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GENETIC VARIANCE, HETEROSIS AND COMBINING ABILITY IN OILSEED RAPE

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James E. Brandle

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ABSTRACT

Reports of substantial heterosis in inter-cultivar hybrids of oilseed rape have led to the development of a large plant breeding effort directed towards to the creation of hybrid oilseed rape cultivars. Early research had assumed that the cultivars that had been used to demonstrate heterosis were genetically homogenous. The effects of genetic variability within cultivars on the expression of heterosis were not considered. The purpose of the work presented herein was to measure levels of genetic variability and to lay some of the basic genetic foundations, necessary for the development of hybrid oilseed rape breeding programs.

It was clearly established that the oilseed rape cultivars used in this study contained significant levels of genetic variability and that these same cultivars were not greatly affected by inbreeding. The presence of significant additive genetic variation implied that selection within cultivars may be effective, and that some short term progress can be made, simply by reselecting within existing cultivars. A corollary of this conclusion is that intra-cultivar genetic variation should not be ignored in future oilseed rape breeding research. The low levels of inbreeding depression suggested that dominance was not a major factor in the genetic determination of the characters investigated. Inbred lines were found that performed as well as, or better than, their source cultivars, illustrating that genetic heterogeneity is not essential to the performance of oilseed rape cultivars.

Intra-cultivar genetic variability was found to have wider implications in terms of hybrid development. Inbred lines, extracted from the parents of heterotic cultivar crosses, were found to vary in terms of their performance in hybrid combination. While most crosses between these inbred lines did exhibit heterosis, some were found to have significantly higher yields and others significantly lower yields than their correspsonding

cultivar hybrids. Given this fact, it follows that hybrid oilseed rape breeding programs should be based on pure line crosses, rather than cultivar crosses.

A diallel cross among seven canola quality inbred lines of diverse origin showed significant heterosis for yield and oil concentration, but not for aliphatic glucosinolate content. Substantial heterosis for yield actually led to the dilution of, and a consequent reduction in levels of, the four alphatic glucosinolates in the seed meal of single cross hybrids. The results of the diallel also indicated that glucosinolate content was mainly controlled by additive gene action. The net result is that heterosis should not have any negative impact on glucosinolates levels in single cross hybrids from canola quality inbreds.

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FOREWORD

This thesis was written using a manuscript format. The first manuscript has been accepted for publication in Genome, the second has been accepted for publication in Crop Science, and the third has been submitted to Zeitschrift für Pflanzenzüchtung.

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INTRODUCTION

Canadian farmers began growing summer oilseed rape (Brassica napus L.) during during World War II and the first licensed cultivar, 'Golden', was released in 1954 (Stefansson 1983). Soon after the release of Golden, plant breeders turned their efforts toward improving the nutritional quality of the oil and meal. In 1974 the first Canadian low euricic acid, low glucosinolate cultivar was released (Stefansson and Kondra 1975). The trademark name, canola, was adopted by the Canola Council of Canada to distinguish the the new, improved quality, oilseed rape from the old oilseed rape. Since that time oilseed rape has gained wide acceptance both here in Canada and in the United States (Gillis 1988), mainly because of the superior nutrional quality of the oil. In 1988, 3,651,800 hectares of were seeded to both B. napus and B. campestris with a total production of 4,218,000 tonnes, making Canada the worlds second largest producer of rapeseed.[1]

Early reports of substantial heterosis (Schuster and Michael 1976, Shiga 1976) coupled with the availability of cytoplasmic male sterility stimulated a large plant breeding effort, during the 1980's, directed towards the development of hybrid oilseed rape cultivars. The exploitation of heterosis has proved very successful in maize (Sprauge 1983) leading to very large yield increases, and it is hoped that similar success may be realized in oilseed rape.

Initial research efforts involving oilseed rape hybrids were mainly concerned with reporting heterosis and developing a functional cytoplasmic male sterility system. The breeding methodology used for hybrids in other crops had not been tested in oilseed rape and the genetic structure of oilseed rape cultivars, in general, had not been investigated. The research presented in the following pages was aimed specifically at oilseed rape, and its purpose was firstly, to fully characterize intra-cultivar variability in genetic terms, and

[1] Canadian Grains Industry, Statistical Handbook 1988

secondly to lay some of the basic foundations necessary for the development of hybrid summer-oilseed-rape breeding programs.

LITERATURE REVIEW

Reproductive Biology of B. napus

Oilseed rape (Brassica napus spp. oleifera L.) is a partially self-pollinated species. Williams et al. (1986) found that oilseed rape plants yield equally well whether self or cross pollinated and concluded that oilseed rape is completely self compatible. Rakow and Woods (1987), using erucic acid content of the seed oil as a genetic marker, found that oilseed rape is approximately 77 % self-pollinated under field conditions in western Canada. When cross pollination occurs, pollen may be dispersed by wind (Williams 1984) or insect activity (Eisikowitch 1981). Although the relative importance of the two mechanisms remains to be clarified, it appears that adequate pollination can occur without the presence of insect vectors (Free and Nutall 1968, Langridge and Goodman 1982).

Differential rates of outcrossing among individual plants necessarily leads to the development of an extensive genotypic array within a given cultivar. Therefore, each individual plant in a population could represent almost any level of inbreeding, ranging from S_0 to S_∞. Gowers (1981) illustrated that partially self-pollinated swede cultivars (Brassica napus spp. rapifera) are differentiated into a number of subgroups, each of which may be at any level of inbreeding.

Heterosis

Shull was the first to correctly interpret hybrid vigor, and later coined the term heterosis to describe the phenomena (Shull 1948). Shull (1948) defined heterosis as the "increase in size or other valuable qualities in the crossbred as compared to the pure biotypes". The genetic basis of heterosis has been studied for over eighty years, but still remains controversial. There are two main nuclear genetic explanations of heterosis, the overdominance and dominance hypotheses.

The first of these two theories of heterosis was first presented by Shull in 1908 and was designated, at that time, as the physiologic stimulation hypothesis (Shull 1948). It was based on the idea that heterozygosity per se was the cause of heterosis. East (1936) further clarified the hypothesis by proposing that the different alleles at a locus were differentiated with respect to their physiologic functions, and that heterozygotes because of this wider range of function, were more physiologically efficient. The concept was closely related to the idea of overdominance proposed by Hull (1945), who felt that heterosis resulted from non-linear interactions between alleles at a locus.

The dominance hypothesis was initially put forward by Keeble and Pellew (1910), who proposed that heterosis for height in peas (Pisum sativum) was due to complementary effects of dominant alleles at two loci. Bruce (1910) presented an algebraic argument, based on a reduction in the number of deleterious recessive alleles in the hybrid, when compared to the parents. Jones (1917) extended the hypothesis to include linkage.

In 1913 Emerson and East (cited in Jones 1917) criticized the dominance hypothesis on the basis of the absence of a skewed distribution in F_2 populations, derived from heterotic F_1's. If dominance were the basis of heterosis, a skewed distribution should result from the expansion of the binomial $(3 + 1)^n$. Collins (1921) showed that skewness due to dominance is not great, if a large number of loci are involved in the expression of the character in question. Jones (1917) cited the inability to obtain inbred lines as productive as single cross hybrids as a major objection to the dominance hypothesis. Bailey and Comstock (1976) illustrated that, when a large number of gene pairs are considered, the probability of finding an F_2 individual with the dominant allele fixed at every locus is extremely small. This low probability would account for the apparent absence of superior inbred lines in the segregating generations of heterotic crosses. Crow (1948) objected to the dominance hypothesis on the grounds that it did not account for heterosis observed in crosses between inbred lines from an equilibrium population. Crow (1948) calculated that 5 % was the maximum possible heterosis under

the dominance hypothesis. Since heterosis often exceeds 5 %, Crow concluded that overdominance must correctly explain hybrid vigor. Hallauer and Miranda (1981) have indicated that maize (Zea mays) hybrids are not often derived from crosses between inbreds from the same population, but instead from crosses between inbreds from two different populations, a fact that invalidates one of Crow's major assumptions. Sprauge (1983) has made the same point. Hull (1945) reasoned that because the yield of hybrids often exceeds the sum of the yields of their inbred parents, overdominance must be the basis of heterosis. Sprauge (1983) has criticized the techniques used for such comparisons and proposed that the use of equivalent leaf areas for both hybrids and their inbred parents is essential, if Hull's (1945) hypothesis is to be properly tested.

Jinks (1981) applied the methodology of biometrical genetics to Nicotiana rustica crosses and concluded that true overdominance is not the cause of heterosis is this species. Jinks (1981) extended his conclusions to include heterosis in maize and barley (Hordeum vulgare) and further indicated that linkage disequilbria and epistasis are the most likely causes of spurious estimates of overdominance. Sprauge (1983) in an extensive review of heterosis in maize, concluded that additive and dominance effects provide an adequate model for heterosis in maize and added that overdominance and epistasis are not important.

The third explanation of heterosis is a byproduct of organelle complementation studies (i.e. McDaniel and Sarkissian 1966) and is based upon interactions between the nuclear and cytoplasmic genomes (Srivastava, 1983). The exact nature of intergenomic interaction and organelle complementation has not been fully interpreted in genetic terms. Srivastava (1983) hypothesized that some important aspects of cellular metabolism, are co-ordinately regulated by more than one genome. Positive interaction between these genomes leads to the elevation of rate limiting steps in many biochemical reactions and results in enhanced vigor. Srivastava (1983) felt that heterosis was associated with higher efficiency of mitochondria and chloroplasts, and that this resulted

from complementation between polymorphic organelles, which may have been transmitted bi-parentally.

Falconer (1981) explained that heterosis in crosses between inbred lines will be expressed only when the following two conditions exist: 1) directional dominance, and 2) relative differences in gene frequency between the two parents. If either or both conditions are not met, then there will be no expression of heterosis. Because gene differences between pairs of inbred lines are unique, Falconer (1981) concluded that the amount of heterosis is specific to a given cross. Furthermore, when each of the two alleles at a locus are fixed as in the inbred parents, differences in gene frequencies are largest and heterosis is maximized.

Early reports of heterosis in oilseed rape inter-cultivar hybrids (Schuster and Michael 1976, Shiga 1976), coupled with the availability of cytoplasmic male sterility (Shiga 1980), stimulated much research into the development of hybrid oilseed rape cultivars. Recent work (Sernyk and Stefansson 1983, Grant and Beversdorf 1985) has demonstrated high (30-60% of mid-parent) levels of heterosis for seed yield in inter-cultivar oilseed rape hybrids. Neither Sernyk and Stefansson (1983) nor Grant and Beversdorf (1985) found any heterosis for oil or protein concentration. Combining ability studies done with cultivar crosses showed significant general (GCA) and specific (SCA) combining ability for seed yield, and oil and protein concentration, but found the importance of SCA to be inconsistent across environments (Grant and Beversdorf 1985). Combining ability studies done with inbred line hybrids have shown significant GCA (Lefort-Buson et al. 1986) and significant SCA (Lefort-Buson et al. 1987). In general, the highest levels of heterosis were found in crosses between geographically diverse parents (Sernyk and Stefansson 1983, Grant and Beversdorf 1985). This is consistent with observations of heterosis in maize cultivar hybrids (Moll et al. 1962).

In an attempt to determine the nature of this heterosis, Lefort-Buson et al. (1987) studied seed yield in crosses between lines of similar and diverse origins. Crosses between lines with similar origins produced low levels of heterosis and a preponderance

of GCA, which the authors interpreted to be the result of additive genetic effects. Diverse crosses resulted in higher levels of heterosis, than crosses between lines of similar origin, and both GCA and SCA were significant. The presence of significant SCA was attributed to the induction of new dominance and epistatic relationships among genes in the hybrids.

Hybrid vigor observed in cultivar derived hybrids represents an average expression of heterosis. The genotypes formed upon crossing are a consequence of the sample of the different individuals taken from each parental cultivar (Hallauer and Miranda, 1981). Early work by Shull in 1910 (Shull, 1952) illustrated that F_1 plants, from cultivar crosses of maize, varied in relative heterosis, some being considerably more heterotic than others. Hybrids produced from cultivar crosses are not strictly repeatable, since their performance is unique to the sample of genotypes used to make the cross. As a consequence, hybrid breeding normally uses pure lines developed through inbreeding, followed by selection among those lines for parental inbreds that exhibit maximum heterosis when crossed (Allard, 1960).

Inbreeding Depression

It was recognized as early as 1908 (Shull, 1952) that inbreeding in maize (Zea mays L.) reduces genetically heterogeneous populations to reproducible pure lines, similar to those described by Johannsen (1903). It was also recognized that continued inbreeding leads to a notable reduction in productivity and vigor of the pure lines, when compared to the original outbreeding population (Shull 1952). The early history of the impact of inbreeding on plant and animal species has been reviewed by Zirkle (1952). Charles Darwin (cited in Zirkle 1952) recognized that among plant species "cross fertilization is generally beneficial and self fertilization injurious". This loss in productivity and vigor upon self-pollination is known as inbreeding depression.

