UNIVERSITY OF ALBERTA

INHERITANCE OF POD LENGTH, AND THE INTERRELATIONSHIPS OF POD AND SEED TRAITS WITH YIELD AND QUALITY IN *BRASSICA NAPUS* L.

by

DENG JIN BING ©

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in

PLANT BREEDING

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "**INHERITANCE OF POD LENGTH, AND THE INTERRELATIONSHIPS OF POD AND SEED TRAITS WITH YIELD AND QUALITY IN *BRASSICA NAPUS* L.**" submitted by **DENG JIN BING** in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Breeding.

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DEDICATION

TO THE MEMORY OF MY GRANDMOTHER WHO TAUGHT ME

"Be truthful and diligent in your conduct"

"Do not feel disgraced in your plain clothes as long as you get yourself educated"

ABSTRACT

Inheritance of pod length, and interrelationships of seed yield, pod length, pod number per plant (PNP), seed set per pod (SSP), seed weight, seed oil, and protein content in *Brassica napus* were investigated.

Three doubled haploid (DH) families were produced through microspore culture of F_1 plants from crosses between the long-podded (approx. 12 cm) and normal-podded (approx. 7 cm) genotypes of spring *B. napus*. F_2, and BC_1 were also produced from F_1 plants. These progeny populations and their parents were grown at the Edmonton Research Station of the University of Alberta, Edmonton, Alberta in 1993 and/or 1994. DH lines were tested in the field at two row spacing and plant densities, i.e. 23 cm row spacing and not thinned, or 46 cm row spacing and thinned to approx. 20 cm between adjacent plants. Nested, completely randomized, randomized complete block (RCB), or split plot in RCB were used in field layouts. In addition, seven genotypes were tested at three locations in Alberta in 1993-94.

These studies showed that the inheritance of pod length fitted a model of three independent nuclear genes with additive effect. In general, pod length had positive associations with SSP and seed weight, and had no relationship with seed oil or protein content. Pod length had a weak compensating relationship with PNP, but this relationship should not be a major concern when selecting for high seed yield. Pod length had a direct negative effect on seed yield, and the long-podded genotypes had no seed yield advantage over genotypes with normal pod length. A high value of SSP was a distinctive characteristic of a high-yielding genotype. Genotypes with small seeds were desirable to produce high seed yield and seed oil under unfavorable environmental conditions. Seed yield and seed oil content were often positively associated. Seed oil and protein content were negatively correlated only when environmental conditions were unfavorable.

Spaced plant density resulted in a marked increase in PNP, and slight increases in pod length and SSP, but slight decreases in 1000 seed weight and seed protein content. Seed oil content was insensitive to changes in plant density.

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LIST OF SYMBOLS AND ABBREVIATIONS

σ_{ij} - covariance of trait i and trait j

σ_i - standard deviation of a trait i

σ_j - standard deviation of a trait j

σ_P^2 - total phenotypic variance of a trait

σ_{BCP1}^2 - variance of a trait in BCP1

σ_{BCP2}^2 - variance of a trait in BCP2

σ_{F1}^2 - variance of a trait in F_1

σ_{F2}^2 - variance of a trait in F_2

σ_{P1}^2 - variance of a trait of parent 1 (P1)

σ_{P2}^2 - variance of a trait of parent 2 (P2)

σ_A^2 - variance of additive effect of gene(s)

σ_D^2 - variance of dominant effect of gene(s)

σ_E^2 - variance of environmental effect

σ_G^2 - total genotypic variance

ANOVA - analysis of variance

b_i - partial regression coefficient

B5 - defined medium for washing microspores and *in vitro* culture of microspore-derived embryos

BC1 - first generation of backcross

BCP1 - first generation of backcross to parent 1

BCP2 - first generation of backcross to parent 2

CMS - cytoplasmic male sterility

DH - doubled haploid

DNA - deoxyribonucleic acid

F_1 - first filial generation

F_2 - second filial generation

G1 - *B. napus* DH line 90-3011 with normal pods (approx. 7 cm)

G3 - *B. napus* DH line 881426K

G4 - *B. napus* DH line 90-3071 with normal pods (approx. 8 cm)

GD - *B. napus* DH line 91-1152 with long pods (approx. 12 cm)

h^2 - heritability

h_B^2 - heritability in the broad sense

h_N^2 - heritability in the narrow sense

HIOIL - *B. napus* DH line 92-21213

HIYLD - *B. napus* DH line 90-20504

LPHIPR - *B. napus* DH line 91-54165NK

L^xL^y - genetic formula for a genotype (see 3.3.2.1 for detail)

M6, M7, M8 - three microspore-culture-derived DH line families

MT - metric tonne

NLN - defined culture medium for *in vitro* culture of microspores (Appendix B)

NMR - nuclear magnetic resonance

P_{0i} - path coefficient from trait i to trait 0 (see 4.2 for detail)

P1 - parent 1

P2 - parent 2

PL - pod length

PNP - pod number per plant

$r(x,y)_g$ - genotypic correlation between trait x and trait y

$r(x,y)_p$ - phenotypic correlation between trait x and trait y

RAPD - random amplified polymorphic DNA

RCB - randomized complete block design

RFLP - restriction fragment length polymorphism

r_{ij} - Pearson correlation coefficient between trait i and trait j

r_s - Spearman (rank) correlation coefficient

S_{lg}^2 - variance component of location x genotype

S_p^2 - total phenotypic variance

S_g^2 - variance component of genotype

S_r^2 - variance component of residual

S_{yg}^2 - variance component of year x genotype

S_{ylg}^2 - variance component of year x location x genotype

S^2 - sample variance

SSP - seed set per pod

χ^2 - Chi-square

CHAPTER 1
INTRODUCTION

In the Cruciferae (syn. *Brassicaceae*) family, *Brassica napus* L., *B. juncea* (L.) Czern, and *B. rapa* L. (syn. *B. campestris* L.) are predominantly cultivated for the production of vegetable oil. These three species are collectively referred to as oilseed brassicas and usually known as rapeseed.

Traditional rapeseed cultivars contain two characteristic components, erucic acid in the extracted oil, and glucosinolates in the residual meal. In many modern cultivars both components have been greatly reduced and such cultivars are referred to as double low cultivars. In Canada, a generic name, canola, has been coined to differentiate double low cultivars from traditional ones (Adolphe 1974). Currently, canola is defined as containing less than 2% erucic acid of the total fatty acids in the extracted seed oil and less than 30 μmol g^{-1} of aliphatic glucosinolates in the air-dried, oil-free residual meal (Downey and Rimmer 1993). Hereafter, rapeseed will be used to describe the oilseed brassicas, and canola will be used to describe double low cultivars.

Rapeseed has played an ever increasing role in the world's agricultural commodities (Fig. 1-1). Currently, it is the third most important vegetable oilseed crop in the world after soybean (*Glycine max*) and cottonseed (*Gossypium* spp.), with an overall production of 29.958 million metric tonnes (MT) in 1994 (Food and Agricultural Organization (FAO) 1994). Given the increasing demand for edible vegetable oil to provide the growing human population with an adequate diet in developing countries, and the growing interest in non-edible uses of oilseed crops in developed countries (Murphy 1994), it can be reasonably expected that rapeseed production in the world will continue to increase.

Rapeseed has become an important crop in Canada since its establishment as a commercial crop in the early 1940s (Canola Council of Canada 1988). Canada has been recognized as a world leader in canola research and production for the last two decades. It ranks among the three largest rapeseed growing countries in the world (Fig. 1-1). The rapeseed growing area in Canada has increased from approximately 1300 hectares (ha) in 1943 (Fig. 1-2) to 5.8 million ha in 1995 (Statistics Canada 1995a), while the production has increased from 1 MT in 1943 (Statistics Canada 1995a) to 7.2 million MT in 1994 (FAO 1994). On average, acreage and production have been increasing at an annual rate of over 84,000 ha. and 136,000 MT from 1943 to 1995, respectively. With the current momentum of research on canola/rapeseed and the increasing international interest in canola production in this country, it is likely that canola/rapeseed production in Canada will continue to increase.

Rapeseed is a crop adapted to various climatic conditions and grown in every continent in the world. Its seed is rich in oil which can be used in the human diet or for industrial purposes. The protein-rich residual meal of canola following extraction of the oil is a desirable feedstock for domestic animals. The amenability of rapeseed to biological and genetic manipulations places this crop in the forefront of current biological and genetic studies among field crops. Haploid production through microspore culture has been routinely used for inbred line production. Transgenic *B. napus* cultivars resistant to the herbicide glufosinate (Oelck et al. 1995) or glyphosate (Stringam, pers. communication) are currently being marketed. Transgenic *B. napus* lines carrying fertility control genes have been used for hybrid seed production (Mariani et al. 1990, Downey and Rimmer 1993). It has been demonstrated that fatty acid profiles of rapeseed can be significantly modified for producing specialized oil through transformation with only a single gene alteration (Knutzon, et al. 1992, Voelker et al. 1992). Genetic maps based on restriction fragment length polymorphism (RFLP) and/or random amplified polymorphic DNA (RAPD) markers are available for *B. rapa* (Song et al. 1990, Chyi et al. 1992, Teutonico and Osborn 1994), *B. napus* (Landry et al. 1991, Uzunova et al. 1995, Ferreira et al. 1994), and the related species *B. oleracea* (Slocum et al. 1990, Landry et al. 1992, Kianian and Quiros 1992) and *B. nigra* (Truco and Quiros 1994). RFLP and/or RAPD markers have been identified in *B. napus* and closely related species for some specific traits, including glucosinolates and their regulation (Magrath et al. 1993, Magrath et al. 1994, Uzunova et al. 1995), vernalization and flowering time (Ferreira et al. 1995), and fertility restorer genes for Ogura type cytoplasmic male sterility (CMS) (Delourme et al. 1994) in *B. napus*, yellow seed color and low seed erucic acid in *B. rapa* (Teutonico and Osborn 1994), self-incompatibility (Boyes et al. 1991) and resistance to clubroot disease (*Plasmodiophora brassicae*) (Figdore et al. 1993) in *B. oleracea*. These genetic maps and molecular markers will greatly facilitate selection for specific trait(s) in rapeseed breeding.

Despite the great success in the improvement of the nutritional value of rapeseed oil and meal, and the exciting advances of biological and molecular studies on rapeseed, improvement in seed yield, oil and protein content of rapeseed has been a gradual process over time. For example, there has been very little improvement of the seed yield in any of the canola/rapeseed production regions in Canada over the 10 year period from 1983 to 1993 (Fig. 1-3). According to the data of Statistics Canada (1995b), on average of all species grown in Canada from 1983 to 1993, the annual seed yield increase rate was only 0.15 kg ha^{-1} in Ontario, 0.11 in Manitoba, 0.09 in Saskatchewan, 0.11 in Alberta, 0.10 in British Colombian (BC), and 0.10 across Canada, respectively. Consequently, emphasis in rapeseed breeding has shifted in recent years from quality improvement to the improvement of seed yield.

Previous studies have shown that rapeseed pods contribute significantly to the developing seeds in nutrients and photosynthate transportation (see Chapter 2, 3 and 4 for details). It was once thought that seed yield could be improved by improving the pod number per plant. However, excess pod number is detrimental to the final seed yield (see Chapter 2). Alternatively, it might be possible to improve seed yield by selection for longer pods with more seeds per pod and increased seed weight.

B. napus germplasm developed at the University of Alberta by crossing Chinese with Canadian *B. napus* germplasm is characterized by long pods (11-13 cm). These long-podded genotypes seem to have more seeds per pod and larger seeds than normal *B. napus* genotypes. However, the inheritance of the long-podded trait and relationships of pod length with seed size, oil and protein content, total seed yield, as well as other agronomic characteristics are not well understood. This project was initiated to address these problems.

The primary objectives of the project were as follows: (1) To study the inheritance of the long-podded character using doubled haploid (DH) populations, and conventional genetic analysis. These studies are presented in Chapter 2 and Chapter 3. (2) To determine the interrelationships of pod length, seed size, oil and protein contents, and other agronomic traits, which are reported in Chapter 2, 3 and 4. (3) To investigate the effects of row spacing and plant density on these traits and their interrelationships, which is reported in Chapter 4 and 5. (4) To investigate the effects of environmental conditions on the performance of the above traits under variable environmental conditions over years and locations in the area of central Alberta, and to understand the nature and degree of genotype x environment interactions for these traits. These studies are presented in Chapter 6. The information from these studies should assist rapeseed breeders in designing future breeding programs and in making selections more effectively. Moreover, it is anticipated that the best lines produced from this study could be used as breeding lines upon the completion of the project. Finally, results from all studies are discussed and summarized in Chapter 7.

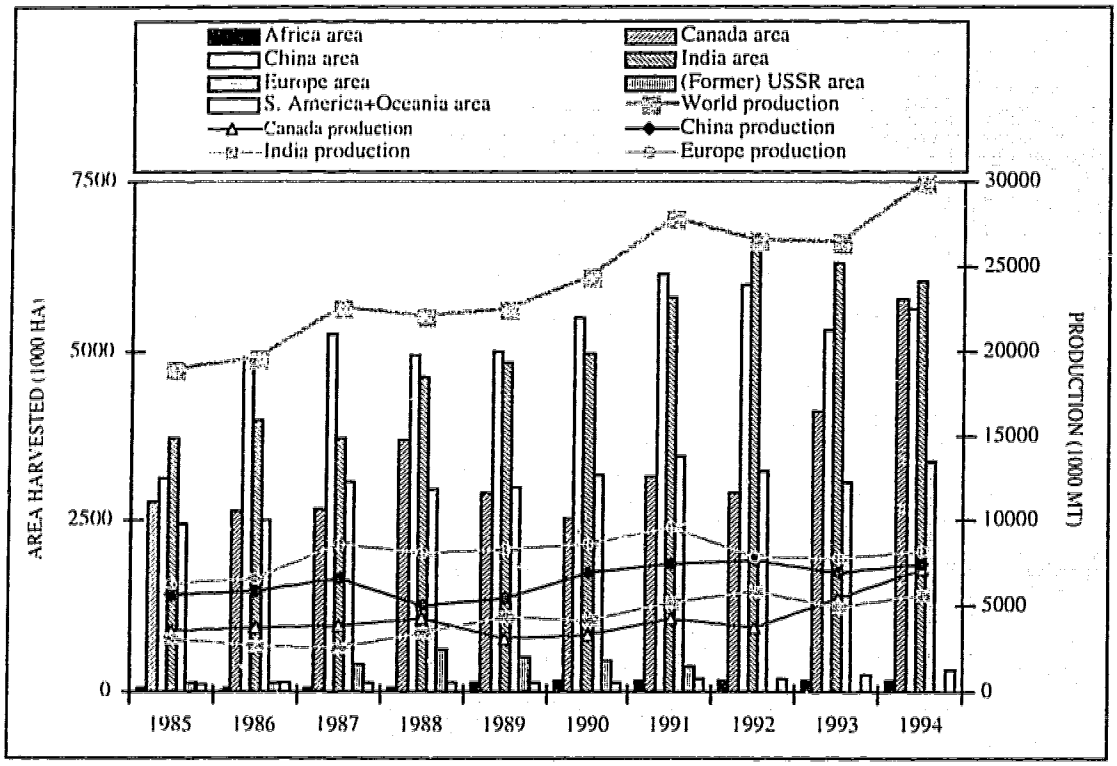

Figure 1-1. Harvested area and total production of rapeseed/canola of the world from 1985 to 1994 (abridged from Table 41, FAO Production Yearbook, Vol. 41-48.)

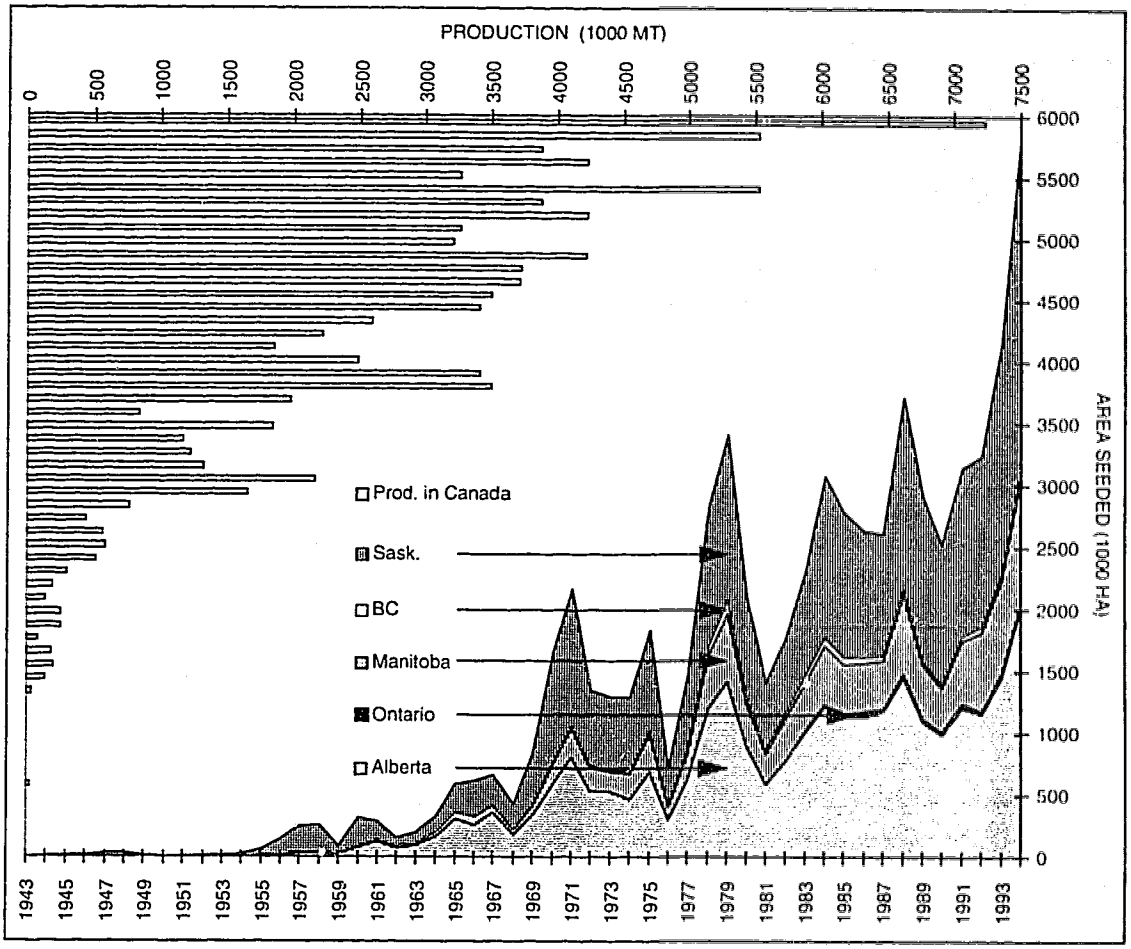

Figure 1-2. Area seeded and production of rapeseed/canola in Canada from 1943 to 1994 (abridged from Statistics Canada CANSIM database, D216568, D229945, D216566, D216569 and D216576, October, 1995).

