material other than HEAR, such as canola type *B. napus* in order to insure that the relationship between genetic distance and hybrid / heterotic performance is not just limited to HEAR cultivars / lines. The results should reconfirm that the utilization of genetic-distance-based-heterosis prediction could greatly improve hybrid cultivar development. The use of molecular markers for the determination of genetic diversity and heterosis prediction within oilseed rape would

allow for assignment of inbred lines to different heterotic groups and potentially predict superior performing hybrids for both seed yield and oil concentration. This would allow for the production of superior hybrids faster especially with the emerging biodiesel market that will require significant increased production of oilseed rape within the next decade or so. Also associating molecular markers with QTLs and using them in order to estimate genetic distance would greatly improve our ability to predict hybrid performance. Therefore, QTL mapping of yield, oil concentration genes would facilitate our ability to accurately predict heterosis.

6.0 Literature Cited

Alwala, S., Suman, A., Arro, J.A., Veremis, J.C., and Kimberg, C.A. 2006. Target region amplified polymorphism (TRAP) for assessing genetic diversity in sugarcane germplasm collections. Crop Science. **46:** 448-455.

Bell, J.M. 1982. From rapeseed to canola: a brief history of research for superior meal and edible oil production in Canada, feeding trials. Poult.Sci. **61**: 613-622.

Benchimol, L.L. Souza, C.L., Garcia, A., Kono, P., Mangolin, C.A, Barbosa, A.M., Coelho, A.S., and Souza, A.P. 2000. Genetic diversity in tropical maize inbred lines: heterotic group assignment and hybrid performance determined by RFLP markers. Plant Breeding. **119**: 491-496.

Bernardo, R. 1992. Relationship between single-cross performance and molecular heterozygosity. Theor. Appl. Genet. **83:** 628-634.

Bernardo, R. 2002. Breeding for quantitative traits in plants. Stemma Press, Woodbury, MN.

Brandle, J.E. and McVetty, P.B.E. 1989. Geographical diversity, parental selection and heterosis in oilseed rape. Can. J. Plant. Sci. **70:** 935-940.

Brandle, J.E. and McVetty, P.B.E. 1989. Heterosis and combining ability in hybrids derived from oilseed rape cultivars and inbred lines. Can. J. Plant Sci. **29:** 1191-1195.

Bruce, A.B. 1910. The Mendelian theory of heredity and the augmentation of vigor. Science. **32:** 627-628.

Bruel, D.C., Carpentieri-Pipoli, V., Gerage, A.C., Fonseca, N., Prete, C., Ruas, C., Ruas, P,. Souza, S.G., and Garbuglio, D. 2006. Genetic distance estimated by RAPD markers and its relationship with hybrid performance in maize. Pesq. Agropec. Bras. Brasilia. **41:** 1491-1498.

Boppenmaier, J., Melchiniger, A.E., Seitz, G., Geiger, H.H., and Herrmann, R.G. 1993. Genetic diversity for RFLPs in European maize inbreds III. Performance of crosses within versus between heterotic groups for grain traits. Plant Breeding. **111:** 217-226.

Burton, W.A., Ripley, V.L., Potts, D.A. and Salisbury, P.A. 2004. Assessment of genetic diversity in selected breeding lines and cultivars of canola quality *Brassica juncea* and their implications for canola breeding. Euphytica. **136:** 181-192.

Charcosset, A., and Moreau, L. 2004. Use of molecular markers for the development of new cultivars and the evaluation of genetic diversity. Euphytica. **137:** 81-94.

Charlton,K.M., Corner, A.H., Davey, K., Kramer, K.G., Mahadevan, S., and Sauer, F.D. 1975. Cardiac Lesions in rats Fed Rapeseed Oils. Can. J. comp. Med. **39**: 261-269.

Cross, J.W. and Schultz, P.J. (1997). Chemical induction of male sterility. In: Shivanna, K.R., and Sawhney, V.K. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press. Pp. 218-237.

Crow, J. F., 1948. Alternative hypotheses of hybrid vigor. Genetics **33**:477-487

Cuthbert, R. 2006. Assessment of Heterosis for Selected Traits in Hybrid HEAR. Unpublished Master's Thesis, University of Manitoba, Manitoba, Canada.