In strictly Mendelian terms, inbreeding leads to an increase in homozygosity and a decrease in heterozygosity. Therefore, inbreeding depression must be due to a difference between the genotypic value of heterozygotes and homozygotes (Falconer 1981). Falconer (1981) has illustrated that a single locus, in a population with an inbreeding coefficient of F, has a mean genotypic value (M_F) of:

$$M_F = M_0 - 2dpqF$$

where M_0 is the mean value of the non-inbred population. The term 2dpqF represents the change in the population mean due to inbreeding, and illustrates that a locus can contribute to changes in mean value only if the dominance deviation (d) is not zero. Therefore inbreeding depression, like its opposite heterosis, depends on the existence of dominance. Furthermore, the magnitude of inbreeding depression is dependent on gene frequencies, so that genes at intermediate frequencies (i.e. p=q=0.5) contribute more to changes in mean value than do genes at high or low frequencies. If a given allele is fixed (i.e. p=1, q=0), there will be no changes in the population mean due to inbreeding. When considering the combined effect of all loci involved in the expression of a character, the population mean is given by the summation of the contributions of the separate loci ($-2F\Sigma pqd$). If a character that is controlled by more than one gene is to show a change in mean value upon inbreeding, dominance must be directional, in order to exclude the possibility of cancellation of effects.

It is important to distinguish the inbreeding depression and heterosis found in populations from that observed in crosses between inbred lines. When a population is inbred at random, the levels of inbreeding depression will be as defined above. When the inbred lines are recrossed, again at random, F returns to zero and the population mean returns to its original value (M_0). The amount of inbreeding depression observed upon selfing will equal the amount of heterosis observed upon recrossing.

When two inbred lines are crossed, the amount of heterosis observed in the F_1 will be in accordance with differences in gene frequency and levels of dominance. Inbreeding depression following random mating in the the F_1 hybrid is equal to one-half the heterosis observed in the original cross. If random mating is maintained in the subsequent generations, then equilibrium will have been reached, and yield levels will stabilize. Conversely, continued selfing of that same hybrid, will reduce yield levels back to those found in the original parents.

Hallauer and Sears (1973) studied inbreeding depression in maize, which was measured as the difference between the mean of a sample of inbred lines and their open pollinated parents. The authors found that inbreeding led to a reduction in size, vigor, yield, and to later flowering. They also observed that a linear regression of yield versus percentage homozygosity accounted for most (92 %) of the variation among the successively inbred generations. The strong linear relationship between the generation means and the percentage homozygosity suggests that a genetic model, based on the cumulative effects of loci with dominance, properly describes inbreeding depression for yield in maize. Since inbreeding exposed recessive alleles, Hallauer and Sears (1973) felt that inbreeding depression was the result of an increase in the frequency of homozygous recessive deleterious loci. Good and Hallauer (1977) concluded from their studies of inbreeding in maize that some level of dominance is required for the expression of inbreeding depression. Their results also showed that genes for larger plant size and high yield were dominant to those for small plant size and low yield, and that early flowering was dominant to late. Epistasis was found to play only a relatively minor role in the inbreeding depression observed by Good and Hallauer (1977), especially when compared to additivity among loci.

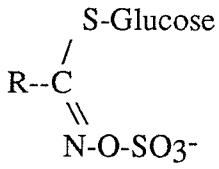
Schuster and Michael (1976) reported inbreeding depression levels in winter oilseed rape that paralleled those found in maize and sunflower. They also indicated that inbred lines could be found that were equal to, or better than, their open pollinated parents. Gowers and Gemmell (1988) also found inbred lines of swede that were

superior to their parental cultivars. In Schuster and Michaels' (1976) experiment S_8 yield, height and oil % were found to be 45%, 94% and 91% of the S_0. Sample size in the successive generations ranged from 1549 in S_0 to 17 in S_8, indicating that their results could be very biased. Meng and Liu (1986) found that inbreeding in winter oilseed rape had a deleterious effect on the early stages of embryo development, resulting in small seeds with low oil content.

Glucosinolates

Oilseed rape meal contains 37 to 38 % protein and has an amino acid profile that compares well with soybean (Glycine max) meal (Clandinin et al. 1986). The use of oilseed rape meal in animal rations had previously been limited, due to excessive levels of glucosinolates (Bowland et al. 1965). Enzymatic degradation of the glucosinolates found in oilseed rape meal leads to the formation of compounds that are both toxic and goitrogeneic. These degradation products cause reduced palatability of the feed and also have a negative impact on animal health. The discovery of the Polish low glucosinolate oilseed rape cultivar 'Bronowski' in 1967, led to the development of the cultivar 'Tower' which had only 10 % of the glucosinolate content found in normal cultivars (Stefansson and Kondra 1975). Present levels of glucosinolates in oilseed rape meal are not considered to have any practical impact on livestock or poultry ration formulation (Clandinin et al. 1986); however, efforts to remove all traces glucosinolates are still underway (Love 1988).

The structural formulae of the R groups of the four major aliphatic glucosinolates found in the seed of oilseed rape are summarized in Table 2.1. Four indole glucosinolates are also present in the meal (Sang et al. 1984), but until the recent development of High Performance Liquid Chromatography techniques (Michinton et al. 1982), the four could not be quantified with sufficient accuracy to allow their manipulation by plant breeders. The basic glucosinolate molecule appears as follows:



Where R represents the side chains that differentiate the various glucosinolates. Underhill (1980) in an extensive review of the plant biochemical aspects of glucosinolates, indicated that all are derived from amino acids and that biosynthesis occurs along a common pathway. Labelling studies have shown that the four major aliphatic glucosinolates found in oilseed rape are derived from the amino acid methionine and repeated chain elongation with the methyl carbon of acetate. The specific enzymes involved in the biosynthetic pathway have not been fully characterized.

Glucosinolates themselves are generally considered non-toxic, however when they are accompanied by the enzyme myrosinase (thioglucoside glucohydrolase), a number of physiologically active compounds result from their enzymatic cleavage. Myrosinase, released when plant tissues are crushed, induces the detachment of glucose to yield a labile aglucone, which upon loss of the sulphate ion spontaneously rearranges to form isothiocyanate (Underhill 1980). This compound can lower the iodine content of the thyroid and lead to goiter formation in livestock or poultry (VanEtten et al. 1969). Supplemental iodine, included in the ration can alleviate this problem. If a hydroxyl group is present on the 2-carbon atom of the side chain, as in 2-hydroxy-3-butenyl and 2-hydroxy-4-pentenyl glucosinolate, then isothiocyanate may further cyclisize to form oxazolidine-2-thione (VanEtten et al. 1969). This compound is also a strong antithyroid agent which acts by inhibiting the organic binding of iodine in the thyroid, its effects cannot be reversed by iodine supplemenation. Conditioning of the meal immediately after the seed has been crushed, denatures the myrosinase, thereby limiting the hydrolysis of any glucosinolates that may be present (Pickard et al. 1986).

Even though most oilseed rape breeders are engaged in the production of low glucosinolate cultivars, the genetic basis of the reduced glucosinolate levels that were found in the cultivar Bronowski are not fully understood. There is evidence that glucosinolate content is controlled maternally and that cytoplasmic factors may be involved (Kondra and Stefansson 1970, Anand 1978). Love (1988) found cytoplasmic effects to be of an extremely minor nature. Kondra and Stefansson (1970) proposed that 3 to 5 dominant or partially dominant loci controlled levels of 3-butenyl, 2-hydroxy-3-butenyl and 4-pentenyl glucosinolate, in crosses between Bronowski and the high glucosinolate cultivar Target. Lein (1970) studied total glucosinolate content and found that high content was dominant to low, but did not indicate the numbers of loci that may have been involved. Zhou and Liu (1987) studied total glucosinolate content and found high levels to be controlled by dominant genes at three loci. Josefsson (1973) conducted a precursor feeding study of the metabolic block established by genes from the cultivar Bronowski and concluded that there was one block early in the synthesis of 3-butenyl glucosinolate and another block at the hydroxylation step of the synthesis of the 2-hydroxy aliphatic glucosinolates. The second block, at the hydroxylation step, has been questioned by Finlayson et al. (1973) and current thinking (Love 1988) favors a single block in the early stages of synthesis.

A comprehensive study done by Love (1988) has confirmed that total glucosinolate content in normal oilseed rape cultivars is controlled by partially dominant alleles at three loci. The "Bronowski block" was found to consists of three homozygous recessive loci, which limit the accumulation of the four aliphatic glucosinolates. Each locus decreased total glucosinolate content by approximately 40 μmol g^{-1} meal and when all three loci are homozygous recessive, total content in the seed is reduced to less than 20 μmol g^{-1} meal. Love (1988) also introduced the concept of product precursor conversion efficiency ratios as a more reliable means of studying the inheritance of certain steps in the biosynthetic pathway of aliphatic glucosinolates. These ratios are apparently free of environmental influence and therefore allow the accurate classification

of genotpyes. Investigation of the conversion of the four to five carbon precursor, in crosses between the high 2-hydroxy-3-butenyl glucosinolate cultivar 'York' and two low glucosinolate cultivars 'Westar' and 'Egra', indicated that this step was controlled by a single dominant gene (EL3). Westar and Egra were homozygous dominant for the EL3 gene and York was homozygous recessive, explaining the predominance of four carbon glucosinolates in this cultivar. The hydroxylation of 3-butenyl glucosinolate was controlled by a single dominant gene at conversion efficiencies between 70 and 95 %. The hydroxylation efficiency of 4-pentenyl glucosinolate exhibited a continuous distribution in the F_2 and therefore the number of loci controlling this trait was not be established.

B. napus is an amphidiploid, consisting of the A genome of B. campestris and the C genome of B. oleracea (U 1935). Röbbelen and Thies (1980) have summarized the the major glucosinolates found in the A and C genome species. B. oleracea contains significant amounts of 3-butenyl and 2-hydroxy-3-butenyl glucosinolate, and high levels of 2-propenyl glucosinolate. B. campestris contains very high levels of 3-butenyl glucosinolate and low levels of the other three aliphatic glucosinolates. Oilseed rape contains almost no 2-propenyl glucosinolate, indicating that interaction between the A and C genomes must suppress the synthesis of this compound. Levels of the remaining four aliphatic glucosinolates found in oilseed rape appear to be in accordance with those found in their respective source genomes.

Table 2.1. The R groups of the four major aliphatic glucosinolates found in Brassica napus (adapted from Röbbelen and Thies 1980).

Trivial Name	Semi-systematic Name	Structural Formula
Gluconapin	3-butenyl glucosinolate	$CH_2=CH(CH_2)_2$
Glucobrassica-napin	4-pentenyl glucosinolate	$CH_2=CH(CH_2)_3$
Progoitrin	2-hydroxy-3-butenyl glucosinolate	$CH_2=CH-CH(OH)-CH_2$
Napoleiferan	2-hydroxy-4-pentenyl glucosinolate	$CH_2=CH-CH_2CH(OH)-CH_2$

THE EFFECTS OF INBREEDING AND ESTIMATES OF ADDITIVE GENETIC VARIANCE WITHIN SEVEN SUMMER OILSEED RAPE CULTIVARS

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Abstract

Seven groups of inbred lines derived from the summer oilseed rape (Brassica napus spp. oleifera) cultivars Westar, Regent, Lergo, Marnoo, Ariel, Karat and R83-11 were evaluated at three sites over two years, for agronomic characters, seed oil and protein concentrations. Estimates of additive genetic variance and heritability were calculated. Comparison of the mean of inbred lines with the mean of their respective source cultivars indicated little, if any, inbreeding depression in all cultivars except Ariel. It was concluded that dominance is not a major factor in the genetic determination of the characters studied. For every trait in every cultivar, individual inbred lines were identified that exceeded or equaled their respective source cultivar means, indicating that neither heterozygosity per se nor genetic heterogeneity are required to maintain maximum performance in summer oilseed rape. Therefore, the oilseed rape cultivars used in this study were considered to be genetically heterogeneous populations, consisting of some number of generally homozygous lines. Significant additive genetic variance was detected for most traits in most cultivars and indicating that superior inbred lines could be extracted from within cultivars.

Introduction

Brassica napus spp. oleifera L. is a partially cross pollinated species. Rakow and Woods (1987), using erucic acid content of the seed oil as a genetic marker, found that summer oilseed rape grown under western Canadian conditions had an average outcrossing rate of 22 %, with individual plants varying from 2 to 75 %. Williams et al. (1986) found that individual plants yield equally well, whether self or cross pollinated and concluded that summer oilseed rape is completely self compatible. Oilseed rape breeders involved in cultivar development generally use open pollinated and/or selfed plants in a pedigree breeding scheme. Inconsistent pollination control combined with differential rates of outcrossing among individual plants, would necessarily lead to an extensive genotypic array within any given oilseed rape cultivar.

In winter oilseed rape, Schuster and Michael (1976) reported significant inbreeding depression for yield, height and oil concentration. Meng and Liu (1986) also found that oil content was reduced upon inbreeding. However, no experiments have yet been conducted to determine the effects of inbreeding or to measure levels of genetic variability in summer oilseed rape cultivars.

Recent research concerning heterosis in oilseed rape (Sernyk and Stefansson 1983; Grant and Beversdorf 1985) has been very promising and the development of hybrid cultivars is now a distinct possibility. Hybrid cultivar development normally relies on the use of inbred lines (Allard 1960), therefore the agronomic performance of inbred lines extracted from summer oilseed rape is also of some interest. The research presented herein attempts to characterize the effects of inbreeding and measure levels of useful genetic variability in seven summer oilseed rape cultivars.