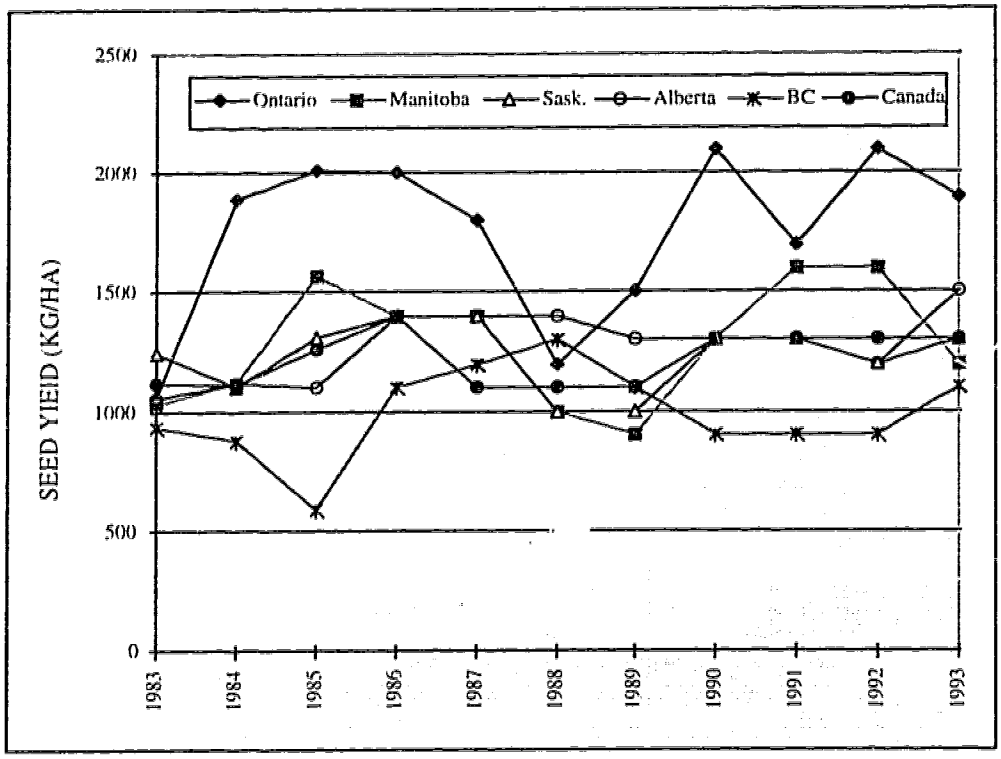

Figure 1-3. Average canola seed yield in four provinces and across Canada from 1983 to 1993 (abridged from Statistics Canada CANSIM database, D229946, D216572-D216575 and D216570, October, 1995).

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CHAPTER 2

INHERITANCE AND RELATIONSHIP OF POD LENGTH AND SEED SET PER POD IN *BRASSICA NAPUS* L.

2.1 INTRODUCTION

Breeding for high seed yield is a primary objective in rapeseed cultivar development. Various breeding methodologies for high seed yield have been developed over the years, and these can be generalized into three categories: 1) exploitation of heterosis of two or more parents with good combining ability; 2) improvement of defect(s) of an otherwise 'good' cultivar by various approaches such as backcrossing, introgression, or somaclonal variation; or 3) screening for high-yielding progenies following hybridization of two parents using conventional pedigree or doubled haploid approaches. However, seed yield improvement of rapeseed has been a very gradual process (see Chapter 1) and alternatives for seed yield improvement remain to be investigated.

Extensive effort has been devoted to identify some simple morphological characters which may be used as a selection criteria for high seed yield. Previous studies in *B. napus* have shown that pods can transport nutrients and photosynthates to the developing seeds (Allen et al. 1971, Norton and Harris 1975, Brar and Thies 1977, Zhang et al. 1991). Similar findings were reported in *B. rapa* (Sheoran et al. 1991). Subrahmanyam and Rathore (1992a, b) reported in *B. juncea* that leaves, stem and pods were equally important as sources of carbon for developing seeds at the ripening stage. In *B. napus* Richards and Thurling (1979) observed high genetic correlation between seed yield and pod number (r = 0.90), and also significant correlation between seed yield and seed number per pod (r = 0.42). A variable but highly positive correlation between pod length and seed weight was observed in *B. napus* (Chay and Thurling 1989a, b), and the authors concluded that seed yield improvement in *B. napus* could be achieved through introgression of long-pod genes into cultivars with an appropriate genetic background. Similar observations were also reported in *B. rapa* where pod length was positively correlated with seed number per pod (Lebowitz 1989).

Given the significant role of rapeseed pods in seed yield, it appeared that seed yield could be improved by improving the pod number per plant. Unfortunately, a large pod density appears to be detrimental to seed yield because a large pod canopy shades the leaves and pods formed earlier and competes for assimilate, leading to the premature detachment of leaves and pods; consequently, final seed yield is reduced (Mendham and Scott 1975, Mendham et al. 1981, Mendham et al. 1984). Studies in *B. napus*, *B. juncea*, and *B. carinata* have indicated that shading pods reduced the seed number per pod and seed weight, resulting in 30 - 90% seed yield reduction (Sharma and Ghildiyal 1992, Singal et al. 1992).

A possible alternative approach for seed yield improvement is to select for genotypes with long pods containing more seeds per pod and large seeds. Chay and Thurling (1989a, b) observed considerable variations of pod length within and between genotypes in *B. napus*, where the long-podded trait was determined by two dominant genes acting in a complimentary manner. Thurling (1991) reported that selection for long pods produced at least five more seeds per pod.

Several long-podded *B. napus* genotypes were developed at the University of Alberta through crossing Chinese *B. napus* germplasm with Canadian germplasm. These genotypes, with an approximate pod length of 12 cm, had longer pods than normal *B. napus* (7 - 8 cm in pod length), and appeared to contain more seeds per pod and larger seeds than normal *B. napus* genotypes. The potential value of these genotypes in rapeseed breeding, particularly in seed yield improvement, needs to be determined. This chapter reports studies on the inheritance of pod length and the associated trait seed set per pod (SSP), and the relationship between these two traits.

2.2 MATERIALS AND METHODS

2.2.1 Production of doubled haploid lines

Doubled haploid (DH) lines were developed from the F_1 progenies of three spring *B. napus* genotypes 90-1152 (GD), 90-3010 (G1) and 90-3078 (G4). Under field conditions, GD was characterized

as long-podded with an approximate pod length of 11 cm, while G1 and G4 had an approximate pod length of 6 and 7 cm, respectively. All three genotypes were DH lines derived from microspore culture of breeding lines available at the University of Alberta.

F_1 hybrids were produced through hand pollination in the greenhouse from reciprocal crosses between GD and G1 or G4. DH lines were produced through microspore culture followed by chromosome doubling in a procedure modified from Coventry et al. (1988) and Swanson (1990). F_1 plants produced from the single crosses were grown in 6-inch plastic pots containing Metro mix (W.R. Grace & Co., Canada) soil-free potting mix and placed in a growth cabinet under 16 hr photoperiod with photon flux density of 425 - 450 µmol $m^{-2}s^{-1}$, and a 24/21 °C day/night temperature regime. The plants were watered twice daily, and fertilized four times a week with a nutrient solution containing 1 g/L 20-20-20 (N:P:K) fertilizer. Buds were harvested at the stage when the stamens were 1/2 to 2/3 the length of the stigma and stored in the refrigerator at 0 - 1 °C for 24 - 48 hrs. The buds were then surface sterilized with the 7% (w/v) sodium hypochlorite for 10 minutes, and rinsed with ice-cold water three times for 5 minutes each. The buds were then crushed with a pestle and mortar containing 10 ml of B5 isolation medium (Appendix A) (Lichter 1982) supplemented with 13% (w/v) sucrose. The homogenate was filtered through 63 and 44 µm nylon mesh (Nytex). The filtrate was then collected into 50 ml Falcon tubes (Fisher Scientific Canada) and centrifuged at 250 g for 10 minutes. The microspore pellet was resuspended with B5 medium in the tubes and centrifuged twice for 10 min each, and then resuspended in a modified NLN medium (Appendix B) (Lichter 1982) containing 13% (w/v) sucrose, dispensed into 100 x 15 mm Petri dishes with 10 ml per dish, sealed with parafilm and placed in an incubator at 32 °C in the dark. After two weeks, the plates were moved onto a rotary shaker at 60 rpm in darkness at room temperature for 2 - 3 weeks.

Well-developing embryos were transferred into 9 cm Petri dishes containing 10 ml of B5 solid culture medium supplemented with 0.15 mg/L gibberelic acid (Appendix C), and placed at 4 °C with 8 hr photoperiod of photon flux density of 30 µmol $m^{-1}s^{-1}$. After 10 days, the plates were incubated at room temperature, with a 12 hr photoperiod of photon flux density of 30 µmol $m^{-1}s^{-1}$ for embryo germination.

Plantlets with true leaves and roots were transplanted to multiwell plastic flats containing moist soil-free Metro mix and grown in the greenhouse at the constant temperature of 20 °C and 16 hr photoperiod supplemented with high intensity discharge 400 W high pressure sodium lamps. Plants were watered and fertilized as previously described.

Plants were identified as being either spontaneous diploid or haploid on the basis of their bud/flower morphology and pollen production. As observed in previous studies (Keller and Armstrong 1978, Siebel and Pauls 1989, Mathias and Röbbelen 1991), diploid plants produced large buds and large flower petals, and abundant pollen. In contrast, haploid plants had smaller buds and flower petals, and anthers with very little or no pollen. As a consequence, diploid plants were fertile upon open pollination, while haploid plants were sterile.

Diploid plants were grown in the greenhouse to produce seeds which were harvested at maturity, while haploid ones were treated with colchicine for chromosome doubling as follows. Haploid plants were washed with tap water to clean the soil-free potting mix from their roots at the beginning of flowering, followed by immersing the roots into 0.34% (w/v) colchicine for 2 hours at room temperature. The plants were rinsed with tap water, then planted in 7.6 cm diameter plastic pots (one plant per pot) containing soil-free Metro mix, and grown in the greenhouse under the conditions described above. Seeds formed on these plants were harvested at maturity and increased in the greenhouse for an additional generation along with the seeds produced from the spontaneous diploids. Three DH line families were produced which were designated as M6 from GD x G1, M7 from the reciprocal cross G1 x GD, and M8 from the cross G4 x GD, respectively.

2.2.2 Field plot design, measurement of pod length, seed set per pod and data analysis

All three DH families were grown in the field at the Edmonton Research Station of the University of Alberta, Edmonton, Alberta in 1993. Each family was grown in a completely randomized block with two replications. Each plot consisted of a single six-meter row with 46 cm spacing between two adjacent plots. Pod length was measured and SSP determined from five randomly sampled plants from each plot. The first five pods formed on the main raceme of each plant were measured and SSP recorded when the pods had reached the full length indicated by the seeds contained therein turning brown in color. Samples were collected from this portion of the raceme because these pods formed first on a plant, and pods at the basal

portion on the main receme have more fertile ovules and total number of ovules per ovary than the pods at apical portion (Bouttier and Morgan 1992). Therefore, these five pods should be less variable in their length and seed set than pods on other parts of a plant. Pod length, defined as the distance between the pedicellar end connecting the pod and the base of the beak, of each sampled pod was measured in centimeter to one decimal point, and seeds in each of these five pods were visually counted. The pod length and SSP of each DH line were determined by averaging the values from 5 pods x 5 plants x 2 replications.

Pod length and SSP within each DH line family were analyzed, and the relationship of SSP and pod length was determined with regression analysis for all three families. Chi-square tests were applied to test the hypothesis of the genetic control system of pod length. Computations were done using SAS/STAT 6.0 (SAS Institute Inc. 1989).

2.3 Results and Discussion

2.3.1 Inheritance of pod length

Pod length exhibited continuous variation in all three families on the basis of one centimeter intervals, varying from 4 to 13 cm (Table 2-1). When grouped in 2 cm intervals with equal spacing from the median, however, they could be classified into four groups within each family (Table 2-2). The lines representing the two extreme groups, i.e. the shortest and the longest pod length, comprised only a small percentage of the lines observed, while the majority of the lines had intermediate pod length.

Because the DH lines were derived from microspores produced on the F_1 plants of two DH parents, the number of genotypes or phenotypes of the DH lines should equal to the number of different types of gametes formed on the F_1 plants. If we assume that pod length is controlled by three independent nuclear genes which may be designated as L_1l_1, L_2l_2 and L_3l_3, respectively, there should be 2^3 types of gametes formed on the F_1 plants. If all three genes are independent and their effect is additive and equal, the frequencies of pod length within the DH line populations (genotypes and phenotypes) can be predicted from the expansion of the binomial distribution $(L + l)^3$, which becomes $1\ L^3l^0 + 3\ L^2l^1 + 3\ L^1l^2 + 1\ L^0l^3$. In this expansion L represents the gene for long pods, and l the gene for short pods. The superscripts of each term are the number of homozygous gene loci for long pods and short pods, respectively. The coefficient of each term is the expected phenotypic frequency of genotype(s) following this coefficient in a population of a DH line family. For example, the second term of this expansion ($3\ L^2l^1$) indicates that three out of eight lines in a DH line family would be expected to be $L_1L_1L_2L_2l_3l_3$, $L_1L_1l_2l_2L_3l_3$, or $l_1l_1L_2L_2L_3l_3$.

Chi-square (χ^2) tests of the three gene hypothesis on the segregation ratios of pod length did not reveal significant differences between the observed and the expected frequencies ($\chi^2_{3,0.05} = 7.81$) (Table 2-2) in any of the three DH line families, supporting the hypothesis that the pod length was controlled by three independent nuclear genes with additive gene action.

It should be pointed out, however, that the classification of the DH lines into four pod-length groups was unavoidably somewhat arbitrary because of the continuity of expression from the shortest to the longest pods. Moreover, pod length is also variable within DH lines. Since each DH line is a genetically homozygous population, the variation in pod length within DH lines can be attributed to environmental factors, and/or to variation in other factors related to pod length. In general, a statistically significant but weak positive association was observed between pod length and its standard error among the DH lines (Fig. 2-1). Thus, it should be expected that after averaging, the frequency of long-podded lines would always be less than expected, as shown in Table 2-2. Nevertheless, it is still interesting to note that all three families could be classified into four similar pod length groups and all had similar average pod length and pod length variability (Table 2-1). Given the number of lines observed in each family, the average pod length and variance should well represent the characteristics of that family. Particularly, the similarity in these characteristics of M6 and M7 suggested that there would be very little cytoplasmic effect on the pod length, since these two families derived from reciprocal crosses.

Together, the data show that the inheritance of pod length of the *B. napus* genotypes studied in this experiment is relatively simple, with three independent nuclear genes with additive gene action involved. Therefore, incorporating the long-podded trait into a rapeseed breeding program should not be difficult. The population size required for screening the long-podded trait is small. Specifically, one out of eight lines or

one out of 64 plants respectively, is expected to have the maximum expression of long pods in a DH line population derived from F_1 plants or in an F_2 population.

2.3.2 Inheritance of seed set per pod and relationship between seed set per pod and pod length

SSP exhibited a typical multigenic characteristic as shown by a continuous variation in each of the three DH families (Fig. 2-2). In addition, there was a weak but statistically significant positive association between SSP and pod length (Fig. 2-3). On average, an increase of one cm in pod length resulted in an increase of at least one more seed per pod. There was, however, considerable variation in this relationship. A DH line with a pod length of 7 cm or shorter, for example, may have more seeds per pod than a line with a pod length of 11 cm or longer. Interestingly, as the SSP increases its variation within lines decreases (Fig. 2-4). Of interest was the fact that a few lines with an approximate pod length of 7 cm had an approximate SSP value of 40. Whether such genotypes with short pods and high SSP genotypes are desirable for high seed yield requires additional investigation.