Cuthbert, J.L. and McVetty, P.B.E. 2001. Plot-to-plot, row-to-row, and plant-to-plant outcrossing studiesin oilseed rape. Can. J. Plant Sci. **81:** 657-664.

Davenport, C.B. 1908. Degeneration, albinism and inbreeding. Science. **28**: 454-455.

Diers, D.W. and Osborn, T.C. 1994. Genetic diversity of oilseed Brassica napus germplasm based on restriction fragment length polymorphisms. Theor Appl Genet. **88:** 662-668.

Diers, D.W., McVetty, P.B.E. and Osborn, T.C. 1996. Relationship between Heterosis and Genetic Distance Based on Restriction Fragment Length Polymorphism Markers in Oilseed Rape (*Brassica napus L.*). Crop Science. **36**: 79-83.

East, E. M., 1908. Inbreeding in corn. Rep. Conn. Agric. Exp. Stn. pp. 419–428.

East, E.M. 1911. The Genotypes Hypothesis and Hybridization. The American Naturalist. **45**: 160-174.

East, E. M., 1936. Heterosis. Genetics **21**:375-397

East. E.M., and Hayes, H.K. 1912. Heterozygosis in evolution and in plant breeding. U.S. Dept. of Agr.,Bur. Of P.I. Bull. **243**: 55 pp.

Emerson, R.A., and East, E.M. 1913. The inheritance of quantitative characters in maize. Nebraska Agr. Expt. Sta. Research Bul. 2.

Felsenstein, J. 2007. Phylip software. Version 3.67. University of Washington. Seattle, WA. USA.

Falconer, D.S. and Mackay, T.F., 1996. Introduction to Quantitative Genetics. Longman LTD. Harlow, England.

Fristensky, B. 2006. Bioinformatics.
http://www.umanitoba.ca/faculties/afs/plant_science/courses/39_769/

Giarrocco, L.E., Marassi, M.A., and Salerno, G.L. 2007. Assesement of the genetic diversity in Argentine rice cultivars with SSR markers. Crop Science. **47:** 853-860.

Grant, I. and Beversdorf, W.D. 1985. Heterosis and combining ability estimates in spring oilseed rape (*Brassica napus L.*). Can. J. Genet. Cytol. **27:** 472-478.

Hallden, C., Nilsson, N.O., Rading, I.M. and Sall, T. 1994. Evaluation of RFLP and RAPD markers in a comparison of *Brassica napus* breeding lines. Theor Appl Genet. **88:** 123-128.

Hasan, M., Seyis, F., Badami, A.G., Pons-Kuhnemann, J., Friedt, W., Luhs, W., and Snowdon, R.J. 2005. Analysis of genetic diversity in Brassica napus L. gene pool using SSR markers. Genetic Resources and Crop Evolution. **53:** 793-802.

Hulan, H.W., Kramer, J.K., Mahadevan, S., Sauer, F.D. and Corner, A.H. 1975. Brassica campestris var Span: II. Cardiopathogenicity of fractions isolated from Spain rapeseed oil when fed to male rats, Lipids **10:** 511-516.

Jain, M.J., Brar, D.S. and Ahloowalia, B.S. 2002. Molecular Techniques in Crop Improvement. Kluwer Academic Publishers. Netherlands. Pg 161-180.

Jones, D. F., 1917. Dominance of linked factors as a means of accounting for heterosis. Genetics **2**:466-479.

Jones, D. F., 1918. The effects of inbreeding and crossbreeding upon development. Conn. Agric. Exp. Stn. Bull. **107**: 100 pp.

Jones, D. F., 1922. The productiveness of single and double first generation corn hybrids. J. Am. Soc. Agron. **14**:242-252.

Jordan, D.R., Tao, Y. Godwin, I.D., Henzell, R.G., Cooper, M. and McIntyre, C.L. 2003. Prediction of hybrid performance in grain sorghum using RFLP markers. Theoretical Applied Genetics. **106:** 559-567.

Joshi, S.P., Bhave, S.G., Chowdari, K.V., Apte, G.S. Dhonukshe, B.L., Lalitha, K., Ranjekar, P.K., and Gupta, V.S. 2001. Use of DNA markers in prediction of hybrid performance and heterosis for a three-line hybrid system in rice. Biochemical Genetics. **39:** 179-200.