Materials and Methods

During 1984 and 1985, 150 seeds (S_0) were chosen at random from the spring oilseed rape cultivars: Westar, Regent, R83-11, Karat, Marnoo, Ariel, and Lergo, representing canola quality cultivars from Australia, Canada and Europe (Table 3.1). Groups of unselected lines from each cultivar were grown in the greenhouse and inbred to S_3 using single seed descent and then increased in the S_4 generation. Forty-eight random lines from the cultivar Lergo and twenty-four random lines from each of the remaining cultivars were used for field trials in 1986. A single random plant from each inbred line was bagged in the field in 1986 to provide S_5 seed for the 1987 field experiments.

In the summers of 1986 and 1987 the seven groups of random inbred lines from each cultivar were machine planted with an eight row belt-cone seeder at two locations, the Point (Riverdale Clay) and the Arboretum (Red River Clay) University of Manitoba campus, Winnipeg. Fertilizer was applied according to soil test and trifluralin (5%) granules were pre-plant incorporated at 0.35 kg a.i. ha^{-1} for broad leaf and grassy weed control. Carbofuran (10 %) granules were banded with the seed at a rate of 1.0 kg a.i. ha^{-1} to control flea beetles (Phyllotreta spp.).

During the 1986 and 1987 growing seasons total rainfall, including supplementary irrigation, was 298 mm and 363 mm respectively. These figures are close to the long term average (297 mm) for this period; however, the month of May was drier than normal in both years. Temperatures were near average during July and August of 1986 and 1987, but higher than average during May and June in both years. The dry and hot conditions early in the 1987 growing season resulted in poor stand establishment at those locations where supplementary irrigation could not be applied. As a result of the adverse conditions, the Arboretum location was not harvested in 1987.

A randomized complete block design with three replications was used for each group of inbred lines at each location. Source cultivars were seeded with their respective inbred line groups, in every third row of each replicate. Plots consisted of single rows, 3 m long, with 61 cm between rows. All plots were seeded at a rate of approximately 40 seeds m^{-1}, harvested by hand, placed in burlap sacks, air dried for three weeks and then threshed using a stationary thresher. Rows were assessed for days to flower (days), height at last flower (cm), days to maturity (days) and yield (tonnes/ha). Seed oil and protein concentrations were determined using oven dried samples from each row. Oil concentration was determined by Nuclear Magnetic Resonance (Robertson and Morrison 1979) and protein concentration was determined by the standard macro Kjeldahl method (N % x 6.25).

The data for the individual environments were analyzed as randomized complete block designs and then combined over environments. All the effects in the model were considered random. Within each group of inbred lines, Barlett's test for homogeneity of error variances (Cochran and Cox 1957) from the three environments was performed for each trait. In cases where the error variances were found to be heterogeneous, a modified procedure suggested by Cochran and Cox (1957) was used. The procedure involves the use of the error degrees of freedom associated with a single location when testing the significance of the line x environment interaction from the combined analysis. For the purpose of comparison of inbred lines and source cultivars, four check rows were chosen at random from each replication and included in the design as control treatments. A single planned contrast, of the mean of inbred lines versus the mean of open pollinated checks was calculated for each trait in each experiment as well as for means (over three environments) of each trait.

For the purpose of estimation of genetic variances, source cultivars were not included in the analysis of variance. Genetic variances were estimated, based on expected mean squares, from the combined analysis of variance (Obilana and Hallauer

1974). In theory when the lines are fully inbred, this estimate of genetic variance is equal to twice the additive genetic variance (Falconer 1981). Assuming 22 % outcrossing (Rakow and Woods 1987) in the source cultivars and given four generations of enforced inbreeding, the inbreeding coefficient should equal 0.97 (Falconer 1981). Therefore, the lines used in this study were assumed to be fully inbred and additive genetic variance values were estimated as one-half the estimate of genetic variance. Narrow sense heritabiliy estimates, on a plot mean basis over three replications and three environments, and their standard errors were calculated using the method outlined by Hallauer and Miranda (1981).

Results and Discussion

Inbreeding Depression

The mean differences between inbred lines and their respective source cultivars for earliness (days to flower and days to maturity), plant height, seed yield, and oil and protein concentration were calculated from the planned contrasts (Table 3.2). If a contrast mean is significant and positive in one environment and significant and negative in another, there is evidence of changes in rank between the two factors being compared. If the contrast is non-significant in one environment and significant in another then the treatments are only weakly interactive and there have been no changes in rank. There were no apparent changes in rank between the mean of inbred lines and the mean of source cultivars, except in the case of oil concentration in the cultivar Ariel. Therefore, it can be concluded that the effects of inbreeding on the characteristics studied in these seven cultivars were generally consistent across the three environments.

Inbred lines were found to be, on average, significantly later flowering and maturing than their source cultivars in all cases, except for Regent and Lergo, where no

significant differences were observed (Table 3.2). Hallauer and Sears (1973) found that inbreeding depression was the result of the increased frequency of homozygous recessive deleterious loci. Falconer (1981) has indicated that inbreeding depression is a function of gene frequency, directional dominance and the number of segregating loci affecting a character. Therefore, in the oilseed rape cultivars which exhibited inbreeding depression, there was degree of dominance affecting the expression of earliness. The low levels of inbreeding depression for days to flower and days to maturity in oilseed rape suggests that there were few segregating loci participating in the expression of earliness and that the recessive alleles at these loci were probably present at very low frequency. No significant inbreeding depression was observed for earliness in Lergo and Regent; therefore, additive genetic effects were implied.

Inbred lines derived from Westar and R83-11 were, on average, significantly (Table 3.2) taller than the mean of their respective source cultivars, indicating that in this case some of the genes for increased height were recessive. Marnoo, Lergo and Ariel inbred lines were significantly shorter than their respective source cultivars, indicating that genes for increased height were dominant. There was no difference between the mean height of inbred lines and the mean height of their respective source cultivars for Regent and Karat, indicating additive gene action for height in these two cultivars. The possibility of oppositional gene effects for height can not be excluded, especially in light of the mixed response to inbreeding observed in the various cultivars. In some respects these results agree with those found for winter oilseed rape by Schuster and Michael (1976), in that the differences in height between the mean of the inbred lines and their respective source cultivars were small. The direction of the difference in this study was not always consistent with the results of those authors. The low levels of inbreeding depression for height observed in this study suggests that there were few segregating loci with dominance influencing the expression of height in this sample of oilseed rape cultivars.

The average yields of Westar, Regent and Karat inbred lines were not significantly different from the source cultivars (Table 3.2), indicating that additive genetic effects predominantly influence yield in these cultivars. The levels of genetic variation for yield within Marnoo were low (Table 3.4), explaining the absence of a difference between the mean of inbred lines and their source cultivar. For R83-11 and Ariel there were highly significant ($P < .01$) differences in yield between inbred lines and their respective source cultivars (Table 3.2). There was also a small but significant ($P < .05$) difference in yield between the mean of inbred lines and the mean of the source cultivars for Lergo. R83-11, Ariel and Lergo inbred lines yielded 12 %, 22 % and 6 % lower than their respective source cultivars (Table 3.3). The presence of significant inbreeding depression for yield in R83-11, Ariel and Lergo indicates that some segregating loci with dominance are present for yield in these cultivars.

The effects of inbreeding depression on yield of Regent, R83-11 and Lergo were not consistent across environments. The differences were large and significant in the 1986 Arboretum environment, but small and non-significant in the other two environments. Inbreeding depression effects for yield were large and consistent across environments for the cultivar Ariel. The somewhat higher levels of inbreeding depression for yield in Ariel indicates that this cultivar probably has more segregating loci with dominance than the other cultivars and that inbreeding exposed deleterious recessive alleles. The average level of inbreeding depression for yield in this study was only 5 %, indicating that the genetic load of the seven cultivars is very low. The results of Schuster and Michael (1976) show even greater levels of inbreeding depression (45 %) than those found in Ariel, Lergo and R83-11 (14 %). The sample size used by Schuster and Michael (1976) was inconsistent across the inbred generations, ranging from 1549 lines in S_0 to 17 in the S_8 generation. Therefore, the results of those authors may reflect sampling error or a greater genetic load in winter oilseed rape.

Inbreeding did not significantly affect oil or protein concentration in Westar, Regent, R83-11 or Lergo (Table 3.3) indicating that additive genetic effects play a major role in the expression of oil and protein concentration in these cultivars. This result confirms the observations of Grami and Stefansson (1977). There was a significant difference between inbred line means and their respective source cultivars for both oil and protein concentration in Karat and for protein alone in Marnoo. Karat inbred lines had, on average, 1.1 % higher oil and 0.5 % lower protein concentration than the mean of the source cultivar (Table 3.2). These results indicate that deleterious recessive factors uncovered during inbreeding reduced protein concentration and in turn, given the inverse relation between oil and protein in oilseed rape (Grami et al. 1977), led to a significant increase in oil concentration. There was no significant additive genetic variation for protein concentration within the cultivar Marnoo (Table 3.4) indicating that the observed difference between the inbred line mean and the source cultivar is a reflection of poor buffering against environmental fluctuations within that group of inbred lines. Inbred lines from the cultivar Ariel did not show any net effect of inbreeding on oil or protein concentrations but did show a strong interaction for oil concentration (Table 3.2). Inbred lines derived from the source cultivar Ariel were significantly lower in oil concentration than the source cultivar in the Point 1987 environment and significantly higher than the source cultivar in the Point 1986 environment, while there was no difference between the two in the Arboretum 1986 trial (Table 3.2). Oil concentration in the Ariel inbred lines must be strongly dependant on environmental conditions during a given season. The differences among Ariel source cultivar means, across the three environments, were not significant. The differences in oil concentration between the mean of inbred lines across environments were significant, suggesting that the inbred lines were fluctuating around a stable Ariel source cultivar mean. It would appear that for oil concentration the genetically homogeneous inbred lines were less able to buffer against environmental fluctuations than the genetically heterogeneous source cultivar.

Genetic Variances and Heritabilities

The combined analysis of variance over the three environments showed that the variation among lines within each group of inbreds was significant ($P < 0.05$) for all traits. The means and ranges of the seven groups of inbred lines illustrate the broad range of variability that exists within most of the cultivars studied (Table 3.3). The estimates of additive genetic variance (Table 3.4) exceeded twice their standard errors in nearly every case, showing that significant genetic variation exists within all the cultivars studied for nearly all traits. Considering the range in the means (Table 3.3) and the narrow sense heritability estimates (Table 3.5), selection within the cultivars used for this study should be effective for most traits. When compared to the other cultivars, the estimates of additive genetic variance and narrow sense heritability for Marnoo were generally low and the estimates for yield and protein were not different from zero. Selection within this cultivar would, as a consequence, probably be less effective. The estimates of additive genetic variance, heritability and their standard errors for the cultivar Lergo were within the range found for the other cultivars, indicating that the larger sample size was probably no more precise than the small sample size in estimating additive genetic variance. The existence of significant additive genetic variation coupled with low levels of inbreeding depression suggests that the cultivars used in this study consist of a mixture of generally homozygous lines that may have some segregating loci with dominance, but differ mainly by additive genes.

Table 3.1. Pedigrees and countries of origin for the seven source cultivars used for inbred line development.

Cultivar	Origin	Cross/Pedigree
Westar	Canada	SD/S68-2895//Midas/3/Tower
Regent	Canada	Liho/2*Turret//Bronowski/2*Turret
R83-11	France	?
Karat	Sweden	Hermes//Bronowski/Gulle
Marnoo	Australia	Chikuzen/Zephyr//Bronowski
Ariel	Denmark	Line/Tower
Lergo	Finland	Hermes//Bronowski/Gulle

Table 3.2. Average differences between the mean of inbred lines and the mean of their respective source cultivars for three environments and combined over environments for days to flower (DAF), days to maturity (DAM), height (HGT, cm), yield (YLD, tonnes/ha), oil (OIL, %) and protein (PRO, %).

Cultivar	Trait	Point 86	Arb 86	Point 87	Combined
Westar	DAF	1.5**	-0.1	2.3**	1.2**
	DAM	1.1**	0.2	2.3**	1.2**
	HGT	4.0	-1.7	5.9**	2.8*
	YLD	0.10	-0.01	0.26	0.11
	OIL	0.41	0.13	0.16	0.23
	PRO	0.02	-0.36	0.42	0.03
Regent	DAF	0.6*	-1.4	0.4	-0.1
	DAM	0.4	0.7	-0.2	0.3
	HGT	2.2	3.8	-6.7**	-0.2
	YLD	0.17	0.47**	-0.10	0.18
	OIL	-0.16	0.13	0.11	0.02
	PRO	0.33	0.12	0.09	0.18
R83-11	DAF	2.4**	2.3**	1.5**	2.1**
	DAM	0.3	0.8**	1.0*	0.7*
	HGT	2.1	7.0**	3.7	4.3*
	YLD	-0.29	-0.70**	-0.19	-0.39**
	OIL	0.16	0.25	-0.67	-0.25
	PRO	-0.16	-0.10	0.21	-0.02
Karat	DAF	3.5**	3.5**	1.9**	3.0**
	DAM	1.5**	2.0**	1.0**	1.5**
	HGT	3.3	0.1	1.8	1.7
	YLD	-0.27	-0.35	0.18	0.15
	OIL	1.37**	1.14**	0.83*	1.12**
	PRO	-0.55*	-0.54**	-0.38	-0.49**
Marnoo	DAF	3.1**	3.9**	0.3	2.3**
	DAM	2.5**	2.3**	0.4	2.1**
	HGT	-5.8**	1.1	-3.5	-2.7*
	YLD	-0.21	-0.03	0.01	-0.08
	OIL	0.41	0.26	0.22	0.30
	PRO	-1.00**	-1.04**	-0.14	-0.73**
Ariel	DAF	3.2**	2.5**	-0.7	1.7**
	DAM	1.1**	1.1**	-0.6	0.6*
	HGT	-9.6**	-7.7**	-14.0**	-10.4**
	YLD	-0.79**	-0.73**	-0.64**	-0.72**
	OIL	0.49*	-0.33	-0.76**	-0.20
	PRO	-0.53*	0.01	0.43	-0.03
Lergo	DAF	-0.4	-0.3	-0.6	-0.4
	DAM	-0.3	-0.2	-0.5	-0.3
	HGT	-4.7**	-5.5**	-1.2	-3.8**
	YLD	-0.09	-0.36*	-0.12	-0.19*
	OIL	-0.04	0.11	0.13	0.06
	PRO	0.09	-0.09	-0.07	-0.02

*,** significant at P=.05 and P=.01 respectively

Table 3.3. Inbred line means, standard errors and ranges, and source cultivar means and standard errors for days to flower (DAF), days to maturity (DAM), height (HGT, cm),yield (YLD, tonnes/ha), oil (OIL, %) and protein (PRO, %) combined over three environments from 1986 and 1987.