It has been reported in winter *B. napus* (Hühn and Schuster 1975) and *B. rapa* (Seiffert and Boelcke 1977) that SSP was insensitive to plant density and competition. SSP along with 1000 seed weight was a relatively consistent seed yield component (Seiffert and Boelcke 1977). A high correlation between SSP and seed yield has been observed (Anderson and Olsson 1961). Thus, it is logical that improving the SSP should result in seed yield improvement. Selection for longer pods might be a more efficient approach than direct selection for high SSP, because these two traits are positively correlated, and fewer genes are involved in pod length than in SSP. Breeding for increased SSP in principle is more difficult than breeding for longer pods. Accordingly, breeding methodologies for these two traits should be different. For instance, long pods could be easily selected from an F_2 progeny or DH line population following a cross between long- and short-podded parents, while genotypes with high SSP should be much less frequent in a population of the same kind. Consequently, in pedigree selection, pod length could be selected in F_2 while selection for SSP should be conducted in later generations. Considering that DH line production through microspore culture from an F_1 generation has become routine in *B. napus* breeding, it might be particularly difficult to greatly improve SSP using this approach. Therefore, it may be necessary to increase the gene frequency for SSP of microspore donor plants prior to DH line production, if the major breeding objective is to increase seed yield. However, it should be possible to select for longer pods while simultaneously selecting for SSP given the positive association of these two traits. In fact, it has been reported that selection for long pods has resulted in an increase of at least five seeds per pod (Thurling 1991). On the other hand, increasing pod length by one cm, on average, could result in only one more seed per pod, and yet there are considerable variations of SSP at the pod length of 7 - 11 cm. Genotypes with pod length greater than 11 cm might not be desirable because the increase in SSP beyond this point may not compensate for the loss of other seed yield components such as pod number per plant as observed in previous studies (Thurling 1991). In addition, when pod length increases, more assimilates have to be distributed for pod wall formation, which requires a greater nutrient supply. Furthermore, when pod length increases with the increased SSP and pod wall, better lodging resistance of plants is required, which is often difficult to achieve. Thus, it appears that in a *B. napus* breeding program for seed yield improvement, selection for genotypes having a pod length of approximately 7 - 10 cm, coupled with a SSP close to 40, would be ideal.

In summary, it has been shown, by using DH populations, that pod length and SSP are controlled by different genetic systems. The former is determined by three independent nuclear genes with additive gene action, while the latter is a multigenic trait. However, both traits varied within DH lines. Pod length variation increases as pod length increases, whereas SSP is inversely proportional to its variation. Pod length and SSP are positively associated, and therefore, they could be simultaneously improved by visual selection for longer pods. On the other hand, there is considerable variation in the relationship of pod length and SSP. Thus, a more preferable approach may be a combined selection for genotypes having a pod length of 7 - 10 cm and an SSP value close to 40.

Although DH lines have been used for genetic analysis of pod length and SSP, such analysis is based on the assumption that there is no selection on the specific trait during microspore culture and subsequent procedures. If this assumption had been violated for any reason, the proportions of different pod lengths or SSP in a DH line population would have been biased accordingly, resulting in misleading conclusions. Therefore, studies on inheritance of these two traits using conventional genetic analysis have been conducted which will be reported in the next Chapter.

Table 2-1. Pod length distribution of three microspore derived DH line families from crosses of long-podded x short-podded parents in *B. napus*

Pod length (cm)	M6 family		M7 family		M8 family	
	No. of DH lines	Percent	No. of DH lines	Percent	No. of DH lines	Percent
4.1 - 5.0			1	1.77		
5.1 - 6.0	1	1.15	2	3.33	1	1.19
6.1 - 7.0	10	11.49	9	15.00	14	16.67
7.1 - 8.0	20	22.99	15	25.00	13	15.48
8.1 - 9.0	24	27.59	12	20.00	17	20.24
9.1 - 10.0	14	16.09	15	25.00	20	23.81
10.1 - 11.0	12	13.79	4	6.67	14	16.67
11.1 - 12.0	5	5.75	2	3.33	2	2.38
12.1 - 13.0	1	1.15			3	3.57
Total	87	100.00	60	100.00	84	100.00
$\bar{x}$	8.10		8.38		8.89	
s^2	2.35		2.09		2.60	

Table 2-2. Chi-square test for the hypothesis of three genes with additive and equal gene action controlling pod length for three DH line families M6, M7 and M8 derived from F_1 plants of long-podded x short-podded parents in *B. napus*.

M6			M7			M8		
Pod length (cm)	Exp.[a]	Obs.[b]	Pod length (cm)	Exp.	Obs.	Pod length (cm)	Exp.	Obs.
5.1 - 7.0	10.88	11	4.1 - 6.0	7.5	3	5.1 - 7.0	10.5	15
7.1 - 9.0	32.63	44	6.1 - 8.0	22.5	24	7.1 - 9.0	31.5	30
9.1 - 11.0	32.63	26	8.1 - 10.0	22.5	27	9.1 - 11.0	31.5	34
11.1 - 13.0	10.88	6	10.1 - 12.0	7.5	6	11.1 - 13.0	10.5	5
No. of DH lines		87			60			84
Chi-square (x^2)	7.50			4.00			5.08	
$\chi^2_{0.05,3}$	7.81			7.81			7.81	

[a] Exp. = Expected frequency.
[b] Obs. = Observed frequency.

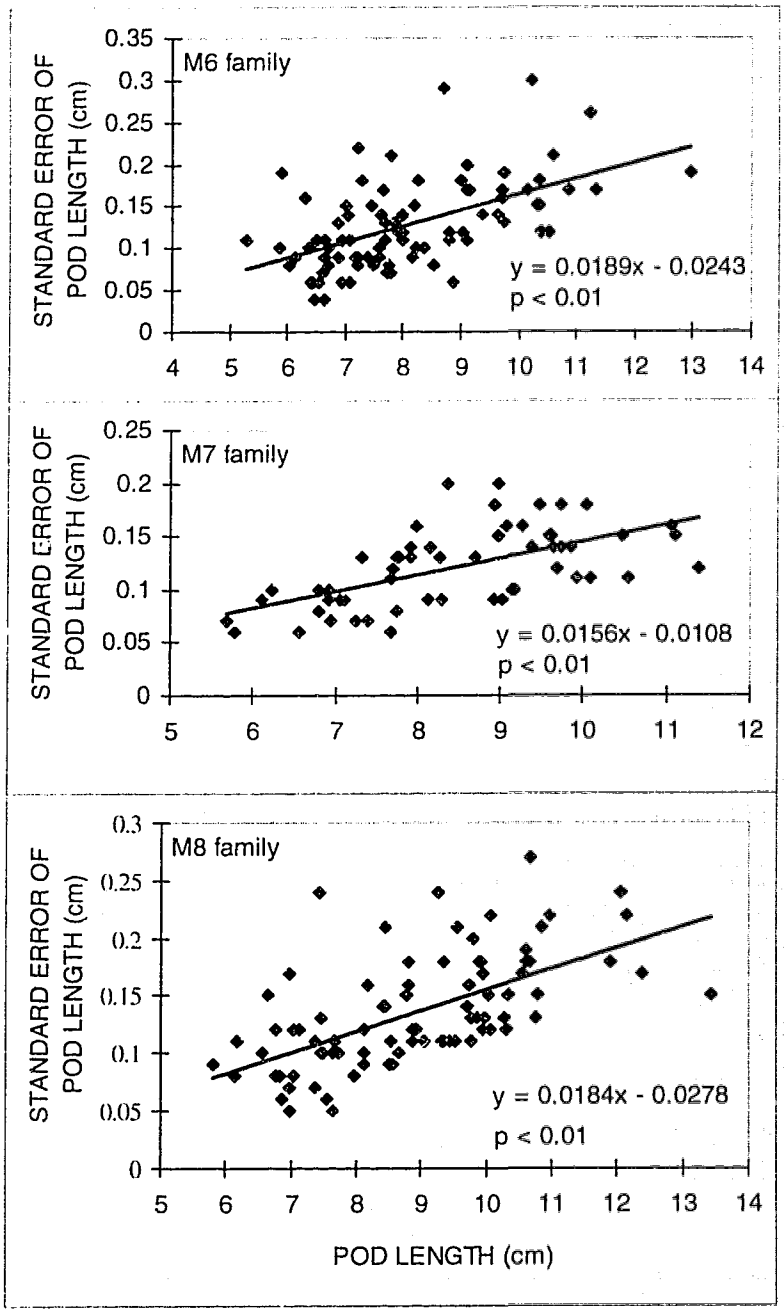

Figure 2-1. Relationship between pod length and pod length variability of three DH line families M6, M7 and M8 in *B. napus*.

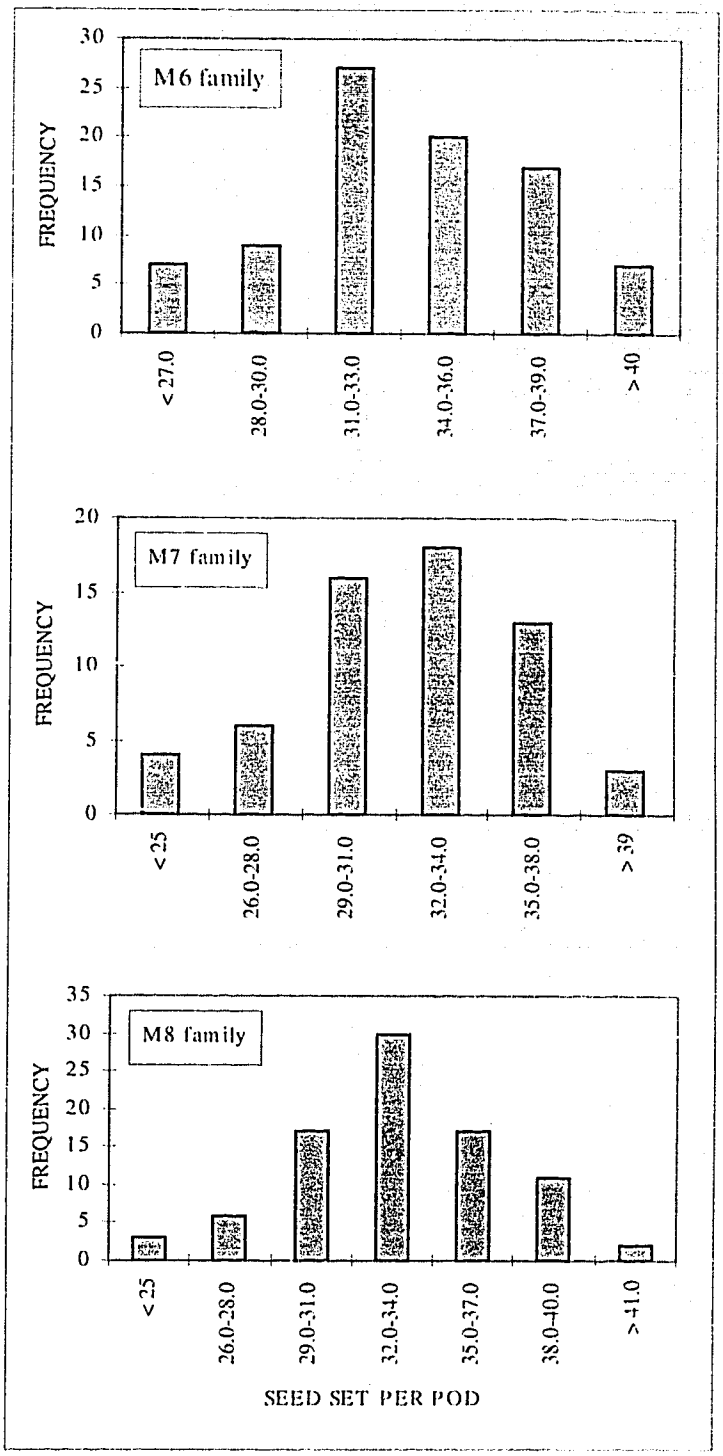

Figure 2-2. Distribution of seed set per pod of three DH line families M6, M7 and M8 *in B. napus*

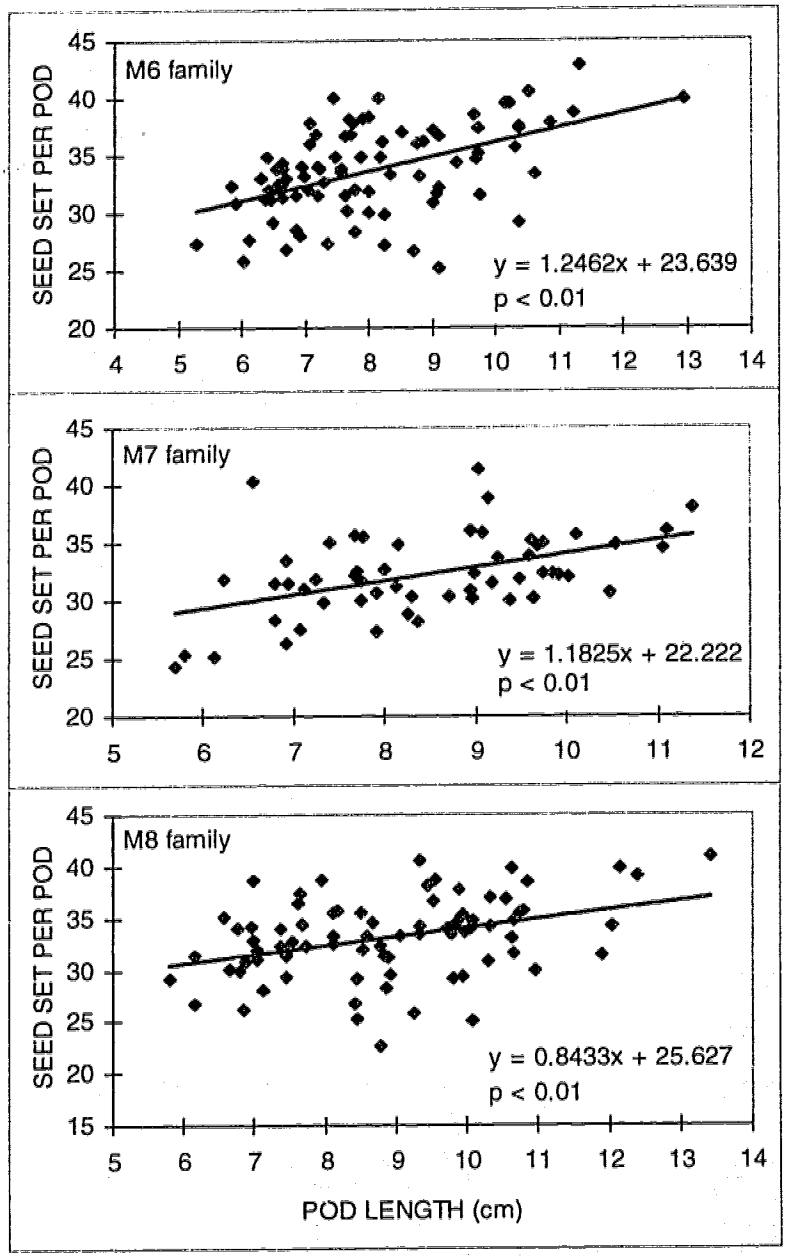

Figure 2-3. Relationship between seed set per pod and pod length for three DH line families M6, M7, and M8 in *B. napus*

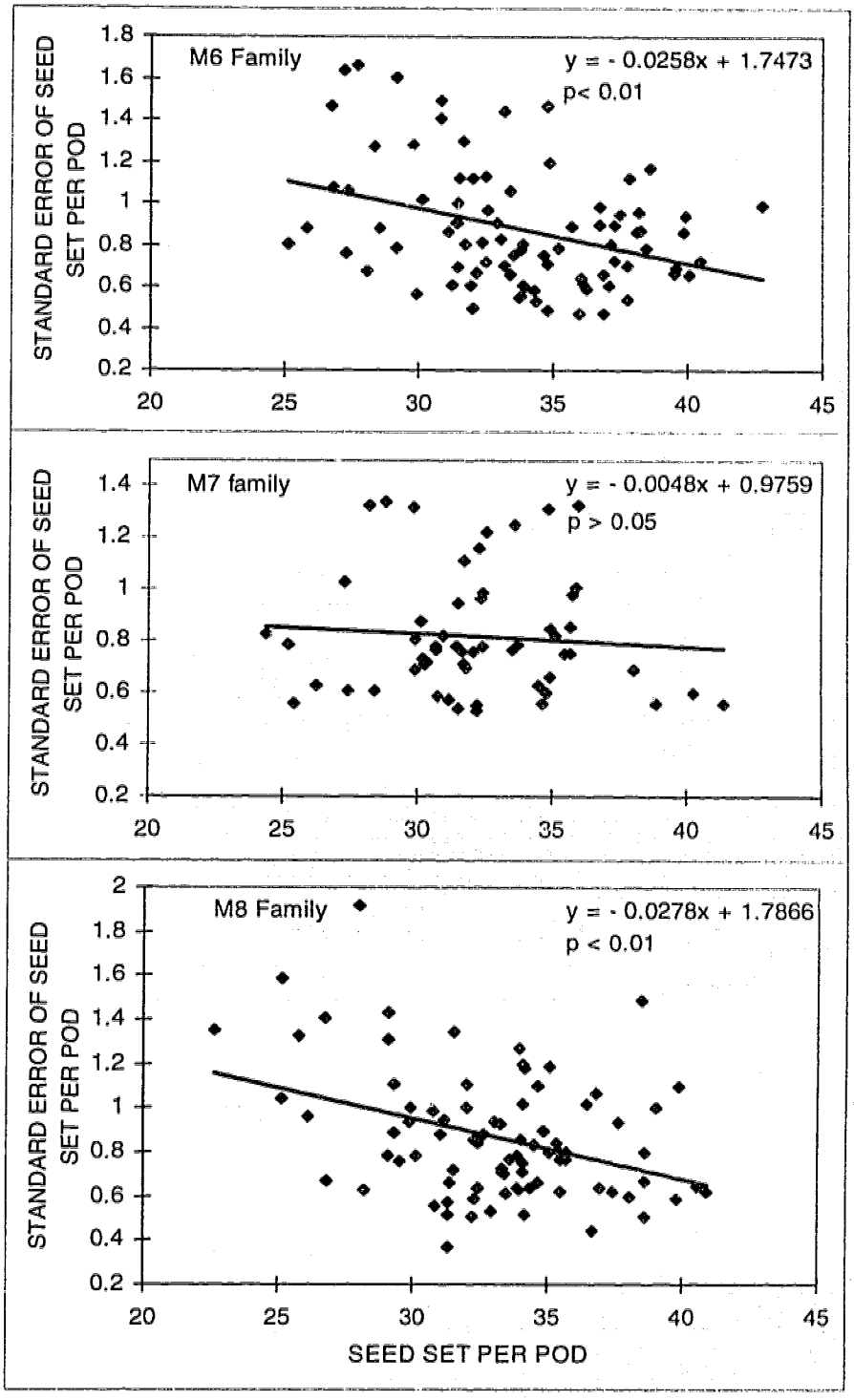

Figure 2-4. Relationship between seed set per pod and its variation for three DH line families M6, M7 and M8 in *B. napus*.