Keeble, F. and Pellew, C. 1910. The mode of inheritance of stature and of time of flowering in peas (Pisum sativum). Genetics. **1**: 47-56.

Lanza, L.L., Souza, C.L., Ottoboni, L.M., Vieira, M.L., Souza, A.p. 1997. Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. Theoritical Applied Genetics. **94:** 1023-1030.

Li, G. and Quiros, C.F. 2001. Sequence –related Amplified Polymorphism (SRAP) a new marker system based on simple, PCR reaction: its application to mapping and gene tagging in Brassica. Theoretical Applied Genetics. 103: 455-461.

Liu, Z.Q., Pei, Y. and Pu, Z.J. 1999. Relationship between hybrid performance and genetic diversity based on RAPD markers in wheat, *Triticum aestivum* L. Plant Breeding. **118:** 119-123.

Liu, X.C. and Wu, J.L. 1998. SSR heterogenic patterns of parents for making and predicting heterosis in rice breeding. Molecular Breeding. **4:** 263-268.

Lombard, V., Baril, C.P., Dubreuil, P., Blouet, F. and Zhang D. 2000. Genetic Relationships and Fingerprinting of Rapeseed Cultivars by AFLP: Consequences for Varietal Registration. Crop Science. **40**: 1417-1425.

Lubberstedt, T., Melchinger, A.E., Duble, C., Vuylsteke, M., and Kuiper, M. 2000. Relationships among early European maize inbreds: IV. Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. Crop Science. **40:** 783-791.

Melchiniger, A.E. 1999. Genetic Diversity and Heterosis. In: The genetics and exploitation of heterosis in crops. American Society of Agronomy, Crop Science Society of America. USA. Pp 99-118.

McCubbin, A, and Dickinson, H.G. (1997). Self-Incompatibility. In: Shivanna, K.R., and Sawhney, V.K. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press. Pp. 199-218.

McVetty, P.B.E. (1997). Cytoplasmic male sterility. In: Shivanna, K.R., and Sawhney, V.K. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press. Pp. 155-183.

Nei, M.L. and Li, W.H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl .Acad. Sci.USA. **10:** 5269 – 5273.

Perumal, R., Krishnaramanujam, R., Menz, M.A., Katile, S., Dahlberg, J., Magill, C.W., and Rooney, W.L. 2007. Genetic diversity among Sorghum Races and Working Groups Based on AFLPs and SSRs. Crop Science. **47:** 1375-1383.

Poehlman, J.M., and Sleper, D.A. 2006. Breeding Field Crops 5th Edition. Blackwell Publishing, Iowa, USA. Pg 171-215.

Prakash, S. 1980. Cruciferous oilseeds in India, p.151-163. In: S. Tsunoda,K. Hinata, and C. Gomez-Campo (eds). Brassica crops and wild allies. Biology and Breeding. Japan Scient. Soc. Press, Tokyo.

Reif, J.C., Xia, X.C., Melchinger, A.E., Warburton, M.L., Hoisington, D.A., Beck, D., Bohn, M., and Frisch, M. 2004. Genetic diversity determined within and among CIMMYT Maize Populations of Tropical, Subtropical and Temperate Germplasm by SSR Markers. Crop Science. **44:** 326 – 334.

Riaz, A., Li,G., Quresh, Z., Swati, M., and Quiros, C.F. 2001. Genetic diversity of oilseed *Brassica napus* inbred lines based on sequence-related amplified polymorphism and its relation to hybrid performance. Plant Breeding. **120**: 411-415.

SAS. 2001. SS Institute Inc. 2001 SAS User's Guide: Statistics. Version 8.1. SAS Institute. Cary NC. USA.

Saunders, G.C. and Parkes, H.C. 1999. Analytical Molecular Biology: Quality and Validation. Royal Society of Chemistry. Great Britain. Pg. 29-47.

Sawhney, V.K. 1997. Genic male sterility. In: Shivanna, K.R., and Sawhney, V.K. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press. Pp. 183-198.

Semagn, K., Bjornstad, A., and Ndjiondjop, M.N. 2006. Review: An overview of molecular marker methods for Plants. African Journal of Biotechnology. **5:** 2540-2568.