Source Cultivar	Trait	--------Inbred Line-------- Mean	Range	Source Cultivar Mean
Westar	DAF	43.7 ± 0.6	42.1 - 47.8	42.4 ± 0.6
	DAM	87.9 ± 1.0	86.6 - 90.7	86.7 ± 0.5
	HGT	123.5 ± 2.4	117.4 -133.1	120.8 ± 2.10
	YLD	2.75 ± 0.17	1.86 - 3.29	2.62 ± 0.15
	OIL	44.2 ± 0.3	42.2 - 45.4	43.9 ± 0.3
	PRO	25.3 ± 0.5	23.6 - 27.3	25.3 ± 0.3
Regent	DAF	44.2 ± 0.5	41.2 - 48.1	44.3 ± 0.5
	DAM	92.9 ± 0.5	91.1 - 94.4	92.6 ± 0.5
	HGT	128.8 ± 2.4	117.0 - 136.6	129.1 ± 2.1
	YLD	2.74 ± 0.16	1.35 - 3.45	2.56 ± 0.14
	OIL	43.6 ± 0.2	41.4 - 44.7	43.6 ± 0.4
	PRO	26.4 ± 0.3	25.3 - 28.2	26.2 ± 0.4
R83-11	DAF	44.5 ± 0.4	39.0 - 52.1	42.5 ± 0.4
	DAM	94.4 ± 0.4	91.2 - 98.8	93.7 ± 0.4
	HGT	140.2 ± 2.5	122.3 - 153.8	135.9 ± 2.7
	YLD	2.67 ± 0.19	1.33 - 3.39	3.06 ± 0.18
	OIL	42.9 ± 0.4	40.5 - 44.2	43.1 ± 0.3
	PRO	26.1 ± 0.3	24.4 - 28.6	26.1 ± 0.3
Karat	DAF	49.1 ± 0.6	45.2 - 52.8	46.2 ± 0.5
	DAM	96.3 ± 0.5	94.2 - 98.9	94.8 ± 0.5
	HGT	143.6 ± 2.1	123.3 - 152.9	141.9 ± 2.5
	YLD	3.03 ± 0.18	1.99 - 3.94	3.18 ± 0.19
	OIL	43.8 ± 0.2	42.2 - 45.0	42.7 ± 0.3
	PRO	26.7 ± 0.2	24.5 - 27.8	27.2 ± 0.3
Marnoo	DAF	48.5 ± 0.5	46.2 - 51.7	46.2 ± 0.4
	DAM	94.7 ± 0.6	93.0 - 97.7	92.7 ± 0.4
	HGT	129.0 ± 2.5	123.1 - 141.0	131.7 ± 2.7
	YLD	3.07 ± 0.15	2.23 - 3.54	3.14 ± 0.18
	OIL	43.9 ± 0.2	41.8 - 45.2	43.6 ± 0.3
	PRO	25.1 ± 0.3	24.4 - 26.1	25.8 ± 0.3
Ariel	DAF	49.5 ± 0.5	42.9 - 58.0	47.8 ± 0.6
	DAM	96.5 ± 0.5	92.9 - 101.3	96.0 ± 0.6
	HGT	149.0 ± 2.5	127.3 - 166.9	159.4 ± 2.3
	YLD	2.51 ± 0.17	1.78 - 3.27	3.22 ± 0.27
	OIL	42.9 ± 0.3	40.4 - 45.2	43.1 ± 0.2
	PRO	25.6 ± 0.3	24.4 - 27.0	25.7 ± 0.3
Lergo	DAF	45.8 ± 0.5	39.8 - 49.4	46.2 ± 0.6
	DAM	93.6 ± 0.5	89.9 - 95.9	93.9 ± 0.4
	HGT	128.9 ± 2.5	103.1 - 141.9	132.7 ± 2.8
	YLD	2.72 ± 0.18	1.56 - 3.88	2.91 ± 0.16
	OIL	42.9 ± 0.4	40.4 - 46.4	42.9 ± 0.3
	PRO	26.8 ± 0.3	24.0 - 29.4	26.9 ± 0.3

Table 3.4. Estimates of additive genetic variance and their respective standard errors for days to flower (DAF), days to maturity (DAM), height (HGT), yield (YLD), oil (OIL) and protein (PRO) of inbred lines, derived from specified source cultivars, grown in three environments during 1986 and 1987.

Cultivar	DAF	DAM	HGT	YLD	OIL	PRO
Westar	0.76 ± 0.07	0.35 ± 0.05	5.1 ± 0.2	0.06 ± 0.02	0.27 ± 0.04	0.21 ± 0.03
Regent	0.96 ± 0.07	0.10 ± 0.03	7.7 ± 0.2	0.28 ± 0.04	0.18 ± 0.03	0.18 ± 0.03
R83-11	3.55 ± 0.13	0.91 ± 0.08	8.9 ± 0.4	0.11 ± 0.02	0.50 ± 0.06	0.47 ± 0.05
Karat	1.87 ± 0.10	0.79 ± 0.07	23.8 ± 0.4	0.05 ± 0.02	0.19 ± 0.03	0.13 ± 0.03
Marnoo	0.41 ± 0.07	0.27 ± 0.05	7.4 ± 0.2	0.02 ± 0.02	0.21 ± 0.04	0.04 ± 0.02
Ariel	6.21 ± 0.17	2.11 ± 0.10	49.8 ± 0.5	0.05 ± 0.02	0.71 ± 0.06	0.26 ± 0.04
Lergo	1.80 ± 0.07	0.62 ± 0.04	26.2 ± 0.3	0.10 ± 0.02	0.61 ± 0.06	0.56 ± 0.05

Table 3.5. Estimates of narrow sense heritabilities (%) and their respective standard errors for days to flower (DAF), days to maturity (DAM), height (HGT), yield (YLD), oil (OIL) and protein (PRO) of inbred lines, derived from specified source cultivars, grown in three environments during 1986 and 1987.

Cultivar	DAF	DAM	HGT	YLD	OIL	PRO
Westar	37.3 ± 3.5	33.2 ± 4.9	27.6 ± 1.2	36.9 ± 12.8	41.3 ± 6.0	41.5 ± 6.8
Regent	40.9 ± 3.2	22.5 ± 7.8	36.1 ± 1.1	40.2 ± 10.0	40.4 ± 5.8	33.7 ± 6.8
R83-11	46.5 ± 1.7	38.9 ± 3.2	39.4 ± 0.6	43.3 ± 9.5	39.7 ± 4.4	42.6 ± 4.6
Karat	43.2 ± 2.3	40.7 ± 3.5	44.4 ± 0.7	30.2 ± 12.9	42.1 ± 7.2	36.3 ± 8.4
Marnoo	23.1 ± 3.9	26.7 ± 5.2	39.0 ± 1.1	24.8 ± 17.1	41.1 ± 6.8	9.2 ± 4.8
Ariel	47.5 ± 1.3	45.1 ± 2.2	46.5 ± 0.5	33.7 ± 13.0	46.1 ± 3.9	40.0 ± 6.0
Lergo	43.3 ± 1.7	40.1 ± 2.8	43.7 ± 0.5	39.6 ± 6.9	44.1 ± 4.1	43.9 ± 4.3

HETEROSIS AND COMBINING ABILITY IN HYBRIDS DERIVED FROM OILSEED RAPE CULTIVARS AND INBRED LINES

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Abstract

Basic genetic information needed to develop hybrid oilseed rape (Brassica napus spp. oleifera) cultivars has not been previously reported. Since recent work had shown oilseed rape cultivars to be genetically heterogeneous, inbred line derived hybrids were compared to cultivar derived hybrids to determine if there were differences among inbred lines in terms of their performance in hybrid combination. For inbred line derived hybrids, mid-parent was compared to general combining ability as a means of predicting hybrid yield. Three inbred lines, from each of three source cultivars (Regent, Marnoo and Karat), were crossed using a factorial mating design and the resultant hybrids were field evaluated in three environments. The inbred lines, source cultivars and cultivar hybrids were included as checks. Yields of inbred line derived hybrids were compared to their respective cultivar derived hybrids and some inbred line crosses were found to be significantly higher and others significantly lower yielding. There were also differences in GCA effects among the inbred lines. This result indicates the presence of variability in breeding values among cultivar derived inbred lines and that hybrid oilseed rape breeding programs should be based on inbred line crosses, rather than cultivar crosses. General combining ability (GCA) was highly significant and accounted for 88 % of the cross sums of squares, while specific combining ability (SCA) was non-significant, indicating that additive genetic effects predominantly influence the expression of yield. Regression of mid-parent on observed hybrid yield was significant, with an r^2 of 0.37. Regression of expected hybrid yield, based on parental GCA, on observed hybrid yield was also significant, but with an r^2 of 0.88. Therefore, GCA was more effective than mid-parent, for describing hybrid yield. There was, however, a significant positive relationship between mid-parent and hybrid yield indicating that, despite the somewhat low r^2, mid-parent may still be a useful guide for the selection of parents for testing in hybrid combination.

Introduction

Early reports of heterosis in oilseed rape (Brassica napus spp. oleifera) cultivar hybrids, coupled with the availability of cytoplasmic male sterility, have stimulated much research into the development of hybrid oilseed rape cultivars (Shuster and Micheal, 1976; Shiga, 1976). Recent work demonstrated high levels of heterosis (30-60% of mid-parent) for seed yield in inter-cultivar oilseed rape hybrids (Sernyk and Stefansson, 1983; Grant and Beversdorf, 1985). In an attempt to determine the nature of this heterosis, Lefort-Buson et al. (1987) studied yield of oilseed rape in hybrids between lines of similar and diverse origins. Hybrids between lines with similar origins resulted in low levels of heterosis and a preponderance of general combining ability, which the authors interpreted to be the result of additive genetic effects. Crosses between diverse genotypes gave higher levels of heterosis, and the authors indicated that in these crosses both general and specific combining ability were significant. The presence of significant specific combining ability was attributed to the induction of new dominance and epistatic relationships among genes.

Hybrid vigor observed in cultivar hybrids represents an average expression of heterosis, as the hybrid is based on a sample of different individuals taken from each cultivar (Hallauer and Miranda, 1981). Early work by Shull in 1910 (Shull, 1952) illustrated that F_1 plants from cultivar crosses of maize (Zea mays) varied in relative heterosis, some being considerably more heterotic than others. Therefore, hybrids produced from cultivar crosses are not generally repeatable. As a consequence, hybrid breeding normally uses pure lines developed through inbreeding, followed by selection among those lines for parental inbreds that exhibit maximum heterosis when crossed (Allard, 1960).

In oilseed rape, cultivars used as parents of hybrids had previously been assumed to be completely homozygous (Grant, 1984). Recent work has illustrated that oilseed

rape cultivars are, in fact, genetically heterogeneous (Brandle and McVetty, 1989). Therefore, it should be possible to select within the two parents of a heterotic cultivar cross, for inbred lines that will give maximum heterosis upon crossing, rather than the average heterosis that had been observed in the original cultivar cross.

The primary purpose of this research was to compare yields of inbred line derived hybrids with those of their respective cultivar derived hybrids, and secondly to establish the presence of variability for combining ability among those inbred lines. In order to find a reliable means of reducing the testing of inbred lines in hybrid combination, possible methods of predicting hybrid performance were also investigated.

Materials and Methods

Genetic Material

Inbred lines were chosen from three groups of random lines extracted from the oilseed rape cultivars 'Regent', Liho/2*Turret//Bronowski/2*Turret; 'Marnoo',Chikuzen/Zephyr//Bronowski; and 'Karat', Hermes//Bronowski/Gulle (Brandle and McVetty, 1989), which had demonstrated high levels of heterosis in inter-cultivar crosses (Sernyk and Stefansson, 1983). Initial selection of inbred lines from each group was done on the basis of average performance in yield trials conducted at two locations during 1986 (Brandle and McVetty, 1989). One high, one low, and one average performing line were selected from the group of inbred lines corresponding to each cultivar. Assuming 22 % outcrossing (Rakow and Woods, 1987) in the source cultivars, four generations of selfing resulted in an estimated inbreeding coefficient of 0.97. Therefore, the lines used in this study were assumed to be fully inbred.