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CHAPTER 3

INHERITANCE AND INTERRELATIONSHIPS OF POD LENGTH, SEED WEIGHT, SEED OIL AND PROTEIN CONTENTS IN *BRASSICA NAPUS* L.

3.1 INTRODUCTION

High seed yield and high seed oil content are primary breeding objectives for oilseed Brassicas. Unfortunately, seed yield improvement has been the most difficult and costly trait to measure accurately. Moreover, breeding for improved quality of seed oil and meal composition has resulted in a rather narrow germplasm base for rapeseed crops, especially in *B. napus* (Downey and Rimmer 1993). Consequently, yield improvement has been very gradual. This can be seen from the rapeseed yield in Canada from the year 1983 to 1993. On average of all rapeseed species grown in Canada in this period, the annual seed yield increase rate was only 0.10 kg ha^{-1} across Canada (Statistics Canada 1995). Thus, there has been an increasing interest in breeding for seed yield improvement in recent years.

Rapeseed pods play significant roles in transporting nutrients and photosynthates to developing seeds (Allen et al. 1971, Allen and Morgan 1972, Norton and Harris 1975, Brar and Thies 1977, Richards and Thurling 1979, Zhang et al. 1991). Variable but highly positive correlations between pod length and seed weight were observed in *B. napus* (Chay and Thurling 1989a, b) and in *B. rapa* (Sheoran et al. 1991). Lebowitz (1989) reported that in *B. rapa* pod length was positively correlated with seed number per pod. Similarly, Subrahmanyam and Rathore (1992a, b) observed in *B. juncea* that pods were important sources of carbon for developing seeds. Seed yield reductions up to 90% have been reported from pod shading in *B. napus*, *B. juncea*, and *B. carinata* (Sharma and Ghildiyal 1992, Singal et al. 1992).

These studies have demonstrated that the rapeseed pod is an important organ influencing seed yield. It was previously thought that increasing the number of pods per plant would result in an increase in seed yield. Unfortunately, a large pod canopy shades the leaves and pods formed earlier and competes for assimilates, leading to the premature detachment of these leaves and pods, and resulting in reduction of final seed yield (Mendham et al. 1981, Mendham et al. 1984).

Large variation in pod length has been reported in *B. napus*, where the long-podded character has been reported to be controlled by two dominant genes acting in a complimentary manner (Chay and Thurling 1989a,b). It was suggested that selection for longer pods with an elevated number of seeds per pod, rather than selection for a heavy pod canopy, would be an alternative for seed yield improvement. Indeed, selection for long pods resulted in an increase of at least five seeds per pod (Thurling 1991).

In addition to high seed yield, high seed oil and protein contents are highly desirable for modern rapeseed cultivars. Unfortunately, intensive selection for oil content usually results in lower protein values (Downey and Rimmer 1993, Daun et al. 1995) because of the negative association between seed oil and protein content (Grami et al. 1977). Grami and Stefansson (1977a,b) observed that oil, protein, the sum percentage of oil, and protein were determined by the maternal genotype, and that gene action was mainly additive with insignificant dominance and absence of epistasis.

Several long-podded *B. napus* genotypes (approx. 12 cm) have also been developed at the University of Alberta. Using microspore-derived doubled haploid (DH) lines, it has been shown that the long-podded trait is positively correlated with seed set per pod (SSP), and that pod length is controlled by three nuclear genes with additive gene effects (Chapter 2). A basic assumption which remains to be tested in using DH lines for studying the inheritance of pod length is the absence of gamete selection for pod length during the microspore culture. In addition, the relationship between the long-podded trait and seed oil, and protein content has not been clearly defined. No information is available in the literature on the relationship between pod length and seed oil or protein content.

The main objective of this study was to investigate the inheritance of the long-podded trait, using conventional genetic analysis. In addition, inheritance of seed weight, seed oil and protein content, the interrelationships of these traits, and their relationships with pod length were also to be investigated.

3.2 MATERIALS AND METHODS

Three DH lines derived from microspore culture in spring type *B. napus*, 90-1152 (GD), 90-3010 (G1) and 90-3078 (G4), were used as parents for producing F_1, F_2, and backcross progenies. Under field conditions, GD had an approximate pod length of 11 cm, while G1 and G4 had an approximate pod length of 7 and 8 cm, respectively. GD was, therefore, referred to as long-podded parent while G1 and G4 as short-podded parents. F_1 hybrids were produced from the reciprocal crosses between GD and G1, or G4, and then selfed and backcrossed to the parental lines to produce F_2 and the first generation backcross progenies. All crosses, self pollination and backcrosses were conducted using hand pollination in the greenhouse.

Two experiments, experiment A and experiment B, were conducted at the Edmonton Research Station of the University of Alberta, Edmonton in 1993.

3.2.1 Experiment A

The genetic relationship between long pods and short pods was investigated from two groups of plant populations. Group one consisted of eight populations, i.e. two parental lines GD and G1, two F_1s of the reciprocal crosses GD x G1 and G1 x GD, and four first generation backcrosses (GD x G1) x GD and (GD x G1) x G1, (G1 x GD) x GD and (G1 x GD) x G1. Group two also consisted of eight similar populations except that the short-podded parent G4 was substituted for G1. All populations were grown in one field block. Each population was grown in one plot consisting of two 6-meter rows with 46 cm row spacing. The plots in the field block were completely randomized. To reduce competition between adjacent plants and to maximize the expression of pod length, plants within each row were thinned to an approximately 20 cm spacing after the seedlings had established in the field. Pod length, as defined in 2.2.2, was measured on the first five pods formed on the main raceme of 10 randomly sampled plants of each population, when the seeds in the pods began to turn brown in color. Methods for determining pod length and SSP were the same as those described in 2.2.2. Data were analyzed using analysis of variance (ANOVA) using the model $Y_{ijk} = \mu + L_i + Pl_{j(i)} + P_{(ij)k}$, where Y_{ijk} is the pod length of the kth pod $[P_{(ij)k}]$ on the jth plant $[Pl_{j(i)}]$ in the ith population $[L_i]$. Pod length of different populations was compared using Duncan's multiple range test (Steel and Torrie 1980). All computations were done with SAS/STAT 6.0 (SAS Institute Inc. 1989).

3.2.2 Experiment B

Six populations, i.e. GD, G4, F_1 of GD x G4, F_2, and two backcrosses F_1 x GD (BCP_1) and F_1 x G4 (BCP_2), were grown in the field. All populations were randomly allocated into one field block. Each population was planted in a four-row plot, six-meter long with 46 cm spacing between two adjacent rows with one exception that F_2 was planted into a six-row plot. After the seedlings had established, plants in each row were thinned to approximately 20 cm between adjacent plants to maximize the expression of pod length and other traits of interest. Pod length was measured on the first five pods formed on the main raceme of randomly sampled plants of each population using the same method described in 2.2.2. The number of plants sampled in each population varied from 25 to 86, depending on specific populations. The plants measured for pod length were harvested individually at maturity, and 1000 seed weight, oil and protein content of the seeds were determined. Two samples of 500 seeds each were weighted from each plant. The mean of the two samples was multiplied by two, and taken as the 1000 seed weight for each plant. Oil content was determined using a Newport NMR with a sample of 1.2 g of seeds per plant. The protein content of the residual meal was analyzed using total combustible N (Leco FP-428) after the extraction of the seed oil. Two samples with 0.05 - 0.1 g of meal each were analyzed for each plant, and the mean of the two samples was used as the protein content for a specific plant.

Data were analyzed with ANOVA using the same model described in section 3.2.1, and pod length of the different populations were compared with Duncan's multiple range test (Steel and Torrie 1980). The interrelationships of pod length, 1000 seed weight, seed oil, and protein content were analyzed with correlation analysis within populations for three segregating populations F_2, BCP_1 and BCP_2. The heritabilities of these traits were estimated using the example of Simmonds (1979) as follows:

$$\sigma^2_E = \frac{\sigma^2_{P1} + \sigma^2_{P2} + \sigma^2_{F1}}{3} \qquad \sigma^2_A = 2\sigma^2_{F2} - (\sigma^2_{wP1} + \sigma^2_{wP2})$$

$$\sigma^2_D = \sigma^2_{F2} - (\sigma^2_A + \sigma^2_E) \qquad \sigma^2_P = \sigma^2_A + \sigma^2_D + \sigma^2_E$$

$$h_B^2 = \frac{\sigma_G^2}{\sigma_P^2} = \left(\frac{\sigma_A^2 + \sigma_D^2}{\sigma_P^2} \right) \qquad h_N^2 = \frac{\sigma_A^2}{\sigma_P^2}$$

In these formula, σ_{P1}^2, σ_{P2}^2, σ_{F1}^2, σ_{F2}^2, σ_{BCP1}^2 and σ_{BCP2}^2 represent variance for the long-podded parent (P_1), short-podded parent (P_2), F_1, F_2 generation of the cross between P_1 and P_2, and the first generation backcross to the P_1, and P_2 respectively. σ_A^2, σ_D^2, and σ_E^2, represent the variance of additive, dominant, and environmental effects, respectively. σ_G^2 and σ_P^2 represent the total genetic and total phenotypic variance, respectively. h_B^2 represents the heritability in broad sense, while h_N^2 represents the heritability in narrow sense.

3.3 RESULTS AND DISCUSSION

3.3.1 Genetic relationship between long pods and short pods from the results of Experiment A

A comparison of pod length of the eight populations within each group showed that the two parents represented the two extremes and differed significantly from each other in pod length (Table 3-1). F_1s from the reciprocal crosses were not significantly different in pod length, but had significantly shorter or longer pods than the parents. There was no significant difference in pod length between two backcross progenies of the reciprocal F_1s to the same recurrent parent. However, there were some differences between two groups. The pod lengths of two backcrosses to the long-podded parent GD in group 1 were not significantly different from those of F_1s, but they were still significantly shorter than the pod length of the recurrent parent GD. Similarly, in group 1 the backcrosses to the short-podded parent G1 had significantly longer pods than the recurrent parent, but their pod lengths were significantly shorter than those of the F_1s. In contrast, three backcrosses in group 2, (G4 x GD) x GD, (GD x G4) x G4 and (G4 x GD) x G4, had similar pod length to the recurrent parents. Meanwhile, (G4 x GD) x GD and (G4 x GD) x G4 had significantly longer or shorter pods than F_1s, whereas the other two backcrosses, (GD x G4) x GD and (GD x G4) x G4, had similar pod lengths to the F_1s.

These results suggested that there was no complete dominant-recessive relationship between long pods and short pods since no F_1 had a pod length similar to the pod length of either parent. Pod length was also clearly controlled by nuclear genes with minimum cytoplasmic influence, as indicated by the similarity in pod length of F_1s produced from reciprocal crosses, and the similarity in pod length of the backcross progenies of the reciprocal F_1s to the same recurrent parent. Moreover, there was an indication that the inheritance of pod length would be relatively simple, as suggested by the recovery of parental genotypes in three of the first backcross progenies in group 2.

3.3.2 Inheritance of pod length from the results of experiment B

Variation in pod length of the individual plants was observed within each of the six populations (Fig. 3-1). Apparently, pod length was influenced by both genetic and environmental factors. For example, the variation in pod length in GD, G4 and F_1 can be mainly attributed to environmental factors since the plants within each of these three populations were genetically identical, whereas the pod length variation in F_2 and two backcross populations were caused by both genetic and environmental factors since these segregating populations consisted of different genotypes. It was also noted that the long-podded parent GD had much larger pod length variation than the short-podded parent G4, although both parents were DH lines. In an earlier study (Chapter 2), it was shown that pod length variation was positively associated with pod length within lines which was in turn positively correlated with SSP. Chay and Thurling (1989a) also observed that long-podded genotypes had larger variation in pod length than short-podded lines. Thus, it would be expected that GD had the larger variation in pod length than G4, which was confirmed by F test ($F = 6.19$, $F_{24,24,0.01} = 2.36$). In addition, it was observed in the field that GD had poorer lodging resistance than G4. It was likely that some of the lodged plants were also sampled for the measurement of pod length because of the random sampling. These lodged plants could have poorer pod development after pollination than

non-lodged plants. This would also increase the variation in pod length. Despite large differences in pod length variation, GD still had the longest pods while G4 had the shortest pods among the six populations (Table 3-2). The pod length of F_1 and F_2 was slightly greater than mid-parent value. Backcrossing shifted pod length towards the value of corresponding recurrent parent, but the pod length of the backcrossing progenies was still significantly shorter or longer than that of the corresponding recurrent parent. Again, there was no complete dominant-recessive relationship between long pods and short pods. The gene effect seemed to be additive, and the expression of pod length was quantitative. These results were in accordance with those presented in section 3.3.1.

Chi-square tests of segregation ratios of pod length for the three segregating populations, F_2, BCP_1 and BCP_2, revealed that a hypothesis of three genes of additive action best fitted the data observed (Table 3-3), with the contribution of each gene to the pod length being equal. With this hypothesis, there should be 27 genotypes and seven phenotypes in F_2, and eight genotypes and four phenotypes in each backcross population, respectively. These genotypes can be expressed with a genotypic formula L^xl^y. In this formula, L represents the gene for long pods and l the gene for short pods, while x and y are the number of alleles. The sum (6) of the x and y is the total number of alleles in a genotype. For example, L^5l^1 indicates the presence of five alleles for long pods, one allele for short pods, and a total of six alleles. There are three possible genotypes in L^5l^1, i.e. $L_1L_1L_2L_2L_3l_3$, $L_1L_1L_2l_2L_3L_3$ or $L_1l_1L_2L_2L_3L_3$.

It is notable that in spring *B. napus* Chay and Thurling (1989a,b) reported that long-podded trait was controlled by two major complimentary genes. In general, the pattern of pod length distribution of six populations observed in the present study was similar to that reported by these two authors in the six similar populations. For instance, the long-podded parent was more variable in pod length than the short-podded parent. The pod length of the F_1 of both studies was slightly greater than the mid-parent value, and the pod length in F_2 displayed a binomial distribution in both studies. However, there are substantial differences between Chay and Thurling's reports and this study. The backcross progeny to the long-podded parent in the present study did not produce longer pods than the recurrent parent, whereas the similar backcross in Chay and Thurling's study produced significantly longer pods than the recurrent parent. The pod length distribution of backcrosses in the present study overlapped by a wide margin, whereas there was virtually no overlaps in Chay and Thurling's study. The majority of the plants in F_2 population in the present study covered a full range within the variability noted in both parents. In contrast, the pod length distribution of F_2 in Chay and Thurling's study was apparently skewed towards the short-podded parent. These differences suggested that genetic control systems of pod length in these two studies were different. The greater variation and overlapping pod length distribution in the segregating populations in the present study support the finding that more genes were involved in the genetic control of pod length for materials in the present study than those in Chay and Thurling's studies.

It is shown in Fig. 3-1 that pod length displayed a continuous variation. Thus, classification of pod length into discrete groups in each population was somewhat arbitrary. There were eight pod length groups in F_2 based on one cm intervals. Since there was certain variation in pod length, one half of the plants each in the range of 9.1 - 10.0 cm could be classified into neighboring groups, resulting in seven groups. Using a similar approach, the plants in BCP_1 could be classified into four groups, where the plants in the range of 6.1 - 8.0 cm were in one group and those from 11.1 to 13.0 cm were in another. One half of the plants each in the range of 9.1 - 10.0 cm were grouped into adjacent classes. Similarly, there were four pod length groups in BCP_2, where plants with pod lengths up to 7.0 cm were in one group, and plants with pod lengths of greater than 10.0 cm were in another, while one half of the plants each in range 8.0 - 9.0 cm were grouped into two neighboring groups.