Seyis, F., Snowdon, R.J. Luhs, W., and Friedt, W. 2003. Molecular characterization of novel resynthesised rapeseed (*Brassica napus*) lines and analysis of their genetic diversity in comparison with spring rapeseed cultivars. Plant Breeding. **122**: 473-478.

Simmonds N.W.1981. Genetic Aspects: Populations and Selection, in, Principles of Crop Improvement, 1st ed., Longman Group, New York. pp. 66-121.

Singleton, R. 1941. Hybrid Vigor and its Utilization in Sweet Corn Breeding. The American Naturalist. **75**: 48-60.

Shull, G. H., 1908. The composition of a field of maize. Am. Breeders Assoc. Rep. **4**: 296-301.

Shull, G. H., 1909. A pure line method of corn breeding. Am. Breeders Assoc. Rep. **5**: 51-59.

Shull, G.H. 1911. The Genotypes of Maize. The American Naturalist. **45**: 234-252.

Shull, G. H., 1948. What is "heterosis"? Genetics **33**: 439-446

Shull, G.H. 1952. Heterosis: A record of researches directed toward explaining and utilizing the vigor of hybrids. Iowa State College Press Ames. Iowa. Pg. 14-48.

Snowdon, R.J., and Friedt, W. 2004. Review: Molecular markers in Brassica oilseed breeding: current and future possibilities. Plant Breeding. **123:** 1-8.

Snowdon, R., Luhs, W., and Friedt, W. 2007. Oilseed rape. In: Chittaranjan, K. Genome Mapping and Molecular Breeding in Plants, Volume 2 : Oilseed Rape. Springer. Berlin. Pp55-114.

Statistics Canada. 2007. Canadian Statistics – Field and specialty crops. http://www40.statcan.ca/l01/cst01/prim11a.htm?sdi=field%20specialty%20crops

Stefansson, B.R. and Kondra, Z.P. 1975. Tower summer rape. Can. J.PlantSci. **55:** 343-344.

Thormann, C.E., Ferreira, M.E., Camargo, L.E.A., Tivang, J.G. and Osborn, T.C. 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theoretical Applied Genetics. **88:** 973-980.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., vandeLee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP. A new technique for DNA fingerprinting. Nucleic Acid Research. **23:** 4407 – 4414.

U, N. 1935. Genome analysis in the *Brassicacea* with special reference to the experimental formation of *Brassica napus*. Jpn. J.BOt. 7: 389-452.

Williams, M.E., Leemans, J, Michiels, F. (1997). Male sterility through recombinant DNA technology. In: Shivanna, K.R., and Sawhney, V.K. Pollen Biotechnology for Crop Production and Improvement. Cambridge University Press. Pp. 155-183.

Xiao, J., Li, J., Yuan, L., McCouch, S.R., and Tanksley, S.D. 1996. Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR-based markers. Theoretical Applied Genetics. **92:** 637-643.

Xu, S., Liu, J., and Liu, G. 2004. The use of SSRs for predicting the hybrid yield and yield heterosis in 15 key inbred lines of Chinese maize. Hereditas. **141:** 207-215.

Yan, Z. 1990. Overview of rapeseed production and research in China. Proceedings of International Canola Conference. April 1990. Atlanta, GA, U.S.A. pp.29-35.

Yu C.Y., Hu, S.W. Zhao, H.X., and Guo, A.G. 2005. Genetic distances revealed by morphological characters, isozymes, proteins and RAPD markers and their relationship with hybrid performance in oilseed rape (*Brassica napus L.*) Theoretical Applied Genetic. **110:** 511-518.

Zhao, M.F., Li, X.H., Yang, J.B., Xu, C.G., HU, R.Y., Liu, D.J., and Zhang, Q. 1999. Relationship between molecular marker heterozygosity and hybrid performance in intra and inter- subspecific crosses of rice. Plant Breeding. **118:** 139 -144.

Zhang, X.Q., Wang, X.D., Jiang, P.D., Hua, S.J. Zhang, H.P. and Dutt, Y. 2007. Relationship between molecular marker heterozygosity and hybrid performance in intra- and interspecific hybrids in cotton. Plant Breeding. **126:** 385 -391.