Crosses between inbred lines were made in the greenhouse using the factorial mating design of Comstock and Robinson (1952). Regent inbred lines (R19, R21, R16)

were used as males while Marnoo (M17, M2, M12) and Karat (K6, K9, K16) inbred lines were used as females. Crosses between the open pollinated cultivars were made in the greenhouse, using ten plants of Karat and of Marnoo as females, and twenty plants of Regent as males. Seed of the inbred lines was increased during this same period.

Experimental Procedures and Data Collection

During mid-May of 1987 and 1988, all entries were machine planted with an eight row belt-cone seeder at two locations, the Point (Riverdale Clay, Mollic Cryofluvent) and the Arboretum (Red River Clay, Vertic Cryoboroll) at the University of Manitoba campus, Winnipeg. Fertilizer was applied according to soil test and trifluralin (5 %) granules were pre-plant incorporated at 0.35 kg a.i. ha^{-1} for broad leaf and grassy weed control. Carbofuran (10 %) granules were banded with the seed at a rate of 1.0 kg a.i. ha^{-1} to control flea beetles (Phyllotreta spp.). Malathion (50% EC) was sprayed at 0.35 kg a.i. ha^{-1} at weekly intervals following flowering, to control diamondback moths (Plutella xyostella).

The trial consisted of 32 entries: 18 inbred line derived hybrids and their 9 inbred line parents, and 2 cultivar derived hybrids and their 3 parents. The entries were seeded at a rate of 40 seeds m^{-1}, in a randomized complete block design with three replications. Plots consisted of three rows, 3 m long, with 30 cm between rows. The two outside rows were seeded to 'OAC Triton' and were not harvested. Yields, at 9 % moisture, were determined from the centre row of each plot. These centre rows were harvested by hand, placed in burlap sacks, air dried for three weeks and then threshed using a stationary thresher.

Rainfall and temperatures for the 1987 growing season were near long term averages. Rainfall was below average for 1988, temperatures averaged 3.8 °C above normal during May, June, July and August. Severe infestations of diamondback moths at

the Arboretum during the 1988 growing season resulted in considerable damage and, as a consequence, that location was not harvested.

The results from the individual environments were first analyzed separately and then combined over environments. Replications and environments were considered random effects and entries were fixed. Bartlett's test for homogeneity of error variances (Cochran and Cox, 1957), from the individual analysis of each environment, was performed. These error variances were found to be heterogeneous, requiring the use of a modified procedure suggested by Cochran and Cox (1957). The modified procedure uses the error degrees of freedom from a single location, when comparing observed F-values from the various interaction mean squares with tabulated values. A protected least significant difference test (LSD) (Steel and Torrie, 1980) was used to compare the individual inbred line hybrids with their respective cultivar hybrids.

The entry sums of squares from the analysis of variance was partitioned into variation among two groups: inbreds (inbred line derived hybrids and parents) and cultivars (cultivar derived hybrids and parents). The inbred sums of squares was further subdivided into variation due to hybrids and parents. The inbred cross sums of squares was again subdivided into variation due to males, females, and males x females. The main effects of males and females are equivalent to general combining ability (GCA), and the male x female interaction is equivalent to specific combining ability (SCA) (Hallauer and Miranda, 1981). Male and female GCA effects were calculated according to Simmonds (1979). Standard errors for the GCA effects [$SE(GCA_m)$ and $SE(GCA_f)$] were calculated following Cox and Frey (1984) and were used to test the significance of those effects.

Expected hybrid yields, based on parental GCA, were compared to mid-parental yields as a means of describing actual hybrid performance. Expected hybrid yield was calculated as follows:

$$X_{ij} = GCA_i + GCA_j + X_{..}$$

Where X_{ij} is the expected hybrid yield of a cross between inbred parents i and j, GCA_i and GCA_j are the GCA effects of parent i and j, and $X_{..}$ is the grand mean of the inbred line derived hybrids. Mid-parent yield was calculated as the mean yield of the two parents involved in a given cross. Both X_{ij} and mid-parent were then regressed on their corresponding hybrid yields. Coefficients of simple determination (r^2) were used to assess the effectivness of GCA and mid-parent as descriptors of hybrid yield.

Results and Disscussion

The analysis of variance (Table 4.1) indicated the presence of highly significant variation among entries, and a significant interaction between entries and environments. The cultivar mean square was highly significant, indicating the presence of variability among cultivar hybrids and their parents. The inbred mean square was highly significant. The contrast between inbred line derived hybrids and their parents was highly significant, accounting for 73 % of the inbred sums of squares, indicating the presence of substantial heterosis. The variation among parents was non-significant and that among crosses was highly significant. The inbred x environment interaction was highly significant. Decomposition of the inbred x environment sums of squares showed that the (F_1's vs. parents) x environments interaction was non-significant, indicating that the expression of heterosis was consistent across environments. The hybrid x environment interaction was non-significant, indicating that the hybrids performed consistently, relative to each other, across the three environments. The parent x environment interaction was highly significant, which may reflect the inability of the inbred parents to buffer against environmental fluctuations and lends some credence to the idea that hybrids are more homeostatic. Shank and Adams (1960) reported a similar result for

comparisons between maize inbred lines and hybrids. These authors also found that hybrids had lower levels of genotype-environment interaction than inbred lines.

Partitioning of the hybrid sums of squares into variation due to males and females, showed that GCA accounted for 88 % of the sums of squares. Both mean squares were significant. The interaction between males and females (SCA) was non-significant and accounted for the remaining 12 % of the hybrid sums of squares. Therefore, the performance of single cross hybrids can be adequately predicted on the basis of GCA alone, and the best hybrids should result from crosses between parents having high GCA effects (Table 4.2). These results further indicate that additive genetic effects, meaning additivity among loci, predominantly influence the expression of yield in this group of oilseed rape hybrids. Lefort-Buson et al. (1987) felt that the presence of significant SCA was a general feature of hybrids involving divergent parents, and was in some way responsible for the higher levels of heterosis observed in these types of hybrids. The presence of significant SCA is a consequence of fluctuations in dominance relationships among the parents (Wassimi et al., 1986). Given the results of the present experiment, the absence of SCA does not appear to preclude the possibility of high levels of heterosis.

Comparisons of the individual inbred line derived hybrids with their respective cultivar hybrids showed that some inbred line derived hybrids were significantly higher and others significantly lower yielding than their respective cultivar derived hybrids (Table 4.3). GCA effects for M12 and K16 were significantly different from zero (Table 4.2), indicating the presence of variation in breeding values among inbred lines. These results confirm that the inbred lines differed in terms of their performance in hybrid combination. The GCA effects were ranked consistently with the average performance of Regent and Marnoo inbred lines (Table 4.2). For Karat, the GCA effect of the inbred line K16 did not conform to the pattern observed in the other two cultivars. The correlation between GCA effects and yield of the inbred parents was positive ($r = 0.58$,

df = 7) but non-significant, indicating that selection of parents based on average performance alone may not be reliable.

Regression of expected hybrid yield on observed hybrid yield resulted in a highly significant regression and an r^2 of 0.88** (Fig. 4.1). This r^2 value is equivalent to the portion of the cross sums of square accounted for by the main effects of males and females (or GCA) in the analysis of variance. The regression of mid-parent on hybrid yield also resulted in a highly significant regression, but gave an r^2 of 0.37** (Fig. 4.2). Therefore, in this study GCA was more effective in describing hybrid performance than was mid-parent. Inspection of the graphical presentation of the regression results indicates that while GCA (Fig. 4.1) better described hybrid yield, there was still a significant positive relationship between midparent and hybrid yield (Fig. 4.2) that could have been used to cull at least a portion of inbred lines. Those combinations of lines, that resulted in very low mid-parent yields, could have been discarded without a great threat of losing potentially useful material. Therefore, mid-parent may be a useful and cost effective guide for the selection of parents for evaluation in hybrid combination.

Table 4.1. Analysis of variance for yield of hybrids and their parents, grown in three environments during 1987 and 1988.

Source	df	Mean Square
ENVIRONMENTS(E)	2	48.7936
REPS within E	6	3.6119
ENTRIES(En)	31	9.5148**
Groups(G)	1	2.0378
Cultivars(Cu)	4	4.7430*
Inbreds(I)	26	10.5365**
Parents vs F_1's	1	201.2368**
Parents(P)	8	2.1245
Hybrids(H)	17	3.2774**
Males(M)	2	8.3006*
Females(F)	5	6.4842**
M x F	10	0.6872
En x E	62	0.8950*
G x E	2	0.1598
Cu x E	8	0.5237
I x E	52	0.9804**
(P vs. F_1) x E	2	0.6371
P x E	16	1.6371**
H x E	34	0.6765
M x E	4	0.8808
F x E	10	1.0000
M x F x E	20	0.4734
ERROR	186	0.5026
MEAN		3.550
C. V. %		19.92

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively

Table 4.2. Estimates of parental yield (t ha^{-1}), and general combining ability effects (t ha^{-1}) measured from hybrids grown in three environments during 1987 and 1988.

Parent	Yield Group	Seed Yield	GCA Effect
		Males	
R19	High	2.618	0.386
R21	Medium	2.287	0.013
R16	Low	1.801	-0.399
$t_{0.025}SE(GCA_m)$			0.449
		Females	
M17	High	2.528	0.208
M2	Medium	2.302	-0.382
M12	Low	1.525	-0.674*
K6	High	3.136	0.281
K9	Medium	2.367	-0.109
K16	Low	2.051	0.676*
$t_{0.025}SE(GCA_f)$			0.452
SE		0.610	

* significantly different from zero at P = 0.05.

Table 4.3. Mean yield (t ha^{-1}) and heterosis, expressed as a percent of the high yielding parent in a cross, of inbred line derived and cultivar derived hybrids, grown in three environments during 1987 and 1988.

Cross	Yield	% Heterosis
M2 x R21	3.986	73.2*
M2 x R19	4.368	66.8*
M2 x R16	3.167	37.5
M17 x R21	4.697	85.8*
M17 x R19	4.536	73.3*
M17 x R16	4.059	60.1*
M12 x R21	3.526	54.2*
M12 x R19	3.954	51.0*
M12 x R16	3.162	75.6*
Marnoo x Regent	4.109	20.3
K6 x R21	4.035	29.0
K6 x R19	5.275	68.2*
K6 x R16	4.201	34.0*
K16 x R21	5.037	120.0*
K16 x R19	5.232	99.8*
K16 x R16	4.429	116.0*
K9 x R21	4.129	74.0*
K9 x R19	4.282	63.6*
K9 x R16	3.927	65.9*
Karat x Regent	4.123	20.5
LSD(0.05)	0.891	

* significantly different from the high parent at P = 0.05, according to LSD

Figure 4.1. Actual yield of hybrids versus expected yield of hybrids, ** indicates regression significant at P=0.01.