In general, the results of the above two experiments were in agreement with the findings in the previously study using DH lines (Chapter 2), which showed that three nuclear genes with additive action were involved in the genetic control of pod length. The genetic analysis using DH lines was based on the average pod length of DH lines, while the genetic analysis used in this chapter was based on the single plants. Both studies were supportive of a three gene model of the inheritance of pod length. The gene action is additive and the contribution of each gene to pod length is equal. In addition, the assumption made in using DH lines for genetic analysis, i.e. there is no gamete selection during the process of DH line production, appears valid, and underscores the superiority of using DH lines for genetic analysis. The advantage of this approach has been well described in the literature and will not be discussed in this thesis.

3.3.3 Heritability of pod length, 1000 seed weight, seed oil and protein contents and the interrelationships of these traits

Estimations of the heritabilities of pod length, 1000 seed weight, seed oil and protein contents indicated that pod length and 1000 seed weight had a medium broad sense heritability (Table 3-4), while the heritabilities of seed oil and protein contents were very low. In addition, most of the narrow sense heritabilities became either negative or greater than the broad sense heritability estimated, resulting from large variation in the data observed. Environmental factors caused some variation in each trait, which was suggested by the presence of variation in each of the four traits investigated in the populations GD, G4 and F_1 (Table 3-5), since each of these three populations consisted of genetically identical individuals. Conceivably, individual plants in the field have different micro-environmental surroundings, which would affect the expression of these traits in varying degrees. Consequently, the heritability estimated on the basis of individual plants would be low. It is noted that variation in seed oil and protein contents was greater than that in pod length and seed weight in all six populations, indicating seed oil and protein contents were more sensitive to environmental variations than pod length and seed weight. In winter *B. napus*, Rakow (1979) also found that oil and protein contents were strongly modified by environmental factors. Thus, the results from the present and previous studies suggest that evaluation of seed oil and protein contents on an individual plant basis in rapeseed breeding programs would be less accurate than the evaluation for seed weight and pod length.

Correlation analysis among pod length, 1000 seed weight, seed oil, and protein contents from three segregating populations showed that pod length and 1000 seed weight were positively associated in BCP_2 and F_2, but were not associated in BCP_1 (Table 3-6). In addition, there was a slight negative relationship between pod length and oil content although this relationship was not statistically significant in BCP_2 and F_2. As expected from their low heritabilities, no consistent relationships were observed among most of these traits, implying again that evaluation on the interrelationships of these traits on an individual plant basis was unreliable.

The studies in this chapter have confirmed that 1) pod length is controlled by three nuclear genes with additive effect and equal contributions. Pod length is, however, responsive to environmental factors with a broad sense heritability of 0.7. 2) On an individual plant basis, pod length has, in general, a positive associated with 1000 seed weight, but pod length and seed oil content seems to have a negative association. 3) Seed oil and protein content are more responsive to environmental variation than pod length and seed weight.

Table 3-1. Comparison of pod length of GD, G1, G4, their F_1 hybrids of reciprocal crosses and first generation backcrosses in *B. napus*.

Population	No. of plants observed	Pod length (cm) [a]	Duncan's group [b]
Group 1			
GD	10	11.4	a
F_1 (GD x G1)	10	9.9	b
(G1 x GD) x GD	10	9.8	b
F_1 (G1 x GD)	10	9.7	b
(GD x G1) x GD	10	9.7	b
(GD x G1) x G1	10	8.5	c
(G1 x GD) x G1	10	8.2	c
G1	10	6.8	d
Group 2			
GD	10	11.6	a
(G4 x GD) x GD	10	10.6	ab
(GD x G4) x GD	10	10.3	bc
F_1 (GD x G4)	10	9.6	cd
F_1 (G4 x GD)	10	9.5	cd
(GD x G4) x G4	10	8.7	de
(G4 x GD) x G4	10	8.2	e
G4	10	8.1	e

[a] Standard error of the mean = 0.3 cm.

[b] The same letter denotes non-significance at p = 0.05.

[c] Standard error of the mean = 0.3 cm.

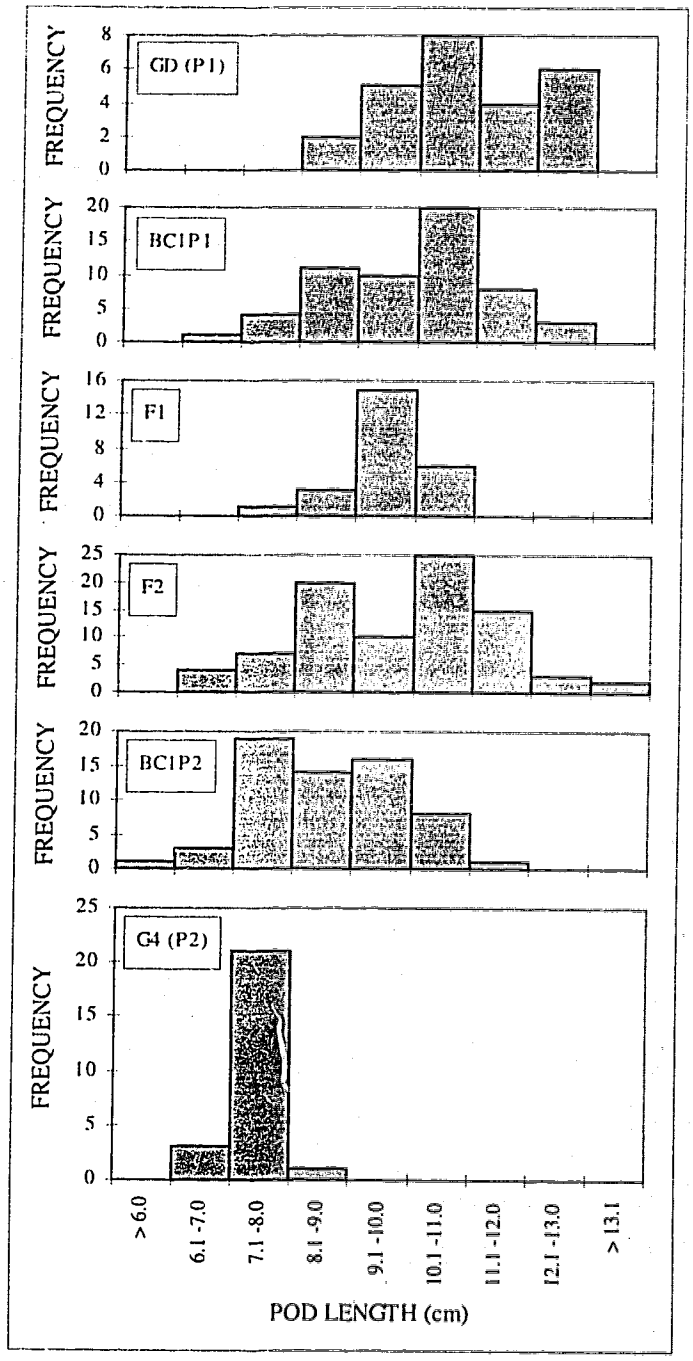

Figure 3-1. Frequency distribution of the pod length of GD, G4, their F_1 and F_2 hybrids, and two backcross generations in *B. napus* under field conditions

Table 3-2. Number of plants observed and the mean pod length of GD, G4, their F_1 and F_2 hybrids and two backcross populations and Duncan's multiple range test of the means in *B. napus*.

Population and generation	No. of plants observed	Mean pod length (cm)	Duncan's group [a]	Standard error	Variance
GD (P_1)	25	10.9	a	0.12	1.67
BCP_1	57	10.0	b	0.08	1.76
F_1 (GD x G4)	25	9.5	bc	0.08	0.71
F_2	86	9.3	c	0.08	2.52
BCP_2	62	8.1	d	0.07	1.42
G4 (P_2)	25	7.4	e	0.05	0.27

[a] p = 0.05, and the same letter denotes non-significance

Table 3-3. Chi-square test of a three gene hypothesis for segregation in pod length in F_2 and two backcross populations of the cross GD (P1) x G4 (P2) in *B. napus*.

Pod length	F_2			BCP_1			BCP_2		
(cm)	Genotype [a]	Exp. [b]	Obs. [c]	Genotype	Exp.	Obs.	Genotype	Exp.	Obs.
6.1-7.0	L^0l^6	1.34	4				L^0l^6	7.75	4
7.1-8.0	L^1l^5	8.06	7	L^3l^3	7.13	5	L^1l^5	23.25	26
8.1-9.0	L^2l^4	20.16	25	L^4l^2	21.38	16			
9.1-10.0							L^2l^4	23.25	23
10.1-11.0	L^3l^3	26.88	30	L^5l^1	21.38	25	L^3l^3	7.75	9
11.1-12.0	L^4l^2	20.16	15	L^6l^0	7.13	11			
12.1-13.0	L^5l^1	8.06	3						
> 13.0	L^6l^0	1.34	2						
No. of plants observed			86			57			62
Chi-square (X^2)		11.74			4.71			2.34	
		$\chi^2_{0.05,6} = 12.59$			$\chi^2_{0.05,3} = 7.81$			$\chi^2_{0.05,3} = 7.81$	

[a] The upper case letter in each genotype is the gene for long pod and the low case letter is the gene for short pod. The exponents of each genotype are the number of gene alleles (see text for details).

[b] Exp. = Expected frequency.

[c] Obs. = Observed frequency.

Table 3-4. Heritabilities of pod length, 1000 seed weight, seed oil and protein contents in *B. napus*.[a]

Heritability	Pod length	1000 seed weight	Oil content	Protein content
h_b^2	0.6591	0.5679	0	0.2557
h_n^2	0	0.1593	0	0

[a] 0 = indicates that estimated values were negative or $h_n^2 > h_b^2$.

Table 3-5. Variance and standard error for pod length, 1000 seed wt. oil and protein contents estimated from individual plants of six populations in *B. napus*.

Population	Traits	No. of plants	Variance	Standard error
GD (P_1)	Pod length (cm)	25	1.47	0.24
	1000 seed wt. (g)	25	0.35	0.11
	Oil content (%)	25	3.66	0.38
	Protein content (%)	25	3.86	0.39
BC_1P_1	Pod length (cm)	23	1.47	0.25
	1000 seed wt. (g)	23	0.83	0.19
	Oil content (%)	23	4.30	0.43
	Protein content (%)	23	4.15	0.42
F_1 (GD x G4)	Pod length (cm)	23	0.39	0.13
	1000 seed wt. (g)	23	0.40	0.13
	Oil content (%)	23	2.32	0.32
	Protein content (%)	23	1.05	0.21
F_2	Pod length (cm)	47	1.95	0.20
	1000 seed wt. (g)	47	0.87	0.14
	Oil content (%)	47	3.21	0.26
	Protein content (%)	47	3.09	0.26
BC_1P_2	Pod length (cm)	25	1.00	0.20
	1000 seed wt. (g)	25	0.77	0.17
	Oil content (%)	25	6.77	0.52
	Protein content (%)	25	6.12	0.49
G4 (P_2)	Pod length (cm)	22	0.14	0.08
	1000 seed wt. (g)	22	0.36	0.12
	Oil content (%)	22	3.75	0.41
	Protein content (%)	22	1.99	0.30

Table 3-6. Pearson correlation coefficients of pod length, 1000 seed weight, seed oil and protein contents from three individual segregating populations in *B. napus*.

Population [a]		Oil content [b]	Protein content	Pod length
BCP1	1000 seed weight	-0.01	0.10	0.25
	Oil content		0.29	-0.52 **
	Protein content			0.13
BCP2	1000 seed weight	0.04	0.10	0.63**
	Oil content		-0.45*	-0.31
	Protein content			0.26
F_2	1000 seed weight	0.32*	0.29*	0.54 **
	Oil content		-0.12	-0.26
	Protein content			0.25

[a] The population size was 50 plants for F_2, 24 plants for BCP1 and 25 plants for BCP2, respectively.

[b] The values followed * or ** indicates $p < 0.05$ or $p < 0.01$, respectively.

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CHAPTER 4

INTERRELATIONSHIPS OF MAJOR AGRONOMIC AND QUALITY TRAITS IN *BRASSICA NAPUS* L.

4.1 INTRODUCTION

High seed yield, seed oil and protein content of residual meal are desirable characters for modern rapeseed cultivars. Various breeding methodologies have been used to improve these traits, which have been discussed in detail by previous reviewers (Thompson 1983, Downey and Rakow 1987, Downey and Röbblen 1989, Thurling 1991, Downey and Rimmer 1993). Most of these methods depend, to a large extent, on resources available, as well as the breeder's personal experience and preference rather than on well established information about the interrelationships of these traits. This results mainly from the complex nature of the inheritance of these traits and the presence of considerable effects on them from environmental factors, and genotype x environment interactions. Thus, multiple location and year tests are routinely conducted in breeding programs to evaluate seed yield, seed oil and protein content. Unfortunately, this approach is very expensive, and is frequently a hit-and-miss process. Understanding the interrelationships of these traits is important for an effective breeding program. The choice of proper parents for making crosses and/or backcrosses, and the design of a proper selection scheme in hybrid progenies, all depend on information about the interrelationships among these traits.

Seed yield of rapeseed cultivars consists of seed yield components, i.e. number of pods per unit area, number of seeds per pod and seed weight. In addition, a number of heritable and environmental factors can indirectly influence final seed yield. These factors include a cultivar's maturity, resistance to diseases and insects, shattering, its tolerance to drought, frost damage and lodging, plant density and branching capacity of plants, the level and duration of retention of green leaves, soil fertility, moisture, different cultivation practices etc. These indirect factors have been discussed in numerous publications and will not be covered here. The emphasis in the present study will centre on the interrelationships of seed yield and its components, and pod length.

The value of rapeseed is virtually determined by its seed oil content and the protein content of the residual meal, and the chemical compositions of these two traits. Significant progress has been made in quality improvement, particularly the reduction of erucic acid in the seed oil and glucosinolates in the residual meal, which have also been discussed generally or specifically in numerous publications and will not be addressed here. The present study will focus on total oil and protein content, and their interrelationships with other traits, with the aim to provide rapeseed breeders with additional information to establish more effective and economic evaluation schemes for improvement of seed yield, and seed oil and protein contents.

4.2 MATERIALS AND METHODS

F_1 hybrids were produced from the cross 90-3010 (G1) x 90-1152 (GD) through hand pollination in the greenhouse. Both parents were doubled haploid (DH) lines of spring *B. napus*. Under field conditions, GD had an approximate pod length of 11 cm, while G1 had an approximate pod length of 6 cm. DH lines designated as M7 family were produced though microspore culture of the F_1 plants using the methods described in 2.2.1. Seeds of each DH line were increased in the greenhouse for an additional generation prior to growing the lines in the field.

In 1993, 25 lines were randomly sampled from the DH line family and grown in the field at the Edmonton Research Station of the University of Alberta, Edmonton, along with the cultivar Alto (spring type *B. napus*). These lines/cultivar were grown in two randomized complete blocks, with each line/cultivar being grown in a plot consisting of four 6-meter rows with 23 cm spacing between rows. The plots were sown with a Fabro drill seeder (Fabro Ltd. Swift Current, Sask., Canada) at a seeding rate of 1.25 g of seeds per row. Five plants were randomly sampled from the two centre rows of each plot to reduce the marginal effect. Pod length was measured and seed set per pod (SSP) recorded from the first five pods formed on the main raceme of each sampled plant, when the pods had reached their full length indicated by seeds contained therein turning brown in color. Samples were collected from this portion of the raceme because

these pods formed first on a plant, and they would be less variable in length and SSP, since previous studies have shown that pods at the basal portion on the main receme have more fertile ovules and total number of ovules per ovary than the pods at the apical portion (Bouttier and Morgan 1992). Pod length, as defined in 2.2.2, and SSP were determined using the methods described in 2.2.2. The means of the pod length and SSP of each line/cultivar from 5 pods x 5 plants x 2 replications were used as the pod length and SSP of each line/cultivar. The number of pods on each sampled plant was counted by hand in the field after the plants had finished flowering, and the means from 5 plants x 2 replications of each line/cultivar were used as the number of pods per plant. Plants from two centre rows from each plot were harvested in bulk at maturity. Seed yield, 1000 seed weight, seed oil and protein contents of each line/cultivar were determined from the bulk seed, using the methods described in section 3.2.2, with the exception that 26.2 g of seeds per sample were used in the determination of seed oil content.

The same lines/cultivar were grown again in the field in 1994 at the same location using the seeds harvested from the field in the previous year. The field plot design was the same as that in 1993 except that there were three replications (blocks) in 1994. Determination of pod length, SSP, the number of pods per plant, seed yield, seed oil and protein contents was the same as in 1993.