Appendix

Appendix Table A: Hybrid HEAR topcross design (Cuthbert 2006)

Parent	Castor	MilleniUM01	MilleniUM03	HR200	HR102	RRHR102	HR199	EU HEAR 1	EU HEAR 2	EU HEAR 3	EUHEAR4	EUHEAR 5
Castor				X	X			X	X	X		X
MilleniUM01					X			X	X	X	X	X
MilleniUM03				X	X			X	X	X	X	X
HR200					X	X	X	X	X	X	X	X
HR102						X	X	X	X	X	X	X
RRHR102							X	X	X	X	X	X
HR199								X	X	X	X	
EUHEAR 1												
EUHEAR 2												X
EUHEAR 3												
EUHEAR 4												
EUHEAR 5												

Appendix Table A1: Nei's Genetic Similarity Matrix based upon 200 polymorphic markers for 12 HEAR cultivars / lines

	EU HEAR 5	HR200	CASTOR	EU HEAR 3	EU HEAR 2	EU HEAR 1	HR102	RRHR102	HR199	MILL03	MILL01	EU HEAR 4
EU HEAR 5	0.000	0.083	0.084	0.070	0.096	0.079	0.132	0.102	0.065	0.080	0.081	0.066
HR200	0.083	0.000	0.046	0.186	0.141	0.171	0.067	0.055	0.045	0.036	0.052	0.049
CASTOR	0.083	0.046	0.000	0.181	0.163	0.182	0.098	0.058	0.054	0.045	0.046	0.045
EU HEAR 3	0.070	0.186	0.181	0.000	0.057	0.023	0.214	0.173	0.150	0.166	0.156	0.151
EU HEAR 2	0.095	0.141	0.163	0.057	0.000	0.060	0.161	0.150	0.147	0.150	0.130	0.131
EU HEAR1	0.079	0.171	0.182	0.023	0.060	0.000	0.201	0.168	0.150	0.161	0.156	0.155
HR102	0.159	0.067	0.098	0.214	0.161	0.201	0.000	0.085	0.109	0.064	0.085	0.085
RRHR102	0.101	0.056	0.059	0.173	0.149	0.167	0.086	0.000	0.064	0.064	0.075	0.071
HR199	0.064	0.046	0.053	0.150	0.147	0.150	0.111	0.064	0.000	0.041	0.055	0.039
MILL03	0.078	0.035	0.046	0.164	0.148	0.159	0.064	0.062	0.039	0.000	0.046	0.051
MILL01	0.081	0.052	0.046	0.156	0.130	0.156	0.085	0.075	0.054	0.045	0.000	0.054
EU HEAR 4	0.065	0.048	0.045	0.151	0.131	0.155	0.085	0.071	0.038	0.051	0.054	0.000

Appendix Table A2: Nei's Genetic Similarity Matrix based upon 400 polymorphic markers for 12 HEAR cultivars / lines

	EU HEAR 5	HR200	CASTOR	EU HEAR 3	EU HEAR 2	EU HEAR 1	HR102	RRHR102	HR199	MILL03	MILL01	EU HEAR 4
EU HEAR 5	0.000	0.083	0.095	0.076	0.104	0.079	0.131	0.095	0.066	0.079	0.078	0.067
HR200	0.081	0.000	0.064	0.184	0.150	0.169	0.074	0.060	0.048	0.045	0.049	0.041
CASTOR	0.094	0.064	0.000	0.205	0.170	0.201	0.089	0.068	0.062	0.055	0.056	0.055
EU HEAR 3	0.075	0.187	0.206	0.000	0.063	0.017	0.207	0.177	0.162	0.167	0.156	0.173
EU HEAR 2	0.104	0.152	0.170	0.063	0.000	0.066	0.170	0.149	0.157	0.154	0.135	0.143
EU HEAR 1	0.078	0.171	0.201	0.017	0.066	0.000	0.193	0.177	0.157	0.161	0.151	0.170
HR102	0.132	0.074	0.089	0.207	0.170	0.193	0.000	0.070	0.091	0.064	0.078	0.074
RRHR102	0.095	0.061	0.069	0.177	0.150	0.176	0.070	0.000	0.062	0.065	0.069	0.060
HR199	0.065	0.047	0.062	0.162	0.156	0.156	0.092	0.062	0.000	0.049	0.061	0.036
MILL03	0.078	0.044	0.056	0.166	0.154	0.158	0.064	0.065	0.048	0.000	0.043	0.050
MILL01	0.077	0.050	0.056	0.155	0.135	0.150	0.078	0.069	0.061	0.041	0.000	0.050
EU HEAR 4	0.067	0.041	0.055	0.174	0.143	0.171	0.075	0.060	0.036	0.050	0.050	0.000