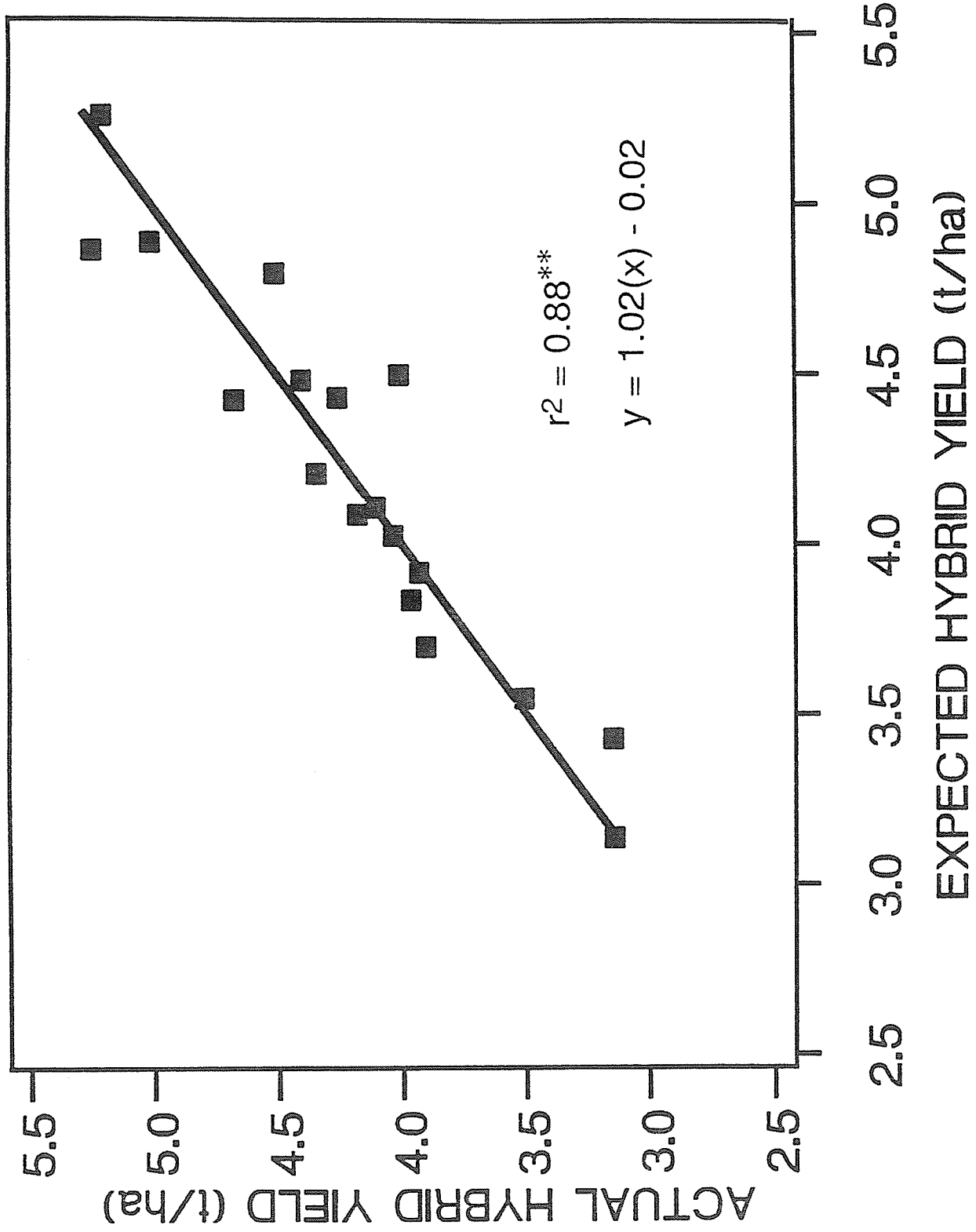
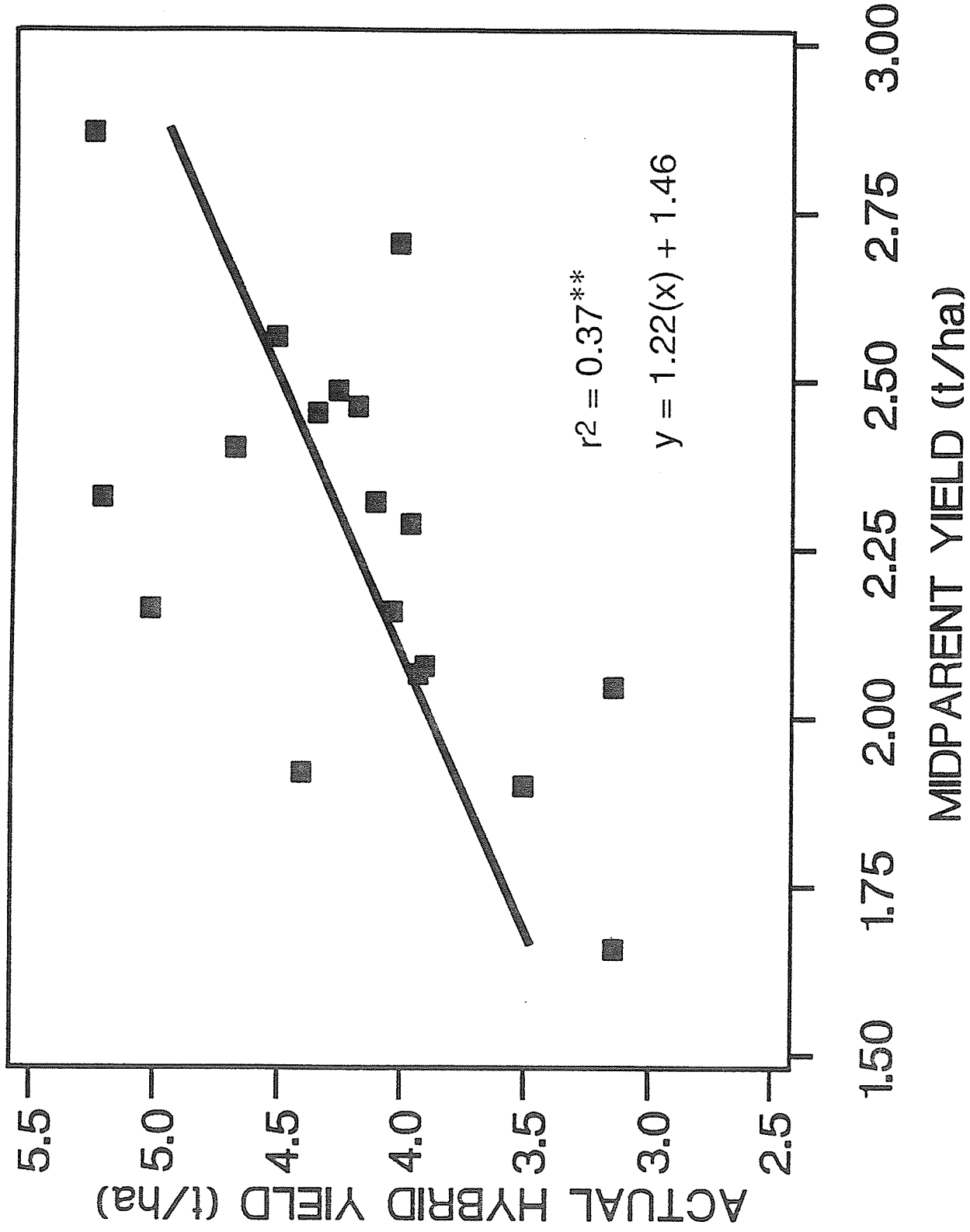

ACTUAL HYBRID YIELD (t/ha)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
$r^2 = 0.88^{**}$
$y = 1.02(x) - 0.02$
2.5
3.0
3.5
4.0
4.5
5.0
5.5
EXPECTED HYBRID YIELD (t/ha)

Figure 4.2. Actual yield of hybrids versus mid-parent yield, ** indicates regression significant at P=0.01.


ACTUAL HYBRID YIELD (t/ha)
5.5
5.0
4.5
4.0
3.5
3.0
2.5
1.50
1.75
2.00
2.25
2.50
2.75
3.00
MIDPARENT YIELD (t/ha)
$r^2 = 0.37^{**}$
$y = 1.22(x) + 1.46$

COMBINING ABILITY ANALYSIS OF SEED ALIPHATIC GLUCOSINOLATES, YIELD, AND OIL AND PROTEIN CONCENTRATION OF A 7 X 7 DIALLEL CROSS IN SUMMER OILSEED RAPE

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With 3 tables

Abstract

The impact of heterosis on glucosinolate levels, and the relationship between seed meal glucosinolate concentration and, yield, oil or protein have not been investigated in spring oilseed rape (Brassica napus spp. oliefera) hybrids, as yet. This study was conducted to assess those factors. Seven inbred lines with less than 20 μmol g^{-1} meal total aliphatic glucosinolates were crossed using a diallel mating design and grown with their inbred parents in three environments, in a randomized complete block design. Yield, oil and protein concentration and the concentration of the four aliphatic glucosinolates were measured. General combining ability was found to be more important than specific combining ability for all characters except yield, where the two were found to be equally important. Heterosis did not act to increase levels of the four aliphatic glucosinolates or protein, but there was significant positive heterosis for both yield and oil concentration. Total glucosinolate concentration was negatively correlated with yield, which appeared to be the result of a dilution effect.

Key words: Brassica napus spp. oleifera, heterosis, correlation

Introduction

Oilseed rape (Brassica napus spp. oleifera) meal contains 37 to 38 % protein and has an amino acid profile that compares well with soybean (Clandinin et al. 1986). Due to excessive levels of glucosinolates, the use of oilseed rape meal in animal rations had previously been limited (Bowland et al. 1965). Enzymatic degradation of the glucosinolates found in oilseed rape meal leads to the formation of compounds that are both toxic and goitrogenic. The discovery of the low glucosinolate cultivar 'Bronowski' in 1967, led to the development of the the cultivar 'Tower' which had only 10 % of the glucosinolate concentration of normal oilseed rape (Stefansson and Kondra 1975). Efforts are currently underway to remove all traces of glucosinolates from the seed meal (Love 1988).

Recent work done by Love (1988) indicated that the metabolic block found in Bronowski was the consequence of three homozygous recessive loci, which were acting in concert to limit the accumulation of the four major aliphatic glucosinolates. Apparently, each locus decreases total glucosinolate concentration of the seed by approximately 40 - 50 μmol g^1 meal, and when all three loci are homozygous recessive, total concentration in the seed is reduced to less than 20 μmol g^{-1} meal. Loves' (1988) results also indicated that the Bronowski genes were only partially dominant. The genetic control of the early stages of glucosinolate synthesis was not investigated. Hybrid oilseed rape breeding would normally rely on crosses between low glucosinolate, low erucic acid, pure lines. Heterosis, affecting the early stages of synthesis, could have a negative impact on levels of glucosinolates found in seed meal of single cross hybrids. Genetic control of glucosinolate concentration, in hybrids between lines homozygous for the Bronowski genes, has not been investigated as yet. The degree of association

between glucosinolate concentration, yield, and oil and protein concentration is also of some practical interest to breeders.

The primary purpose of this research was to investigate the genetic control of, and the impact of heterosis on, glucosinolate levels in hybrids derived from crosses among low glucosinolate inbred lines. Heterosis and combining ability for yield, and oil and protein concentration was also investigated. The relationships between the four aliphatic glucosinolates, yield, and oil and protein concentration was determined using correlation analysis.

Materials and Methods

Genetic Material

One S4 inbred line was chosen from within each of seven groups of inbred lines, that had been extracted from the spring oilseed rape cultivars: 'Westar', 'Regent', 'R83-11', 'Marnoo', 'Karat', 'Ariel' and 'Lergo' (Brandle and McVetty, 1989a). Selection was based on yield performance in trials conducted at two locations during 1986. Assuming 22 % outcrossing (Rakow and Woods, 1987) in the source cultivars and given four generations of selfing, the inbreeding coefficient of these lines was 0.97. Therefore, the lines were considered to be completely homozygous. All selected lines contained less than 20 μmol g^{-1} meal of aliphatic glucosinolates and were assumed to be homozygous for the Bronowski genes. Crosses between inbred lines were made in the greenhouse using a diallel mating design (Griffing 1956). Seed of the inbred lines was increased during this same period.

Experimental Procedures and Data Collection

During mid-May of 1987 and 1988, all entries were machine planted with an eight row belt-cone seeder at two locations, the Point (Riverdale Clay) and the Arboretum (Red River Clay) University of Manitoba campus, Winnipeg, Canada. Fertilizer was applied according to soil test and trifluralin (5 %) granules were pre-plant incorporated at 0.35 kg a.i. ha^{-1} for broad leaf and grassy weed control. Carbofuran (10 %) granules were banded with the seed at a rate of 1.0 kg a.i. ha^{-1} to control flea beetles (Phyllotreta spp.). In the summer of 1988, malathion (50 % EC) was sprayed at 0.35 kg a.i. ha^{-1} at weekly intervals following flowering, to control diamondback moths (Plutella xyostella).

The trial consisted of 28 entries: 21 inbred line hybrids and their 7 inbred line parents. The entries were seeded at a rate of 40 seeds m^{-1}, in a randomized complete block design with three replications. Plots consisted of three rows, 3 m long, with 30 cm between rows. The two outside rows were seeded to 'OAC Triton' and were not harvested. Yield data was collected from the centre row of each plot. The plots were harvested by hand, placed in burlap sacks and air dried for three weeks, then threshed using a stationary thresher. Seed samples were taken from each plot for oil, protein and glucosinolate analysis. Oil concentration was determined using Nuclear Magnetic Resonance (Robertson and Morrison 1979) and protein concentration was measured using the standard macro Kjeldahl procedure. The concentrations of the four major seed aliphatic glucosinolates, 3-butenyl (BUT), 4-pentenyl (PENT), 2-hydroxy-3-butenyl (HOBUT) and 2-hydroxy-4-pentenyl (HOPENT) glucosinolate, were determined using a modification of the standard method of Daun and McGregor (1981). The method is based on gas-liquid chromatography, which allows separation and quantification of the trimethylsilyl derivatives of the desulfated glucosinolates present in the seed. An apparatus for the rapid preparation of oil-free meal for glucosinolate analysis was used to

increase the speed of sample preparation (Raney et al. 1987). A Perkin-Elmer 8320B capillary gas chromatograph, equipped with a flame ionization detector and a Perkin-Elmer AS-300 autosampler was used for all glucosinolate determinations. Product precursor conversion efficiency ratios, which measure the efficieny of the hyroxylation of 3-butenyl (BHER) and 4-pentenyl glucosinolate (PHER), were calculated as outlined by Love (1988). The formulae appeared as follows:

$$BHER = [HOBUT/(HOBUT + BUT)] \times 100$$

$$PHER = [HOPENT/(HOPENT + PENT)] \times 100$$

Rainfall and temperatures for the 1987 growing season were near long term averages. Rainfall was below average for 1988 and temperatures averaged 3.8 °C above normal during May, June, July and August. Severe infestations of diamondback moths at the Arboretum during the 1988 growing season resulted in considerable damage and that location was not harvested. Each year-location combination was treated as an individual environment in the combined analysis.

The results from the individual environments were first analyzed separately and then combined over environments. Replications and environments were considered to be random and entries fixed. Bartlett's test for homogeneity of error variances (Cochran and Cox, 1957) from the analysis of each of the three environments was performed. In the cases where the error variances were found to be heterogeneous, a modified procedure suggested by Cochran and Cox (1957) was used when testing the significance of the various interaction mean squares. Concentrations of BUT and HOBUT were transformed using natural logarithms, and those of PENT and HOPENT using $\ln(x + 1)$. Diallel analysis was performed on F_1 data, using Griffings' (1956) Method 4, Model 1 for fixed effects. Pearson product moment correlations were calculated among the various chacters for each environment, using all 28 entries. The correlations were tested for

homogeneity and then pooled over the three environments. Each pooled correlation coefficient had $[(n_1 - 2) + (n_2 - 2) + (n_3 - 2)] = 78$ degrees of freedom.