The phenotypic correlations of pod length, SSP, the number of pods per plant, 1000 seed weight, seed oil content and the protein content of the residual meal were calculated as $r_{ij} = \frac{\sigma_{ij}}{\sigma_i \sigma_j}$, where σ_{ij} is the covariance of trait i and j, and σ_i and σ_j are standard deviations of i and j, respectively. The rank correlation coefficient (r_s) of each trait in two years was calculated with the same formula using the ranks of DH lines/cultivar on each trait in two years. Analysis of variance of each trait was performed using a random model for all factors, i.e. year, replication within year, line/cultivar, line x year interaction. Computations for variances and covariance, correlations and regressions, and ranks of each trait were done using SAS/STAT 6.0 (SAS Institute Inc. 1989). The direct and indirect effects on seed yield from pod length, SSP, pod number per plant and 1000 seed weight, diagrammed in Fig. 4-1, were analyzed with path coefficient analysis, using the principles described by Li (1977). Briefly, path coefficient analysis permits the separation of a correlation coefficient between two variables into components of direct and indirect effects. In Fig. 4-1 for example, the direct effect of trait i on seed yield (0) is measured by the path coefficient (P_{0i}) from i to 0, which is a standardized partial regression coefficient and calculated as $P_{0i} = b_i \frac{\sigma_i}{\sigma_0}$, where b_i is the partial regression coefficient of seed yield on component i when all components, i.e. pod length, SSP, number of pods per plant, and 1000 seed weight, are considered. σ_i and σ_0 are standard deviation of component i and seed yield, respectively. The indirect effect of a component i on seed yield (0) via component j is $P_{0ij} = r_{ij} P_{0j}$, where r_{ij} is the correlation coefficient between i and j, and P_{0j} is the path coefficient from j to 0. For example, the phenotypic correlation coefficient (r_{10}) between pod length (1) and seed yield (0) in Fig. 4-1 can be partitioned into the direct effect from pod length to seed yield (P_{01}) and three indirect effects $r_{12}P_{02}$, $r_{13}P_{03}$, and $r_{14}P_{04}$, where the sum of the direct and indirect effects equals to the value of r_{10} ($r_{10} = P_{01} + r_{12}P_{02} + r_{13}P_{03} + r_{14}P_{04}$).

4.3 RESULTS

Two consistent results were observed over two years: Pod length was positively correlated with 1000 seed weight (Table 4-1), and protein content had no relationship with any other traits. Except for these two findings, no consistent relationships were observed among other traits. For instance, pod length was closely related to SSP in 1993 but there was no significant relationship between these two traits in 1994. Similar associations occurred between seed yield and SSP, between SSP and seed weight, as well as between pod length and PNP. Apparently, there were significant interactions between certain traits and years which were confirmed by the analysis of variance (Table 4-2). Of the seven traits investigated, pod length had the least variation over years, followed by 1000 seed weight, oil content and SSP, which were indicated by the highly significant rank correlations of these traits between two years (Table 4-3).

Path coefficient analysis (Table 4-4) revealed that there was a direct negative effect of pod length on seed yield, but indirectly, pod length had a positive effect on seed yield either through SSP or seed

weight. In 1993, an indirect positive effect on seed yield via SSP (0.452) was greater than the direct negative effect (-0.298) of pod length. However, in 1994 the direct negative effect of pod length on seed yield (-0.652) was three times as large as the indirect positive effect via 1000 seed weight (0.214). Averaged over two years, there was no positive contribution of pod length to seed yield.

The effect of 1000 seed weight on seed yield also varied with years. In 1993, its positive effect mainly came from an indirect positive effect via SSP (0.401) and was greater than the indirect negative effect via pod length (-0.210). The negative indirect effect on seed yield via pod length (-0.492) was nearly twice as great as the positive direct effect (0.284) of seed weight in 1994.

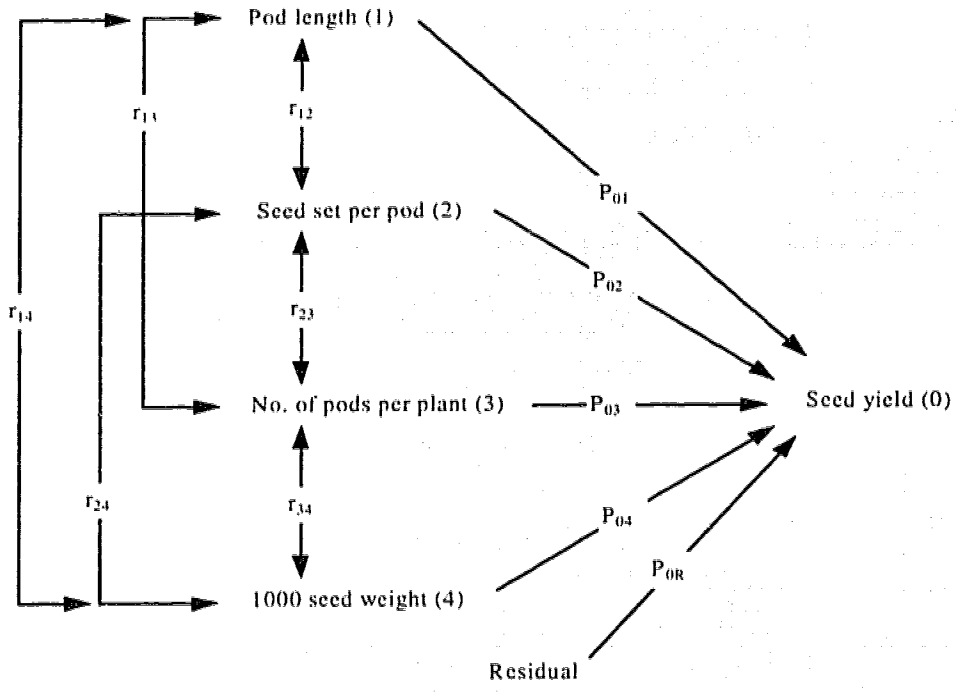

Figure 4-1. Cause-effect relationship between seed yield and four seed yield related traits in *B. napus*. P_{0i} between a single-headed line represents the path coefficient from that trait (i) to seed yield (0), and the r_{ij} between a two-headed line represents the correlation coefficient between the two components (i and j) linked by that line.

While the number of pods per plant had no apparent effect on seed yield, SSP was the only trait having positive effect on seed yield in two years among pod length, SSP, the number of pods per plant and 1000 seed weight (Table 4-4).

4.4 DISCUSSION

4.4.1 Relationships between seed yield and its components

4.4.1.1 Seed yield vs. the number of pods per plant

Significant differences in the number of pods per plant were observed among the lines in the present study (Table 4-3), but the ranks of this trait between two years were poorly correlated (Table 4-3). This suggested that the number of pods per plant was a highly variable trait responsive to other factors. In fact, previous studies have shown that the number of pods per plant can be influenced by both environmental factors and physiological conditions of plants (Andersson and Olsson 1961). Allen and Morgan (1975) found that pod number was positively correlated with the leaf area index at the onset of flowering.

Induced shading often resulted in fewer flowers and fewer pods developing to maturity (Tayo and Morgan 1979). Sowing dates also influenced the number of pods per plant, with early sowings often producing more pods per plant than late sowings (Richards and Thurling 1978, Mendham and Scott 1975, Mendham et al. 1981a,b). Given these findings, selection for the number of pods per plant in rapeseed breeding would be very difficult without the effective control of other factors.

Despite the large differences in both the number of pods per plant and seed yield (Table 4-3) among the lines investigated, no relationship between these two traits was found in the present study (Table 4-1). Path coefficient analysis did not identify any significant direct or indirect contribution from the number of pods per plant to seed yield either (Table 4-4). This was somewhat surprising, considering that pod number is one of the seed yield components. In addition, positive correlation between seed yield and the number of pods has been frequently reported (Andersson and Olsson 1961, Allen et al. 1971, Allen and Morgan 1972). In spring *B. napus and B. rapa*, Richards and Thurling (1978 1979) observed high genetic correlation (r = 0.90) between these two traits, where the number of pods per plant was one of the determinants of genotype variation in seed yield. Using path coefficient analysis, Shabana et al. (1990) found that the number of pods per plant had the highest direct effect on seed yield of individual plants. Foss (1977) obtained high seed yield in *B. juncea* by selection for a large number of pods on the terminal branches. On the other hand, high pod canopy does not always produce high seed yield. In winter *B. napus*, Mendham et al. (1981a) obtained high seed yield from late sowings. Compared with plants of early sowings, late sown plants initiated inflorescence and flowered later, had approximately half the number of pods m^{-2}, but produced higher seed yield (Mendham et al. 1981b). This yield advantage over early sowings was the result of greater seed number per pod which was related to the reduced seed abortion in the lower portion of the pod canopy.

The exact reason for the difference between this and most of the previous studies in the relationship between the number of pods and seed yield is unclear. Considering that the number of pods per plant was highly variable, the sample size in the present study might be too small to represent a line's true value of this trait, since only five plants from each line were sampled from two (in 1993) or three (in 1994) replications for measuring this trait, and the mean of the sampled plants was used to represent the number of pods per plant for a line. Moreover, the number of pods per plant was counted on the plants in the field and sampled plants were left to grow to maturity. There should have been some reduction, probably with varying degrees among the different plants, in the number of pods per plant from time of the data collection to the time of harvesting. The number of pods per plant could also be compensated for by the other yield components. For instance, a significant negative correlation was observed between the number of pods per plant and 1000 seed weight in 1994 (Table 4-1). In addition, the genotypes used in the present study were DH lines except cv. Alto. These lines were genetically homozygous and have not been selected for any traits. Whereas most, if not all, of the genotypes used in previous studies were released open pollinated cultivars. It is possible that in such released cultivars high seed yield and high number of pods per plant have been selected together during the course of cultivar development. These possibilities could have resulted in the difference between the present and previous studies in the relationship between the number of pods per plant and seed yield. However, the information on the relationship between these two traits in nonselected populations would be more interesting than the information from established cultivars for practical breeding purposes.

Thus, it appears from the present and previous studies that the number of pods per plant is a highly variable trait, and its relationship with seed yield is still unclear. This makes it very difficult to use the number of pods per plant alone as a selection criteria for high seed yield in *B. napus* breeding programs.

4.4.1.2 Seed yield vs. seed set per pod

A positive correlation between seed yield and SSP was observed in 1993 but not in 1994 (Table 4-1) in the present study. Path coefficient analysis showed that SSP had a direct positive effect on seed yield in both years (Table 4-4). Averaged over two years, SSP had the highest positive effect on seed yield among the four traits investigated. In previous studies a positive correlation between these two traits has also been reported in *B. napus* (Andersson and Olsson 1961, Allen et al. 1971, Allen and Morgan 1972), *B. juncea* (Rawat and Anand 1977), and *B. rapa* (Kler et al. 1992). It appears from these studies that a high SSP would be an important character of a high seed-yielding genotype. Selection for high SSP should result in increased seed yield.

Large variation in SSP was also observed in the present study among the DH lines (Table 4-3), suggesting there is a potential for the further improvement of this trait.

It should be noted that SSP, as other yield components, is also influenced by environmental factors. This was indicated by a medium rank correlation (0.44) of SSP between two years. Indeed, a significant line x year interaction (Table 4-2) suggested that there was strong genotype x environment interaction for this trait. For instance, Morgan (1981) observed that added nitrogen fertilizer increased SSP. Mendham et al. (1981a,b) found that SSP was influenced by the pod canopy of the plants which was related to sowing date. Therefore, when using SSP as a reference for seed yield, one should also consider the environmental conditions which might cause changes in expression of this trait.

4.4.1.3 Seed yield vs. 1000 seed weight

Previous studies have shown that selection for high seed weight can improve seed yield. Morice (1960) concluded that the best character for selection for seed yield was seed weight per pod. In four years of trials with winter *B. napus* Musnicki (1975) observed that the highest yielding variety, Brilland, showed a yield advantage over other native and foreign varieties mainly because of its higher 1000 seed weight. However, no apparent direct relationship between seed yield and 1000 seed weight was observed in the present study, but indirectly the effect of seed weight on seed yield came from either SSP or pod length. As indicated by the path coefficient analysis, without the changes in other traits, longer pods in general would have a negative effect, while increasing SSP would have a positive effect. Clearly, such indirect effects varied with years as indicated by the different path coefficient values in two years. These results suggested the effect of seed weight on seed yield is interrelated to other seed yield related traits and environmental factors. It would be very difficult to clearly define the relationship between seed yield and seed weight. Again, the exact factor(s) causing the difference between the present and previous studies in the relationship between seed weight and seed yield is unknown. Differences in the genotypes (selected cultivars vs. non-selected lines) investigated and/or in environmental conditions, as discussed in 4.4.1.1 for the relationship between seed yield and the number of pods per plant, would also apply to the arguments for the difference between the present and previous studies in the relationship of seed yield and seed weight.

However, 1000 seed weight was the most stable trait among the number of pods per plant, SSP, and 1000 seed weight, which was indicated by its very low variance attributable to replications within year and year x line interactions (Table 4-2). The high stability of 1000 seed weight was also demonstrated by its highly significant rank correlation between two years (r_s=0.88) (Table 4-3). Thus, in a rapeseed breeding program, seed weight can be evaluated accurately with relatively few replications and years, and be selected at early generations.

4.4.2 The value of long pods in rapeseed crops

Early studies have shown that rapeseed pods can transport nutrients and photosynthates from pods to the developing seeds in *B. napus* (Allen et al. 1971, Norton and Harris 1975, Brar and Thies 1977, Zhang et al. 1991), *B. rapa* (Sheoran et al. 1991) and *B. juncea* (Subrahmanyam and Rathore 1992). However, a excessively large pod canopy would lead to seed abortion on the lower pods of the canopy and reduced seed yield (Mendham and Scott 1975, Mendham et al. 1981a,b, Mendham et al. 1984). This led to the increasing interest in developing cultivars with longer pods. Such cultivars may have either increased seeds per pod or/and seed weight with optimum number of pods per plant. Chay and Thurling (1989a, b) observed considerably variable but highly positive correlations between pod length and seed weight in *B. napus*. Later, it was reported that long-podded lines produced at least five more seeds per pod than short-podded lines (Thurling 1991). However, the long-podded lines had no advantage in seed yield over others, since long-podded lines had reduced pod number per plant (Chay and Thurling 1989b, Thurling 1991).

Large differences in pod length were observed among the DH lines in the present study (Table 4-3). In addition, previous studies have shown that the pod length of the materials tested in the present study was determined by three nuclear genes (Chapter 2 and 3), indicating pod length was an easily selected trait. This conclusion was supported by the results of the studies in this chapter. The highly significant rank correlation of pod length between two years (r_s = 0.93) suggested that the relative pod length of the lines varied very little between two years. Together, these studies have shown that pod length is a highly heritable and simply inherited trait. Of major interest in the present study were the relationships of pod length with other agronomic and quality traits.

No studies on the relationship between pod length and seed oil, or seed protein content have been reported earlier. In the present study, pod length was not related to seed oil or protein content (Table 4-1), despite the large variation observed in all three traits. Thus, it may not be possible to improve seed oil or

protein content through indirect selection for pod length. However, lack of association between pod length with seed oil, or protein content suggests that it should be possible to develop long-podded cultivars with high seed oil and protein content.

Pod length had a positive correlation with 1000 seed weight and seemed to be positively associated with SSP, but path coefficient analysis revealed a negative direct effect of pod length on seed yield. Thus, it is important to know whether the indirect contributions of pod length to seed yield is greater or less than its direct negative effect. Path coefficient analysis did not show any significant indirect positive contribution of pod length to seed yield either (Table 4-4). Detailed analysis on seed yield and pod length also did not show any advantage of long-podded lines over some of the DH lines with normal pod length. For example, cultivar Alto ranked 22 and 20 in pod length in 1993 and 1994, respectively (Table 4-3), but it ranked second in seed yield in both years. Similarly, the DH lines with the longest pod length in both years did not produce the highest seed yield. Within the range of DH lines investigated, the overall negative effect of pod length on seed yield was close to, or greater than its indirect positive effect. Therefore, although longer pods may increase SSP and/or seed weight, it in general has a slight negative effect on seed yield. Considering that an increase in pod length by 1 cm can, on average, result in only one more seed per pod (Chapter 2), and there is large variation in the relationship, the indirect positive effect of pod length on seed yield through SSP would be small, and indeed, the path coefficient analysis showed that such an indirect effect varied with years. In 1993 this indirect effect (0.45) made pod length have a very weak positive correlation with seed yield (0.178), but there was no positive indirect contribution from pod length to seed yield through SSP in 1994. Similarly, the indirect contribution of pod length to seed yield via seed weight was also very limited and inconsistent. Thus, data from the present study did not provide any solid evidence for the advantage of long pods in seed yield.

It should be mentioned, however, that the DH lines tested in the present study were derived from the cross G1 x GD. These two parents were DH lines and chosen mainly for their contrasting pod lengths and homozygosity of this trait for genetic studies on pod length, but not for their seed yield potential. In fact, they had not been selected for any agronomic or quality traits. Therefore, the yield and the yield components of these two parents and their DH line progenies may have limited genetic potential even though there were large differences in pod length. This could limit the possibility of producing long-podded lines with good yield advantage from this germplasm. It would be interesting to study the yield performance of the progeny from crosses between long-podded genotypes and high-yielding genotypes with normal pod length. In addition, since longer pods will naturally require more assimilates for pod wall formation, proper soil fertility should be maintained. Furthermore, with the increase in pod length, lodging resistance should also be improved in order to support the heavier dry matter production. All these factors should be considered when exploring the possible potential of long pods in rapeseed breeding.