Appendix Table A3: Nei's Genetic Similarity Matrix based upon 600 polymorphic markers for 12 HEAR cultivars / lines

	EU HEAR 5	HR200	CASTOR	EU HEAR 3	EU HEAR 2	EU HEAR 1	HR102	RRHR102	HR199	MILL03	MILL01	EU HEAR 4
EU HEAR 5	0.000	0.079	0.098	0.074	0.104	0.080	0.118	0.090	0.059	0.076	0.076	0.062
HR200	0.077	0.000	0.064	0.162	0.135	0.151	0.060	0.053	0.046	0.042	0.047	0.041
CASTOR	0.098	0.063	0.000	0.191	0.156	0.191	0.082	0.068	0.067	0.060	0.063	0.055
EU HEAR 3	0.073	0.164	0.192	0.000	0.060	0.021	0.176	0.156	0.147	0.145	0.146	0.153
EUHEAR 2	0.104	0.135	0.155	0.060	0.000	0.063	0.143	0.136	0.137	0.134	0.124	0.131
EU HEAR 1	0.079	0.153	0.191	0.021	0.063	0.000	0.168	0.155	0.143	0.140	0.145	0.154
HR102	0.118	0.060	0.082	0.176	0.144	0.168	0.000	0.063	0.083	0.056	0.072	0.069
RRHR102	0.091	0.055	0.068	0.156	0.135	0.155	0.063	0.000	0.057	0.058	0.066	0.055
HR199	0.059	0.046	0.067	0.147	0.137	0.143	0.083	0.057	0.000	0.050	0.058	0.036
MILL03	0.076	0.042	0.059	0.147	0.134	0.141	0.056	0.058	0.051	0.000	0.041	0.047
MILL01	0.076	0.048	0.064	0.147	0.124	0.147	0.073	0.067	0.059	0.043	0.000	0.043
EU HEAR 4	0.061	0.041	0.056	0.153	0.131	0.153	0.068	0.054	0.036	0.047	0.043	0.000

Appendix Table A4: Nei's Genetic Similarity Matrix based upon 786 polymorphic markers for 12 HEAR cultivars / lines

	EU HEAR 5	HR200	CASTOR	EU HEAR 3	EU HEAR 2	EU HEAR 1	HR102	RRHR102	HR199	MILL03	MILL01	EU HEAR 4
EU HEAR 5	0.000	0.082	0.103	0.073	0.104	0.079	0.112	0.091	0.063	0.078	0.077	0.065
HR200	0.079	0.000	0.062	0.165	0.134	0.156	0.057	0.050	0.044	0.041	0.047	0.040
CASTOR	0.102	0.061	0.000	0.195	0.153	0.197	0.078	0.066	0.064	0.060	0.061	0.055
EU HEAR 3	0.074	0.165	0.195	0.000	0.058	0.019	0.174	0.160	0.153	0.150	0.147	0.160
EU HEAR 2	0.105	0.134	0.153	0.058	0.000	0.062	0.136	0.136	0.140	0.131	0.120	0.134
EUHEAR 1	0.079	0.158	0.197	0.019	0.062	0.000	0.170	0.159	0.151	0.143	0.147	0.162
HR102	0.112	0.057	0.078	0.175	0.136	0.170	0.000	0.064	0.078	0.051	0.065	0.067
RRHR102	0.090	0.051	0.067	0.159	0.135	0.158	0.063	0.000	0.056	0.057	0.064	0.055
HR199	0.062	0.044	0.063	0.153	0.140	0.151	0.079	0.057	0.000	0.050	0.055	0.036
MILL03	0.077	0.041	0.060	0.149	0.131	0.141	0.051	0.057	0.049	0.000	0.040	0.047
MILL01	0.077	0.047	0.060	0.146	0.120	0.146	0.064	0.064	0.054	0.039	0.000	0.041
EU HEAR 4	0.065	0.040	0.054	0.161	0.134	0.162	0.067	0.055	0.036	0.047	0.041	0.000