Results and Discussion

Combining Ability

The analysis of variance showed that there was significant variation among entries for all characters measured (Table 5.1). The entry x environment interaction was significant for all characters except yield. For BUT, the variation among hybrids was significant and that among parents non-significant. The contrast, F_1's vs. parents, was also significant, but accounted for only 3 % of the entry sums of squares, indicating that heterosis and therefore average dominance was only a minor component of the variation among entries. Inspection of hybrid and parent means (Table 5.2) confirms this observation. The mean concentration of BUT in the hybrid seed meal was 0.32 μmol g^{-1} meal lower than that of the parents. The GCA mean square was highly significant (Table 5.1). GCA accounted for 92 % of the cross sums of squares, illustrating the importance of additive genetic effects in the control of BUT levels in this sample of oilseed rape hybrids. The parents, hybrids and GCA x environment interactions were all significant, indicating that levels of BUT were not expressed consistently across environments, which confirms observations made by Josefsson and Åppelqvist (1968) who also found glucosinolate levels to be affected by environment. SCA was significant, but accounted for only 8 % of the cross sums of squares, and was therefore of far less consequence than GCA.

For PENT, the parent mean square and the contrast F_1's vs parents were both non-significant, but the variation among hybrids was highly significant (Table 5.1). GCA was highly significant and accounted for 91 % of the cross sums of squares, again illustrating the importance of additive genetic effects. SCA was significant, accounting

for the remaining 9 % of the cross sums of squares. The parents, hybrids, GCA and SCA x environment interactions were all highly significant illustrating the failure of PENT levels to be expressed consistently across environments, and again confirming the significant effect of genotype x environment interaction that was observed by Josefsson and Åppelqvist (1968).

The parents and hybrids mean squares and the contrast F_1's vs. parents were highly significant for both HOBUT and HOPENT (Table 5.1). The contrast accounted for only 3 % and 16 % of the HOBUT and HOPENT sums of squares respectively, illustrating that while heterosis was significant, it was only a minor component of the variation among entries. The differences between the mean of the parents and the mean of the hybrids were 0.46 and 0.06 μmol g^{-1} meal respectively (Table 5.2). Both GCA and SCA were highly significant, but GCA was more important, accounting for 89 % and 83 % of the variation in HOBUT and HOPENT among hybrids. Therefore, additive genetic effects had the greatest influence on the expression of HOBUT and HOPENT concentrations in this sample of oilseed rape hybrids. The hybrids, parents and GCA x environment interactions were all significant, indicating again that glucosinolate levels were strongly dependent on environmental conditions.

The variation among parents for total aliphatic glucosinolates was non-significant (Table 5.1). The variation among hybrids and the contrast, F1's vs. parents, were both highly significant. The contrast accounted for about 4 % of the entry sums of squares and the hybrids had 0.87 μmol g^{-1} meal less aliphatic glucosinolates than the parents (Table 5.2). The results of the contrast indicate that heterosis, and therefore dominance, does not play a role in the determination of aliphatic glucosinolate levels in this group of hybrids. The hybrids were consistently lower in glucosinolate concentration than the parents (Table 5.2) and given the high levels of seed yield heterosis found in the hybrids, these observations indicate that the difference between hybrids and parents may have been the result of dilution. Heterosis was probably not acting to reduce glucosinolate

concentrations in this sample of hybrids. The significant negative correlation between yield and total aliphatic glucosinolates (Table 5.3) serves to confirm this observation. The hybrids, parents and GCA x environment interactions were all highly significant, a result consistent with that found for the individual glucosinolates.

The two product precursor conversion efficiency ratios exhibited similar results in the analysis of variance (Table 5.1). Variation due to parents, hybrids and GCA were all highly significant, SCA was significant for BHER only. GCA accounted for 87 % and 95 % of the BHER and PHER cross sums of squares respectively, illustrating that the majority of the variation among hybrids was due to additive genetic effects. The interaction mean squares were significant in every case, with the exception of the SCA x environment interaction for PHER. This indicates that the conversion efficiency ratios are as sensitive to environmental fluctuations as the individual glucosinolates.

For oil and protein concentration, the parent mean square was non-significant, but the hybrids mean square and the F1's vs. parents contrast were both highly significant. The contrast accounted for 21 % and 7 % of the oil and protein entry sums of squares respectively. The hybrids were, on average, 0.80 % higher in oil and 0.50 % lower in protein (Table 5.2). The higher oil concentration was likely the result of heterosis and the reduced protein the result of the inverse relation between oil and protein (Table 5.3). GCA and SCA were highly significant for both oil and protein (Table 5.1). GCA made up 79 % and 75 % of the oil and protein hybrid sums of squares, illustrating the importance of additive genetic effects in the control of these characters, in this sample of hybrids. Grant and Beversdorf (1985) found that SCA was more important than GCA, for oil and protein concentration in their sample oilseed rape hybrids, reflecting the fact that estimates of GCA and SCA are relative to the sample of lines used to make the hybrids.

For yield, all the main effects were significant. The contrast F_1's vs parents, accounted for 66 % of the cross sums of squares which indicates that heterosis was a

major component of the variation among entries. The yields of the hybrids were, on average, 80 % higher than their parents (Table 5.2), confirming the results of the analysis of variance. GCA and SCA made up 39 % and 61 % of the cross sums of squares respectively. The prescence of significant SCA indicates that there are fluctuations in dominance relations among the parents. Grant and Beversdorf (1985) also found a perponderance of SCA in their experiments. Brandle and McVetty (1989b) found GCA to predominate in their work, indicating that the dominance relations among the parents in their crosses were consistent and also reflecting the fact that the estimates GCA and SCA are relative to each set of parents and hybrids. The (F_1's vs. parents) x environments interaction was significant indicating that heterosis was not expressed consistently across environments.

Correlations

BUT and HOBUT, and PENT and HOPENT were positively correlated with each other (Table 5.3), as were BHER and PHER reflecting the common hydroxylation step that has been proposed for both BUT and PENT (Gland 1982). The positive correlation between both BUT and PENT, and HOBUT and PENT reflects the origin of BUT and PENT from a common amino acid substrate (Underhill 1980). The positive correlation between BUT, PENT, HOBUT and HOPENT, and total aliphatic glucosinolates reflects the fact that the individual glucosinolates are a linear function of the total.

Yield was negatively correlated to HOBUT and total aliphatic glucosinolates, and positively correlated to oil concentration (Table 5.3). The negative correlations may be the result of dilution of a constant amount of glucosinolates in an increased amount of seed. The increased amount of seed having resulted from heterosis in the hybrids. The positive correlation between yield and oil was probably the consequence of vigorous hybrids, having efficient biosynthetic systems, being capable of producing both high

yields and high oil concentrations. Oil and protein concentration were strongly negatively correlated, a result similar to that found by Grami et al. (1977). PENT and HOPENT were positively correlated to protein concentration, possibly the result of linkage. BUT was negatively correlated to protein concentration which may reflect competition for substrate. PENT and HOPENT were negatively correlated with oil concentration which may be a function of their positive relationship to protein concentration and the strong negative correlation between oil and protein concentration. The net result of this relationship is that selection for lower levels of five carbon glucosinolates may result an increase in oil concentration.

The results of this investigation indicate that glucosinolate concentration in crosses among this sample of low glucosinolate, low erucic acid inbred lines is mainly controlled by additive gene action and that heterosis did not have any negative impact on glucosinolate levels in these single cross hybrids. Glucosinolate concentration was found to exhibit some detrimental correlations with yield, oil and protein, but the relationships are probably not sufficiently strong to be of great consequence in a breeding program.

SUMMARY

General combining ability for seed meal glucosinolates, oil and protein concentration was found to be more important than specific combining ability in all cases except yield, where the two were found to be equally important. Heterosis did not act to increase levels of the four aliphatic glucosinolates or protein concentration, but there was significant positive heterosis for both yield and oil concentration. Total glucosinolate concentration was negatively correlated with yield, which appeared to be the result of a dilution effect.

The results of this investigation indicate that glucosinolate concentration in crosses among low glucosinolate, low erucic acid, inbred lines is mainly controlled by additive gene action. Heterosis did not have any negative impact on glucosinolate levels in this sample of single cross hybrids. Glucosinolate concentration was found to be detrimentally correlated with yield, oil and protein, but the relationships were not suffuciently strong to be of great consequence in a breeding program.

Table 5.1. Analysis of variance for 3-butenyl (BUT), 4-pentenyl (PENT), 2-hydroxy-4-butenyl (HOBUT), 2-hydroxy-4-pentenyl (HOPENT) and total aliphatic glucosinolates (TOTAL), butenyl (BHER) and pentenyl (PHER) hydroxylation efficiency ratio, yield (YLD), oil and protein (PRO).

Source	df	BUT	PENT	HOBUT	HOPENT	TOTAL	BHER	PHER	YLD	OIL	PRO
		Mean Squares									
Rep(R)	6	0.038	0.007	0.011	0.001	0.019	8.24	18.92	3.67	27.41	40.51
Env(E)	2	1.335	0.443	2.325	0.115	2.063	79.70	298.69	16.81	131.53	124.36
Entry(En)	27	0.434**	0.196**	0.796**	0.136**	0.556**	184.33**	774.31**	6.47**	5.26**	6.85**
P v. F_1	1	0.299**	0.016	0.580**	0.598**	0.531**	17.18	79.58	114.48**	29.91**	13.60**
Parent(P)	6	0.399	0.309	1.230**	0.254**	0.685	378.20**	1546.57**	1.04*	7.27	13.38
Hybrid(H)	20	0.451**	0.171**	0.678**	0.105**	0.520**	135.07**	579.24**	2.62**	3.41**	4.53**
GCA	6	1.386**	0.518**	2.003**	0.292**	1.539**	392.85**	1841.12**	3.40**	8.99**	11.24**
SCA	14	0.051**	0.022**	0.110**	0.025**	0.083**	24.57**	38.43*	2.29**	1.02**	1.65**
En x E	54	0.028**	0.013**	0.038**	0.006**	0.031**	9.57**	23.99**	0.66**	1.30**	1.41**
P v F_1 x E	2	0.004	0.001	0.370**	0.007**	0.049	76.94**	73.01**	1.53**	0.12	0.49
P x E	12	0.240**	0.125**	0.125**	0.054**	0.309**	122.13**	436.41**	0.318	18.47**	23.33**
H x E	40	0.025*	0.011**	0.027**	0.005**	0.025**	6.10**	21.82**	0.70*	0.92	1.17*
GCA x E	12	0.037**	0.015**	0.083**	0.007**	0.043**	12.57**	30.51**	0.97**	2.61**	2.64**
SCA x E	28	0.020	0.009**	0.003	0.004**	0.017	3.34**	18.10	0.59	0.20	0.54
Error	160	0.017	0.003	0.017	0.001	0.016	1.87	14.63	0.50	0.54	0.80
CV %		10.16	12.26	7.19	19.93	5.40	2.09	17.93	22.68	1.64	3.41

*,** significant at P = 0.05 and 0.01 respectively.

Table 5.2. Hybrid and parental means for 3-butenyl (BUT), 4-pentenyl (PENT), 2-hydroxy-4-butenyl (HOBUT), 2-hydroxy-4-pentenyl (HOPENT) and total aliphatic glucosinolates (TOTAL) (mmol g-1 seed), butenyl (BHER) and pentenyl (PHER) hydroxylation efficiency ratio, yield (YLD, t ha^{-1}), oil (%) and protein (PRO, %).