To conclude, there were large but highly heritable variations in pod length in *B. napus*. However, no apparent advantage of long pods for seed yield was obtained within the lines investigated. Of the yield components, seed weight can be reliably selected, but it does not have an apparent positive association with seed yield, and it alone can not be used as a selectable trait for high seed yield. The number of pods per plant had no significant relation with seed yield either in this study. SSP appeared to be the only trait having a positive direct effect on seed yield. Therefore, more attention should be given to SSP than to any other yield components when selecting for high seed yield. When the interrelationships of seed yield and yield related traits are taken into account, it is still very difficult to choose a single, or a few, yield components as a selection criteria for seed yield. This difficulty partially arises from compensating effects among the seed yield components, as observed from present and previous studies. An increase in one component is often accompanied by reduction of another (Andersson and Olsson 1961, Thurling 1974, Musnicki 1975, Mendham et al. 1981a,b, Gou and Yuan 1987, Tommey and Evans 1992). Apart from such compensating effects, the presence of large environmental and physiological effects on seed yield and yield related traits make any indirect evaluation on seed yield very unreliable. In fact, the contributions from so-called yield components are very limited. For example, in the present study a large proportion of the effect on seed yield comes from factors which can not be explained by the traits investigated. This is indicated by the values of determinations of 0.4 in 1993 and 0.3 in 1994 (Table 4-4), when the seed yield was regressed on pod length, SSP, pod number per plant and 1000 seed weight. When all findings from many studies on seed yield and yield related traits are considered, it seems, as has been reported by Grosse et al. (1992) in winter *B. napus*, that high seed yield could be obtained by different combinations of three yield components.

4.4.3 Interrelationships of seed oil content, protein content, and seed yield

4.4.3.1 Seed oil content vs. protein content of residual meal

Oil percentage has a relatively higher heritability than seed yield (Olsson 1960). In addition, oil content can be quickly and accurately measured with wide-line nuclear magnetic resonance (NMR) (Conway and Earle 1963) or near infrared (NIR) (Starr et al. 1985). Therefore, more rapid progress can be made in selecting for seed oil content than for seed yield in rapeseed breeding. Unfortunately, intensive selection for oil content usually results in lower protein values (Downey and Rimmer 1993, Daun et al. 1995) because seed oil and protein contents are negatively associated (Grami et al. 1977). Grami and Stefansson (1977a,b) observed that oil, protein and the sum percentage of oil and protein were determined by the maternal genotype, and that gene action was mainly additive with insignificant dominance and absence of epistasis. The heritability of the sum of oil and protein was higher than that of individual traits. Therefore, selection for the sum of oil and protein would be more effective than selection for either one without consideration of the other. Studies have also shown that the correlation between oil contents of individual plants and those of their progeny plots is poor (Olsson 1960, Thompson 1983). This may be due to environmental effects. Rakow (1979) found in winter *B. napus* that oil and protein contents were strongly modified by environmental factors. With field trials in *B. napus*, Zhao et al. (1993) found that seed protein and oil contents were influenced by the level of fertilizers N and S, but the effects were dependent on soil type. When the soil was S-sufficient, application of S had no significant effect on seed protein or oil contents, whereas application of N increased protein content, but decreased oil content concurrently. In S-deficient soil, there were significant interactions between S and N on protein. Thus, it appears that selection for oil and protein content should be based on plot rather than on individual plants, and the environmental conditions, particularly soil fertility, should be considered.

Large variation in seed oil and protein content was observed in the present study (Table 4-3) but no relationship between these two traits was observed over two years. This suggests that it may be possible to simultaneously improve seed oil and protein content. It should be remembered that in the present study, the evaluation of seed oil and protein was based on replicated plots rather than on individual plants. In addition, all lines except cv. Alto are DH lines which had not been selected for either oil or protein content. Therefore, these lines/cultivar were genetically more uniform than plants from conventional pedigree progenies. These factors might be related to the finding that oil and protein were not negatively associated as had been reported earlier. However, since this study was conducted at only one location, a study of these relationships over a larger range of different environmental conditions would be desirable, and should clarity the initial results.

4.4.3.2 Seed yield vs. seed oil or protein content

It is surprising that the information on the relationship between seed yield and seed oil, or seed protein content is limited in the literature, given that high seed yield, seed oil and protein contents are primary requirements for a rapeseed cultivar. In *B. juncea* Rawat and Anand (1977) found a positive correlation between seed oil content and seed yield per plant. It has been mentioned earlier that the correlation of oil and protein contents of single plants and those of progeny plots are usually poor (also see Chapter 2). It was also known that seed yield evaluation based on individual plants was very unreliable. The reported positive correlation of seed yield and oil content has very little practical importance. By testing five genotypes of spring *B. napus* in three sowing dates, three sowing rates over two years and two locations, Degenhardt and Kondra (1984) did not find any association between seed yield and percentage seed oil or protein. Apparently, the relationship between seed yield and oil or protein content is still unclear.

Except for the positive correlation between seed yield and seed oil content observed in 1993 (Table 4-1), no positive or negative association was found between seed yield and seed oil or protein content in the present study over two years. Such inconsistency suggests it would be very difficult to clearly define the relationship between seed yield and these two quality traits. Nevertheless, no negative relationship was observed between these two quality traits and seed yield or any yield component. Improvement of oil and protein with any combinations of yield components or seed yield might be possible. Again, this conclusion is based on data from the replicated plots of homozygous genotypes at one location. Studies on the interrelationships of these traits from pedigree progenies and/or DH lines over a wider environmental range remains to be investigated.

Most of the traits studied in the present study are quantitative in nature, so they might perform quite differently in different environments. Differences in plant densities due to uneven germination are common in fields which might be a factor affecting expression of traits. Variation in one trait might cause changes in its relationships with others. For breeding programs, the information on the interrelationships of selected traits under different environments is important for proper experimental design and genotype evaluations. Accordingly, an experiment was specifically conducted to study the interrelationships of pod length, seed yield, yield components, and the seed oil and protein contents under different row spacings and plant densities. This study will be reported in the following chapter.

Table 4-1. Pearson correlation coefficients of seed yield, pod length, seed set per pod (SSP), pod number per plant (PNP), 1000 seed weight (seed wt), seed oil and protein content from 25 DH lines/cultivar in *B. napus* tested over two years[a].

	Year	Pod length (1)[b]	SSP (2)	PNP (3)	Seed wt (4)	Oil	Protein
Seed yield (0)	1993	0.18	0.59**	-0.08	0.24	0.55**	0.08
	1994	-0.43*	0.18	0.18	-0.18	0.18	0.07
Pod length (1)	1993		0.61**	0.10	0.70**	-0.02	-0.06
	1994		0.20	-0.42*	0.75**	0.25	-0.12
SSP (2)	1993			0.05	0.54**	0.33*	0.00
	1994			-0.35	0.25	0.35	0.00
PNP (3)	1993				0.04	-0.03	0.20
	1994				-0.39*	-0.16	-0.07
Seed wt (4)	1993					-0.20	0.33
	1994					0.00	0.03
Oil	1993						0.00
	1994						-0.16

[a] The values followed by * denotes $p < 0.05$ and those followed by ** denotes $p < 0.01$.

[b] The numbers in the parenthesis are the variable numbers corresponding to the variables in Fig. 4-1

Table 4-2. Analysis of variance for pod length (PL), seed set per pod (SSP), pod number per plant (PNP), seed yield (yield), 1000 seed weight (Seed wt), seed oil (oil) and protein content in *B. napus* over two years.

Source of variation	DF	Mean square[a]						
		PL	SSP	PNP	Yield	Seed wt	Oil	Protein
Year	1	0.10	8.32**	29654.21*	15.64	17.03**	0.86	0.55
Replication /year	3	0.20	0.12	2260.61	31.77	0.05	4.44**	13.21**
Line	24	5.51**	31.06**	3703.15*	71.67**	1.96**	12.72**	8.49*
Year x Line	24	0.41**	15.06**	1553.94	45.60**	0.15*	1.96**	3.80

[a] The values followed by * denotes $p < 0.05$ and those followed by ** denotes $p < 0.01$.

Table 4-3. The means of pod length, seed set per pod (SSP), pod number per plant (PNP), seed yield, 1000 seed weight, seed oil and protein content of 26 DH lines/cultivar, and the Spearman correlation coefficients (r_s) of each trait between two years in *B. napus*.

DH lines /	Pod length (cm)		SSP		PNP		Yield (100 kg/ha)		1000 Seed wt (g)		Oil (%)		Protein (%)	
cultivar	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994	1993	1994
Alto	6.8	7.3	31	31	136	154	35	33	4.4	3.6	44.8	42.2	38.3	37.8
M7DH01	9.4	9.2	34	34	129	107	23	22	5.6	4.6	42.4	40.8	38.2	36.8
M7DH02	9.9	8.8	31	29	127	179	15	21	5.1	4.7	38.2	40.0	36.4	37.2
M7DH03	7.9	8.9	23	31	121	113	15	22	5.2	4.7	38.2	40.7	40.5	41.1
M7DH04	8.0	7.9	29	28	92	162	17	23	4.8	4.4	37.9	39.4	35.7	37.6
M7DH05	9.8	9.3	36	32	138	133	37	22	5.6	4.6	44.0	43.5	34.7	36.9
M7DH06	10.4	9.4	34	29	185	186	24	22	6.3	5.3	39.3	38.9	41.1	37.8
M7DH07	8.3	8.2	31	29	107	114	23	18	4.9	4.4	40.8	40.3	37.0	38.7
M7DH08	9.1	8.6	37	32	142	135	27	26	6.4	5.3	39.2	40.2	37.4	37.3
M7DH09	5.6	6.8	22	29	103	182	23	25	4.7	4.1	38.8	38.9	38.2	36.9
M7DH10	7.2	7.3	32	29	109	108	24	25	4.0	3.9	42.2	40.7	34.6	38.7
M7DH11	8.1	8.5	24	29	145	175	18	26	4.6	4.3	37.9	40.7	36.0	36.7
M7DH12	9.7	9.1	32	30	92	127	24	26	5.6	4.5	40.2	41.0	36.0	38.1
M7DH13	6.0	6.7	27	30	180	245	19	21	3.5	3.1	42.3	40.9	38.6	38.8
M7DH14	9.6	9.1	30	30	126	169	23	18	5.4	4.1	42.3	42.3	36.5	35.7
M7DH15	7.8	7.8	30	29	164	181	22	20	4.6	3.6	37.8	37.3	36.7	37.2
M7DH16	8.9	9.2	33	36	99	122	26	22	5.3	4.9	43.2	43.5	38.0	38.2
M7DH17	8.8	8.9	30	31	109	106	25	23	5.6	4.7	39.6	41.6	38.2	37.0
M7DH18	7.7	7.7	34	35	63	112	31	26	4.9	4.3	39.3	39.7	40.0	38.1
M7DH19	9.9	9.4	33	29	130	173	33	23	5.6	4.9	41.2	41.0	39.5	37.4
M7DH20	7.4	7.4	31	33	124	178	28	30	5.7	4.7	39.1	40.1	40.8	40.1
M7DH21	6.8	6.9	27	29	130	160	19	25	3.7	3.5	41.6	40.3	36.2	35.2
M7DH22	8.0	8.4	33	36	188	149	22	23	5.6	4.4	39.1	39.7	39.7	36.1
M7DH23	7.0	7.8	30	32	78	187	22	34	5.0	4.3	41.5	42.3	37.1	36.5
M7DH24	7.8	8.4	30	33	113	174	20	24	6.1	5.0	40.1	40.4	38.7	36.9
M7DH25	7.3	7.2	30	30	125	209	23	30	4.4	3.5	41.6	42.1	38.5	39.3
Range	4.8	2.7	14	8	125	139	20	16	2.9	2.2	7.0	6.2	6.4	5.9
r_s	0.9255		0.4357		0.2766		0.2166		0.8791		0.7646		0.3254	
$Pr > \|r\|$	0.0001		0.0295		0.1807		0.2984		0.0001		0.0001		0.1125	

Table 4-4. Path coefficients to seed yield from four seed yield-related traits pod length, seed set per pod, pod number per plant and 1000 seed weight in *B. napus*

Pathways of associations [a]	Path coefficients in the year	
	1993	1994
Seed yield (0) vs. pod length (1)		
Direct effect (P_{01})	-0.298	-0.652
Indirect effect via seed set per pod ($r_{12}P_{02}$)	0.452	0.055
Indirect effect via pod number per plant ($r_{13}P_{03}$)	-0.010	-0.047
Indirect effect via 1000 seed weight ($r_{14}P_{04}$)	0.033	0.214
Total r_{10}	0.178	-0.430
Seed yield (0) vs. seed set per pod (2)		
Direct effect (P_{02})	0.746	0.275
Indirect effect via pod length ($r_{12}P_{01}$)	-0.181	-0.130
Indirect effect via pod number per plant ($r_{23}P_{03}$)	-0.004	-0.040
Indirect effect via 1000 seed weight ($r_{24}P_{04}$)	0.025	0.071
Total r_{20}	0.587	0.178
Seed yield (0) vs. pod number per plant (3)		
Direct effect (P_{03})	-0.081	0.113
Indirect effect via pod length ($r_{13}P_{01}$)	-0.031	0.272
Indirect effect via seed set per pod ($r_{23}P_{02}$)	0.034	-0.097
Indirect effect via 1000 seed weight ($r_{34}P_{04}$)	0.002	-0.110
Total r_{30}	-0.080	0.178
Seed yield (0) vs. 1000 seed weight (4)		
Direct effect (P_{04})	0.047	0.284
Indirect effect via pod length ($r_{14}P_{01}$)	-0.210	-0.492
Indirect effect via seed set per pod ($r_{24}P_{02}$)	0.401	0.069
Indirect effect via pod number per plant ($r_{34}P_{03}$)	-0.003	-0.044
Total r_{40}	0.235	-0.182
Path coefficient of residual (P_{0R})	0.773	0.838
Total value of determination (R^2)	0.402	0.297

[a] The number and symbols following each trait are corresponding to those indicated in Fig. 4-1.

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CHAPTER 5

EFFECTS OF ROW SPACING AND PLANT DENSITY ON AGRONOMIC AND QUALITY TRAITS IN *BRASSICA NAPUS* L.

5.1 INTRODUCTION

Most of the agronomic and quality traits of rapeseed, such as seed yield and its components, and seed oil and protein contents, are sensitive to variation in macro- and micro-environmental conditions. The performance of such traits under one set of environmental conditions could be very different from that under other conditions. In addition, variation in one trait might cause changes in its relationships with others. The information on the interrelationships of such traits under different environments is important for proper experimental design and genotype evaluations. While macro-environmental effects on the performance of breeding materials at this time can only be roughly estimated using tests with multiple locations and years, effects of micro-environmental factors can be specifically tested with proper experimental design.

Among various micro-environmental factors, variation in plant density due to uneven germination or various survival rates after winter is very commonly observed in field test plots. Breeders very often have to evaluate the performance of their breeding materials and make inferences about them based on such varying plant densities. Unfortunately, conclusions based on one plant density might not be applicable to other plant densities. Roy and Paul (1991) observed in field trials with three *B. rapa* cultivars grown at four densities that plant height, number of branches and number of pods per plant, number of seeds per pod and harvest index decreased with increase in population density. Similar results were reported from field trials with *B. juncea* (Singh et al. 1989) where three cultivars were grown at two row spacings and three plant densities. In *B. napus*, McGregor (1987) observed from two cultivars in a field with hail injury that reduced plant density compensated for the yield loss through increasing the number of seeds per pod and seed weight in some instances, but yield compensation at low plant density was mainly due to an increase in pod number. These studies indicate that row spacing and plant density influence seed yield and yield related traits. Few studies have dealt in depth with the interrelationships of various traits at variable row spacing and plant densities. For genotype evaluations, interrelationships of specific traits and relative performances of genotypes at different row spacing and plant densities are equally important to the absolute values of individual traits under such conditions. This chapter reports the experiments which were conducted to study the influences of row spacing and plant density on pod length, seed yield and seed yield related traits, seed oil and protein contents in *B. napus* and interrelationships of these traits.

5.2 MATERIALS AND METHODS

F_1 hybrid plants were produced from the cross 90-3078 (G4) x 90-1152 (GD) in the greenhouse using the methods described in 2.2.1. Both G4 and GD were doubled haploid lines of spring type *B. napus* from the breeding program of the University of Alberta. Under field conditions, GD was characterized as a long-podded genotype with an approximate pod length of 11 cm, while G4 had an approximate pod length of 7 cm. The F_1 plants were grown in the growth chamber and doubled haploid (DH) lines designated as M8 family were produced from the F_1 plants through microspore culture, which has been described in 2.2.1. The seeds of each DH line were increased for an additional generation in the greenhouse prior to growing them in the field. Two different field plot layouts were used in 1993 and 1994.

5.2.1 Field layout in 1993

Twenty DH lines randomly sampled from the DH line family and cv. Alto (spring type *B. napus*) were grown at the Edmonton Research Station of the University of Alberta, Edmonton. Each DH line/cultivar was grown at two different row spacings and plant stands within rows, i.e. normal row spacing and plant stand (normal density) and doubled row spacing and spaced plant stand within rows (spaced density). In the normal density, the DH lines/cultivar were grown in a completely randomized field block. Each plot consisted of four 6-meter rows with 23 cm spacing between two adjacent rows. The plots were sown with a Fabro drill seeder (Fabro Ltd. Swift Current, Sask., Canada) at a seeding rate of 1.25 g of seeds

per row. The plants within rows were not thinned. In the spaced density, the same DH lines/cultivar were grown in another completely randomized field block on the same sowing date as the normal density, with each plot consisting of two 6-meter rows with the doubled row spacing (46 cm). The plots were also sown with a Fabro drill seeder (Fabro Ltd, Swift Current, Sask., Canada) at the rate of 1.25 g seeds per row, and the plants within each row were thinned to an approximately 20 cm spacing between two adjacent plants, after the seedlings had established.