CROSS	BUT	PENT	HOBUT	HOPENT	TOTAL	BHER	PHER	YLD	OIL	PRO
				Hybrids						
WST/MNO	2.75	0.33	5.20	0.12	8.40	65.3	25.6	4.183	45.2	25.2
WST/KRT	2.47	0.40	3.58	0.05	6.51	59.0	10.5	3.884	45.3	26.2
WST/RGT	3.11	0.24	6.94	0.05	10.34	69.1	17.3	2.513	44.5	26.2
WST/ARL	3.41	0.41	6.69	0.08	10.59	66.5	17.3	3.501	45.4	25.2
WST/LGO	2.83	0.41	5.32	0.11	8.67	65.2	21.3	4.372	44.9	25.9
WST/R83	3.77	0.34	6.42	0.07	10.60	63.0	17.4	3.849	45.6	25.2
MNO/KRT	2.70	0.65	4.52	0.22	8.09	62.2	24.1	3.839	43.7	27.1
MNO/RGT	2.98	0.33	5.83	0.12	9.27	66.1	26.8	4.105	43.9	26.3
MNO/ARL	3.80	0.86	8.23	0.37	13.26	68.2	30.6	3.306	43.7	26.6
MNO/LGO	3.77	0.90	8.57	0.65	13.89	69.2	42.4	3.661	43.5	27.2
MNO/R83	4.96	0.52	8.21	0.25	13.94	62.4	31.7	3.181	45.0	24.6
KRT/RGT	2.91	0.41	3.84	0.03	7.19	56.9	6.7	3.827	44.7	26.9
KRT/ARL	3.19	0.61	4.46	0.08	8.34	58.1	11.9	2.857	45.0	26.6
KRT/LGO	3.01	0.78	4.74	0.21	8.74	60.8	20.8	2.874	44.3	27.1
KRT/R83	4.00	0.82	5.11	0.13	10.06	55.9	13.2	3.642	45.4	25.6
RGT/ARL	3.62	0.44	7.46	0.09	11.61	67.2	17.5	2.326	45.1	25.7
RGT/LGO	3.42	0.38	6.00	0.11	9.91	63.6	22.9	3.918	44.7	26.5
RGT/R83	5.08	0.31	7.80	0.07	13.26	60.6	17.2	2.932	45.2	26.0
ARL/LGO	3.64	0.83	7.39	0.26	12.12	66.7	23.7	3.905	44.5	26.6
ARL/R83	4.67	0.89	8.02	0.20	13.77	63.1	18.5	3.343	45.3	25.6
LGO/R83	5.65	0.74	9.15	0.26	15.80	61.7	26.1	4.051	45.1	25.6
SE	0.34	0.04	0.34	0.02	0.34	0.8	1.6	0.279	0.3	0.4
MEAN	3.47	0.58	6.36	0.17	10.49	63.4	21.1	3.53	44.8	26.1
				Parents						
WST	3.03	0.24	5.96	0.04	9.28	66.1	14.2	2.064	44.6	25.8
MNO	3.43	0.69	8.15	0.58	12.85	70.4	45.5	1.693	43.1	27.0
KRT	3.02	0.75	3.31	0.08	7.17	52.1	9.8	1.595	43.2	28.4
RGT	3.93	0.30	7.50	0.05	11.78	65.9	13.0	1.950	43.7	27.1
ARL	4.90	1.00	10.94	0.23	17.07	68.7	18.2	1.623	44.3	26.0
LGO	3.81	0.83	7.77	0.50	12.90	66.9	37.4	2.474	43.7	27.3
R83	5.09	0.35	7.11	0.09	12.64	57.9	19.5	2.289	45.5	24.6
SE	0.16	0.12	0.12	0.08	0.19	3.7	7.0	0.188	1.4	1.6
MEAN	3.79	0.60	6.82	0.22	11.36	64.0	22.5	1.96	44.0	26.5

Table 5.3. Pooled correlation coefficients between various traits measured from hybrids and parents, grown in three environments during 1987 and 1988.

Trait	BUT	PENT	HOBUT	HOPENT	TOTAL	BHER	PHER	OIL	PRO
YIELD	-0.18	-0.14	-0.27*	-0.08	-0.26*	-0.10	0.03	0.32*	-0.18
BUT		0.29*	0.74*	0.20	0.86*	-0.02	0.15	0.17	-0.32*
PENT			0.38*	0.67*	0.48*	0.08	0.34*	-0.30*	0.30*
HOBUT				0.52*	0.97*	0.63*	0.50*	-0.14	-0.17
HOPENT					0.53*	0.48*	0.88*	-0.48*	0.27*
TOTAL						0.46*	0.47*	-0.10	-0.17
BHER							0.59*	0.35*	-0.02
PHER								-0.32*	0.11
OIL									-0.82*

* significantly different from zero at P = 0.05, df = 78.

GENERAL DISCUSSION

Gowers (1981) illustrated that swede cultivars may be differentiated into subgroups, at various levels of inbreeding. Given the similar reproductive biology of swede and oilseed rape, this same type of differentiation probably applies to oilseed rape as well. The need for pure or inbred lines in breeding and genetic studies in swede has already been recognized (Bradshaw 1986). However, the variability within oilseed rape had not been characterized nor had its wider significance in terms of hybrid oilseed rape breeding been considered. The first portion of the research presented herein addresses these questions.

Oilseed rape was found to contain significant levels of genetic variation, mostly due to additive differences among genotypes within cultivars. This situation is very much different than that found in maize, where segregating genes with dominance play an important role (Hallauer and Sears 1973) in the expression of most agronomically significant characters. The results of this work also showed that inbred lines could be extracted from oilseed rape cultivars that were as good or better than their source cultivars, a similar result was found for swede (Gowers and Gemmell 1988). It would seem apparent, in the face of this evidence, that there exists some potential for short term improvement of oilseed rape by simply re-selecting existing cultivars.

Considering the genetic variability that exists within cultivars, it appeared to be wise to consider the effect of this variability on the breeding of hybrid oilseed rape cultivars. This was done by measuring the variability among crosses, between inbred lines extracted from parents of known heterotic cultivar crosses. The results of these experiments did confirm that the genetic variability found in the first experiments, could be extended to include variation in general combining ability and to the levels of heterosis found in inbred line derived hybrids. A similar result was found for maize in 1910 (Shull 1952), where F_1 plants from heterotic cultivar crosses were found to vary in

relative heterosis. In general, this indicates that oilseed rape cultivar hybrids, like those in maize, are only expressing average heterosis. Heterosis for seed yield can only be increased by selecting within the parents of heterotic cultivar crosses, for inbred lines that will give maximum heterosis upon crossing.

Love (1988) showed that genes involved in the Bronowski block were partially dominant. If the remaining loci responsible for glucosinolate synthesis were also dominant, then heterosis may have a negative impact on glucosinolate concentration in hybrids from low glucosinolate parents. A diallel cross among low glucosinolate inbred lines showed that the glucosinolate content in the hybrids was mainly controlled by additive gene action and that heterosis, and therefore dominance, was not a factor. There was however substantial heterosis for seed yield and oil concentration, indicating that seed meal quality restrictions should not present a barrier to the production of hybrid oilseed rape cultivars. Breeders can feel free to utilize the genetic variability found within cultivars to initiate their hybrid breeding programs. Sustained progress would however, require the development of some type of population improvement program.

SUMMARY/CONCLUSION

Reports of substantial heterosis in inter-cultivar hybrids of oilseed rape have led to the development of a large plant breeding effort directed towards to the creation of hybrid oilseed rape cultivars. Early research had assumed that the cultivars that had been used to demonstrate heterosis, were genetically homogenous. The effects of genetic variability within cultivars on the expression of heterosis were not considered. The effects of heterosis on glucosinolate content in seed meal of hybrids had not been investigated. The purpose of the work presented herein was to measure levels of genetic variability and to lay some of the basic genetic foundations, necessary for the development of hybrid oilseed rape breeding programs.

It was clearly established that the oilseed rape cultivars used in this study contained significant levels of gentic variability and that these same cultivars were not greatly affected by inbreeding. The presence of significant additive genetic variation implied that selection within cultivars may be effective, and that some short term progress can be made, simply by reselecting existing cultivars. A corollary of this conclusion is that intra-cultivar genetic variation should not be ignored in future oilseed rape breeding research. The low levels of inbreeding depression suggested that dominance was not a major factor in the genetic determination of the characters investigated. Inbred lines were found that performed as well as, or better than, their source cultivars, illustrating that genetic heterogeneity is not essential to the performance of oilseed rape cultivars.

Intra-cultivar genetic variability was found to have wider implications in terms of hybrid development. Inbred lines, extracted from the parents of heterotic cultivar crosses, were found to vary in terms of their performance in hybrid combination. While most crosses between these inbred lines did exhibit heterosis, some were found to have significantly higher yields and others significantly lower yields than their corresponding

cultivar hybrids. Given this fact, it follows that hybrid oilseed rape breeding programs should be based on pure line crosses, rather than cultivar crosses. In general, cultivar crosses could be used to establish heterotic patterns, followed by the development of inbred lines from within the cultivars, then top-crossing to identify good general combiners. The selected inbred lines can then be crossed, to establish which combinations give maximum heterosis. Mid-parent yield may be a useful guide to the selection of parents to be tested as hybrids.

A diallel cross among seven canola quality inbred lines of diverse origin showed significant heterosis for yield and oil concentration, but not for aliphatic glucosinolate content. Substantial heterosis for yield actually led to the dilution of, and a consequent reduction in levels of, the four alphatic glucosinolates in the seed meal of single cross hybrids. The results of the diallel also indicated that glucosinolate content was mainly controlled by additive gene action. The net result is that heterosis should not have any negative impact on glucosinolates levels, in single cross hybrids from canola quality inbreds.

RECOMMENDATIONS FOR FURTHER STUDY

While this research has provided some of the information necessary to establish an effective hybrid breeding program, a significant effort is still required before the production of hybrid oilseed rape cultivars becomes routine. The existence of heterosis in oilseed rape is well known. The requisite techniques needed to evaluate and exploit heterosis have been long established in other crops and could be transferred, with some modification, to oilseed rape. Cytoplasmic male sterility (CMS) is available, but is imperfect and remains as a significant impediment to the success of hybrid oilseed rape. Incomplete sterility of the female parent is a serious problem in seed production, as is the biological cost of this cytoplasm. A substantial research effort is essential in order to fully characterize these problems and to formulate practical solutions.

Alternative pollination control systems, such as self-incompatability (SI) and genetic male sterility (GMS), need to be investigated and their biological cost compared to that of CMS. Practical methods of seed production, based on SI and GMS are essential before any of these systems could replace CMS.

If heterosis in oilseed rape were due to repulsion phase linkage of dominant genes alone, then selection within segregating populations from heterotic crosses may give some very productive pure lines. The yields of these pure lines should be comparable to their hybrid parents. However, the probability of obtaining a pure line with all the dominant genes is very low. Recombination of superior lines and reselection of the subsequent populations, better known as recurrent selection, may improve the probability of obtaining pure lines equal to the hybrid. This hypothesis needs to be tested.

Microspore culture has great potential to reduce the time required for the creation of inbred lines, provided that certain genotypes are not favoured in culture, and the breeder is left with a non-representitive sample of lines. The efficacy of emergent techniques, such as microspore culture, require investigation.

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Appendix I. Expected mean squares for an analysis of variance of inbred lines repeated over environments, and examples of narrow sense heritability and standard error calculations.

Source	df	MS	Expected Mean Square
Environment(E)	e-1		
Reps/E	e(r-1)		
Inbreds	g-1	M_3	$\sigma^2_e + r\sigma^2_{ge} + re\sigma^2_g$
I x E	(e-1)(g-1)	M_2	$\sigma^2_e + r\sigma^2_{ge}$
Error	e(r-1)(g-1)	M_1	σ^2_e

- where e, r and g are the number of environments, replications and inbred lines respectively.

$h^2 = \sigma^2_a/[(\sigma^2_e/re)+(\sigma^2_{ge}/e)+(\sigma^2_g)]$, where $\sigma^2_a = 0.5(\sigma^2_g)$

$$SE(h^2) = 0.5\sqrt{\frac{[(2/(re)^2)\ ((M2_3/g+1)+(M2_2/((e-1)(g-1)+2)))]}{(\sigma^2_e/re)+(\sigma^2_{ge}/e)+(\sigma^2_g)}}$$

Appendix 2. Analysis of variance and expected mean squares for a factorial mating design combined over environments.

Source	df	Expected Mean Square
ENVIRONMENTS(E)	e-1	
REPS within E	e(r-1)	
ENTRIES(N)	n-1	$\sigma^2_e + r\sigma^2_{ne} + er\phi_n$
Groups(G)	g-1	$\sigma^2_e + r\sigma^2_{ge} + er\phi_g$
Cultivars(C)	c-1	$\sigma^2_e + r\sigma^2_{ce} + er\phi_c$
Inbreds(I)	i-1	$\sigma^2_e + r\sigma^2_{ie} + er\phi_i$
Parents vs F1's(V)	1	$\sigma^2_e + r\sigma^2_{ve} + er\phi_v$
Parents(P)	p-1	$\sigma^2_e + r\sigma^2_{pe} + er\phi_p$
Hybrids(H)	mf-1	$\sigma^2_e + r\sigma^2_{he} + er\phi_h$
Males(M)	m-1	$\sigma^2_e + rf\sigma^2_{me} + erf\phi_m$
Females(F)	f-1	$\sigma^2_e + rm\sigma^2_{fe} + erm\phi_f$
M x F	(m-1)(f-1)	$\sigma^2_e + r\sigma^2_{mfe} + er\phi_{mf}$
En x E	(n-1)(e-1)	$\sigma^2_e + r\sigma^2_{ne}$
G x E	(g-1)(e-1)	$\sigma^2_e + r\sigma^2_{ge}$
Cu x E	(c-1)(e-1)	$\sigma^2_e + r\sigma^2_{ce}$
I x E	(i-1)(e-1)	$\sigma^2_e + r\sigma^2_{ie}$
(P vs. F1) x E	(e-1)	$\sigma^2_e + r\sigma^2_{ve}$
P x E	(p-1)(e-1)	$\sigma^2_e + r\sigma^2_{pe}$
H x E	(mf-1)(e-1)	$\sigma^2_e + r\sigma^2_{he}$
M x E	(m-1)(e-1)	$\sigma^2_e + rm\sigma^2_{me}$
F x E	(f-1)(e-1)	$\sigma^2_e + r\sigma^2_{fe}$
M x F x E	(m-1)(f-1)(e-1)	$\sigma^2_e + r\sigma^2_{mfe}$
ERROR	e(n-1)(r-1)	σ^2_e

Appendix 3. Calculation of general combining ability effects and standard errors for a factorial mating design combined over environments.

	A	B	C	D	
P	x	x	x	x	$X_{p.}$
Q	x	x	x	x	$X_{q.}$
R	x	x	x	x	$X_{r.}$
	$X_{.a}$	$X_{.b}$	$X_{.c}$	$X_{.d}$	$X_{..}$

$GCA_A = X_{.a} - X_{..}$

$SE\ GCA_{(male)} = \sqrt{\{MS_{me}\ [(f-1)/mfer]\}}$

$SE\ GCA_{(female)} = \sqrt{\{MS_{fe}\ [(m-1)/mfer]\}}$