For the normal density, pod length and seed set per pod (SSP) were determined from five plants randomly sampled from the two central rows of each plot. Pod length was measured, and seeds contained recorded from the first five pods formed on the main raceme of each sampled plant, when the pods had reached their full length as indicated by the seeds turning brown in color. The reason that samples were collected from this portion of the raceme was that these pods were formed first on a plant. In addition, studies have also shown that pods at the basal portion on the main receme have more fertile ovules and total number of ovules per ovary than the apical pods (Bouttier and Morgan 1992). Therefore, pod length and SSP at this portion of raceme should be less variable than those on other parts of a plant. The means of pod length and SSP of each line/cultivar from 5 pods x 5 plants were used to represent the pod length and SSP of each line/cultivar. The number of pods of each sampled plant was counted in the field after plants had finished flowering, and the mean of the five plants was calculated to represent the number of pods per plant of each line/cultivar. At maturity, only plants of two central rows of each plot were harvested in bulk. Seed yield, 1000 seed weight, seed oil and protein contents of each line/cultivar were determined from the seed bulk. Seed yield, 1000 seed weight, seed oil and protein contents of each line/cultivar were determined from the bulk seed, using the methods described in section 3.2.2, with the exception that 26.2 g of seeds per sample were used in the determination of seed oil content.

For the spaced plant density, five plants from each plot were randomly sampled to determine pod length, SSP and the number of pods per plant, using the method described earlier. All plants were harvested in bulk at maturity. Seed yield, 1000 seed weight, seed oil and protein contents of each line/cultivar were determined from the seed bulk, using the same methods for the normal plant density.

5.2.2 Field layout in 1994

A split plot in randomized complete block design was employed to get more accurate estimation of the effects of plant density on seed yield and yield related traits, oil and protein contents, and interrelationships of these traits. Nine DH lines which showed good potential in seed yield and seed oil content in 1993 and cv. Alto were grown in four randomized complete blocks at the Edmonton Research Station of the University of Alberta, Edmonton, using the seeds harvested in the previous year, where the lines/cultivar were whole-plots and plant densities were subplots. Two plant densities as defined in 5.2.1 were studied, where each plot of the normal density consisted of four 6-meter rows with 23 cm row spacing, and each plot of spaced density consisted of two 6-meter rows with 46 cm row spacing. All plots were sown with a Fabro drill seeder (Fabro Ltd. Swift Current, Sask., Canada) at the rate of 1.25 g of seeds per row. After the seedlings had established in the field, the plants in the plots of the spaced density were thinned into an approximately 20 cm spacing within rows. Five plants were randomly sampled from each subplot to measure pod length, SSP and number of pods per plant, using the same methods described previously. At maturity, all plants of each subplot, i.e. four rows in normal density and two rows in spaced density, were harvested in bulk. Seed yield, oil and protein contents were determined using the methods described earlier.

5.2.3 Data analysis

For the data in 1993, the phenotypic correlations were calculated from the means of pod length, SSP, the number of pods per plant, 1000 seed weight, seed oil content and protein content of each DH line/cultivar within each plant density, using the formula $r_{ij} = \frac{\sigma_{ij}}{\sigma_i \sigma_j}$, where σ_{ij} is the covariance of traits i and j, and σ_i and σ_j are standard deviations of i and j, respectively. The rank correlation coefficient (r_s) of each trait in two years or at two plant densities was calculated with the same formula using the ranks of DH lines/cultivar in two years, or at two plant densities for the data in both years. ANOVA was performed for each trait for the data in 1994, using the model $Y_{ijk} = \mu + \rho_i + \alpha_j + \gamma_{ij} + \beta_k + (\alpha\beta)_{jk} + \varepsilon_{ijk}$ (Steel and Torrie 1980), where Y_{ijk} is the observation in the ith block on jth whole-plot with the kth subplot.

Computations were done using SAS/STAT 6.0 (SAS Institute Inc. 1989).

5.3 Results and Discussion

5.3.1 Effects of row spacing and plant densities on individual traits pod length, the number of pods per plant, seed set per pod, 1000 seed weight, seed yield, seed oil and protein contents

The effects of row spacing and plant density on individual traits varied with the different traits. In general, pod length, the number of pods per plant and SSP increased at the spaced plant density, with the increase in the number of pods per plant being the most significant (Fig. 5-1 and Fig. 5-2). Pahkala et al. (1994) found in a previous study that with the increase in plant density the number of branches decreased as did the number of pods on the branches and on the main raceme. Similar findings were also reported by Roy and Paul (1991). These results showed that the number of pods per plant was very sensitive to the variation in row spacing and plant density.

Although there was no apparent difference in 1000 seed weight between the two plant densities in 1993 (Fig. 5-1), data in 1994 showed a slight (Fig. 5-2) but statistically significant (Table 5-1) decrease in 1000 seed weight at spaced plant density. The different results in these two years might be related to the different field layouts. The normal and spaced plant densities were planted in two different blocks in 1993, but they were planted in the same whole-plot in 1994. Conceivably, the soil fertility for the same DH line/cultivar at two plant densities, and possibly some other factors as well, would be more variable in 1993 than in 1994. Unfortunately, the block effect, if it existed, could not be statistically analyzed for the data in 1993.

The spaced plant density appeared beneficial for a high seed yield. In 1993, plants at the spaced density took twice as much row space as that of plants at the normal density (46 cm vs. 23 cm), but there were fewer plants within rows at the spaced density than at the normal density because of thinning. Because only two centre rows were harvested from each plot with normal density, the seed yield of each plot at the normal density was doubled. Nevertheless, most of the DH lines at the spaced plant density still produced higher seed yield than they did at the normal density (Fig 5-3). This indicated that plants at the normal density might be too densely populated, resulting in the reduction of seed yield. Although the possible block effect, which could not be statistically detected, might have been involved in this difference between the two plant densities in 1993, the data in 1994 showed that the normal plant density had no advantage in seed yield over the spaced plant density. In 1994, all four rows at the normal plant density were harvested. Therefore, within each whole-plot there should have been more than twice the number of plants at the normal density than at the spaced density. Interestingly, the seed yield at the normal plant density was not significantly higher than at the spaced density (Fig. 5-2 Table 5-1).

Given the fact that there was only a slight decrease in seed weight, but dramatic increase in the number of pods per plant, coupled with increases in pod length and SSP, a proper plant density was essential for producing high seed yield. A large reduction in plant density might be detrimental to seed yield, but a slight reduction from the normal density as defined in this experiment would be favorable. Thus, an optimum plant density at which to achieve the maximum number of pods per plant without significant adverse effects on other traits, such as maturity and lodging resistance of plants, is essential for high seed yield. Additional studies are needed to identify such an optimum plant density.

Because the genotypes tested in this study were DH lines except Alto which was an adapted cultivar in this area, the variation observed within each line/cultivar at different plant densities could be attributed to environment (plant density), and possibly genotype x environment interactions (plant density x line). ANOVA for the number of pods per plant revealed significant difference in plant density (Table 5-1), suggesting that the number of pods per plant mainly depended on the resource available. Thus, it is likely that cultivation practices, such as changes in seeding rate or row spacing, would have a greater effect on pod number per plant than breeding approaches.

Pod length, SSP and seed weight were much less responsive to the variation in plant density than the number of pods per plant. This conclusion was based on the fact that the ranks between the spaced and normal plant densities were highly correlated for pod length, SSP and 1000 seed weight (Table 5-2, Table 5-3), but poorly correlated for the number of pods per plant. Thus selection for pod length, SSP and seed weight in breeding programs would be more effective than for the number of pods per plant.

Accordingly, in breeding for improved seed yield, more emphasis should be given to SSP and seed weight with reference to pod size than to the number of pods per plant. Since row spacing and plant density affect the absolute values of pod length, SSP, and 1000 seed weight, but have little effect on the relative values of these traits, these traits may be evaluated at either high or low plant density provided the genotypes under study are grown at the same plant density. Variable plant densities due to uneven germination or different survival rates after winter often occur in test plots in rapeseed breeding programs. Such variation would affect absolute values of traits under study, which would lead to a biased conclusion if the experiment had limited replication. Thus it is necessary to study the minimum number of replications required for an accurate estimation of the traits under study in areas, such as the western Canadian prairie, where varying plant densities are unavoidable.

Of the two quality traits seed oil and protein content, no apparent differences in seed oil content were observed between the two row spacings and plant densities in either 1993 (Fig. 5-1) or 1994 (Fig. 5-2). The analysis of variance for the data in 1994 confirmed that there was no significant difference in the seed oil content between the two plant densities (Table 5-1). This is similar to a previous report (Morrison et al. 1990) which showed that row spacing had no significant effect on seed oil content of *B. napus* cv. Westar. The ranks of seed oil content at two plant densities also exhibited high correlation in both years (Table 5-2, Table 5-3). Apparently, seed oil content was very tolerant to the variation in plant density. Therefore, oil content can be evaluated at any plant density in rapeseed breeding. This finding could be used to advantage. When there are not enough seeds for the evaluation of certain traits, seed oil content can still be evaluated at a spaced plant density, and then only those genotypes with a satisfactory level of oil content be advanced for evaluation of other traits.

In contrast to seed oil content, protein content was more sensitive to the variation in plant density. Data in 1993 indicated that relative values of protein contents of the 21 genotypes were significantly different at different plant densities indicated by the low value of Spearman correlation coefficient (0.33) (Table 5-2). This poor correlation might be due to the presence of genotype x plant density interaction on protein content. It might be also related to variable environmental conditions in two blocks since two plant densities were grown in two separate blocks. The 1994 experiment, using a split plot in randomized complete blocks with four replications, should be more accurate than the experiment in 1993 for measurement of the protein content. Indeed, although the ranks of protein contents of nine DH lines and cv. Alto in 1994 were highly correlated (Table 5-3), there were significant differences in seed protein content between the two plant densities indicated by a highly significant mean square value (10.96, $p < 0.01$) (Table 5-1). It was also clearly shown in Fig. 5-1 and Fig. 5-2 that most lines/cultivar had lower protein content at the spaced plant density than at the normal density. This was different from the result by Morrison et al. (1990), where no significant difference in protein content was found between two row spacings. However, studies by Shrief et al. (1990) also showed that a reduced plant density resulted in the decrease in seed protein content, when four *B. napus* cultivars were grown at two row spacings and three plant densities. Thus, although the protein content at different plant densities was well correlated, the absolute values at a high plant density was still higher than at a low plant density. Together, the information from the present study suggests that it may be possible to evaluate protein contents of different genotypes at either high or low plant density, but plant density should be as uniform as possible, and the field plots of genotypes under evaluation should be as close as possible.

5.3.2 Effects of row spacing and plant densities on the interrelationships of pod length, the number of pods per plant, seed set per pod, 1000 seed weight, seed yield, seed oil and protein content

The effect of row spacing and plant density on the interrelationships of the traits investigated also varied with the traits under study. Correlation analysis of the data in 1993 showed positive associations between pod length and SSP, and 1000 seed weight (Table 5-4), regardless of plant density. Similar results were obtained from experiments of DH lines of M7 family in 1993 (Chapter 4), where longer pods were always positively correlated with larger seeds and more seeds per pod. Thus, row spacing and plant density had no significant effect on the relationship between pod length and SSP, or seed weight.

The relationship between pod length and the number of pods per plant differed with plant densities. No relationship between these two traits was observed at the normal plant density (Table 5-4). This result was in agreement with the observation using DH lines of M7 family (Chapter 4) in 1993 also at the

normal plant density. At the spaced plant density, however, pod length was negatively associated with the number of pods per plant. With DH lines of M7 family in 1994 at the normal plant density (Chapter 4), a negative correlation between pod length and the number of pods per plant was also observed. Chay and Thurling (1989) also reported a similar negative correlation between these two traits at normal plant density, but not at reduced plant density. Taking account of all these studies, it appeared that there was a compensating relationship between pod length and the number of pods per plant. The magnitude of this relationship was reduced at the normal plant density but increased at the spaced plant density. It was likely that at the normal plant density, the potential for plants to produce a heavy pod canopy and develop long pods was not fully expressed because of the limited resources available, hiding the compensating relationship. When more nutrients were available at the spaced plant density, this compensating relationship was more clearly expressed than at the normal plant density. Probably, plants primarily distribute more resources to the initiation of pods than to the growth of pods at spaced plant density, which was indicated by a much greater increase in the absolute value of the number of pods per plant than that of pod length (Fig. 5-1 and Fig. 5-2). Conceivably, within the framework of growth period and the available nutritional supplies there would still be a limit in overall resources. The resource divergence to pod initiation would reduce the resource supplies to the growth of pods, resulting in the number of pods per plant being increased at the expense of increase in pod length.

Similarly, the number of pods per plant and SSP had a negative correlation at the spaced plant density but they had no relationship at the normal plant density (Table 5-4). Again, there might be a compensating relationship between these two traits, which was hidden at the normal plant density.

Apart from these relationships described, no significant relationship was observed among seed yield and its related traits.

Consistent positive association between pod length and SSP, and seed weight at both spaced and normal plant densities suggested that long pods could be used as a reference for high SSP and large seeds in rapeseed breeding. It is known that seed yield is directly or indirectly related to the number of pods per plant, SSP and pod length. The compensating relationship between the number of pods per plant and pod length, and between the number of pods per plant and SSP indicates that there should be an optimum combination among these traits to achieve the highest seed yield. Since plant density influences the magnitude of this compensating relationship, an adequate plant density would be required to obtain this optimum combination. As discussed earlier, there were increases in the absolute values of the number of pods per plant, pod length and SSP at the reduced plant density. It appears that a slightly reduced plant density would be beneficial for producing high seed yield by significantly increasing the number of pods per plant, although an increase in the number of pods per plant would reduce the plants' potential to produce longer pods and more seeds per pod. However, an excessively high pod canopy, which could result in a reduction of SSP, and consequently final seed yield, should be avoided.

Seed yield was positively correlated with seed oil content regardless of plant density in 1993 (Table 5-4). A positive correlation ($r = 0.55$) between these two traits was also observed with 25 DH lines of M7 family in 1993 at the normal plant density (Chapter 4). However, no significant relationship between these two traits was found at any plant density in 1994 (data not shown), which might be due to the limited genotypes (10) investigated, consequently limited variation in both traits, since these 10 genotypes were chosen for their good yield and seed oil potential in 1993. Nevertheless, the data from the present study indicate it should be possible to improve seed yield and seed oil content simultaneously since they have either a positive correlation or no relationship. As a matter of fact, a recent report (Daun et al. 1995) on the best Canadian rapeseed/canola cultivars grown from 1956 to 1994 demonstrated that both seed oil and seed yield have been increased, and there is a positive correlation ($r = 0.69$) between these two traits.

Correlation analysis did not show any significant relationship between seed protein content and seed yield in 1993 (Table 5-4) and 1994 (data not shown) at any plant densities, indicating that plant density had no apparent effect on the relationship of these two traits.

It was notable that seed protein content was positively correlated with seed oil content at the normal plant density but not at the spaced plant density in 1993 (Table 5-4). The correlation analysis for the data in 1994, however, did not show any relationship between these two traits at any plant densities (data not shown), despite the significant differences in each trait. In all situations, no negative association between seed protein and oil content was found. This is contrary to previous studies in which seed oil and protein were negatively correlated (Grami et al. 1977, Daun et al. 1995). The reason for this difference is unclear.

The information from the present study suggests that it might be possible to improve the seed yield, seed oil and protein content simultaneously.

The present study showed that row spacing and plant density influenced the expression of traits and in some cases their interrelationships to varying degrees, depending on the specific traits. When the traits under study were individually considered, reduced plant density resulted in a dramatic increase in the number of pods per plant and slight increases in pod length and SSP, but a slight decrease in seed weight. While seed oil content was insensitive to variation in plant density, there was a significant decrease in seed protein content at reduced plant density. Plant density also had significant influence on seed yield, with reduced plant density being favorable for a high seed yield. Thus in rapeseed breeding programs, evaluation of seed oil content does not require a uniform plant density, because at normal and spaced plant densities not only seed oil contents would be highly correlated, but they would not be significantly different as well. A uniform plant density is essential for an accurate estimation of protein content, since protein content can be dramatically different at different plant densities. A uniform plant density would also be preferable for evaluation of pod length, 1000 seed weight and SSP. Although plant density would influence the absolute values of pod length, 1000 seed weight and SSP, the differences would not be very large, and the relative values of these traits would be highly correlated at varying plant densities. In contrast, a uniform plant density is essential for an accurate estimation of the number of pods per plant and seed yield, because at varying plant densities these two traits would be markedly different, and their relative values would be poorly correlated.

While row spacing and plant density had no apparent effect on the relationship between pod length and SSP and seed weight, increased row spacing and reduced plant density within rows increased the magnitude of the compensating relationship between the number of pods per plant and SSP, and also between the number of pods per plant and pod length. Regardless of row spacing and plant density, no negative association was observed between seed oil and protein content. In addition, seed yield was positively correlated with seed oil content in some cases. Thus, it might be possible to improve seed yield, seed oil and protein contents simultaneously without significantly reducing one or the other.

It should be noted that the information from this experiment comes from DH lines, except Alto which is a highly inbred cultivar. It is unclear whether the interrelationships of these traits, or responses of these traits to variation in plant density, are similar to those from genotypes in the early generations of pedigree progenies. Therefore, additional studies are required to address this issue.

The information presented in this and the previous chapters is obtained from studies conducted at one location. It is known from the previous discussion that genotype (G) x environment (E) interaction is common for most of the traits investigated. It is of interest to understand the degree of environmental effects and/or G x E interaction on seed yield and seed yield related traits, seed oil and protein content. Studies on these issues with tests in multiple locations and years will be reported in the following chapter.

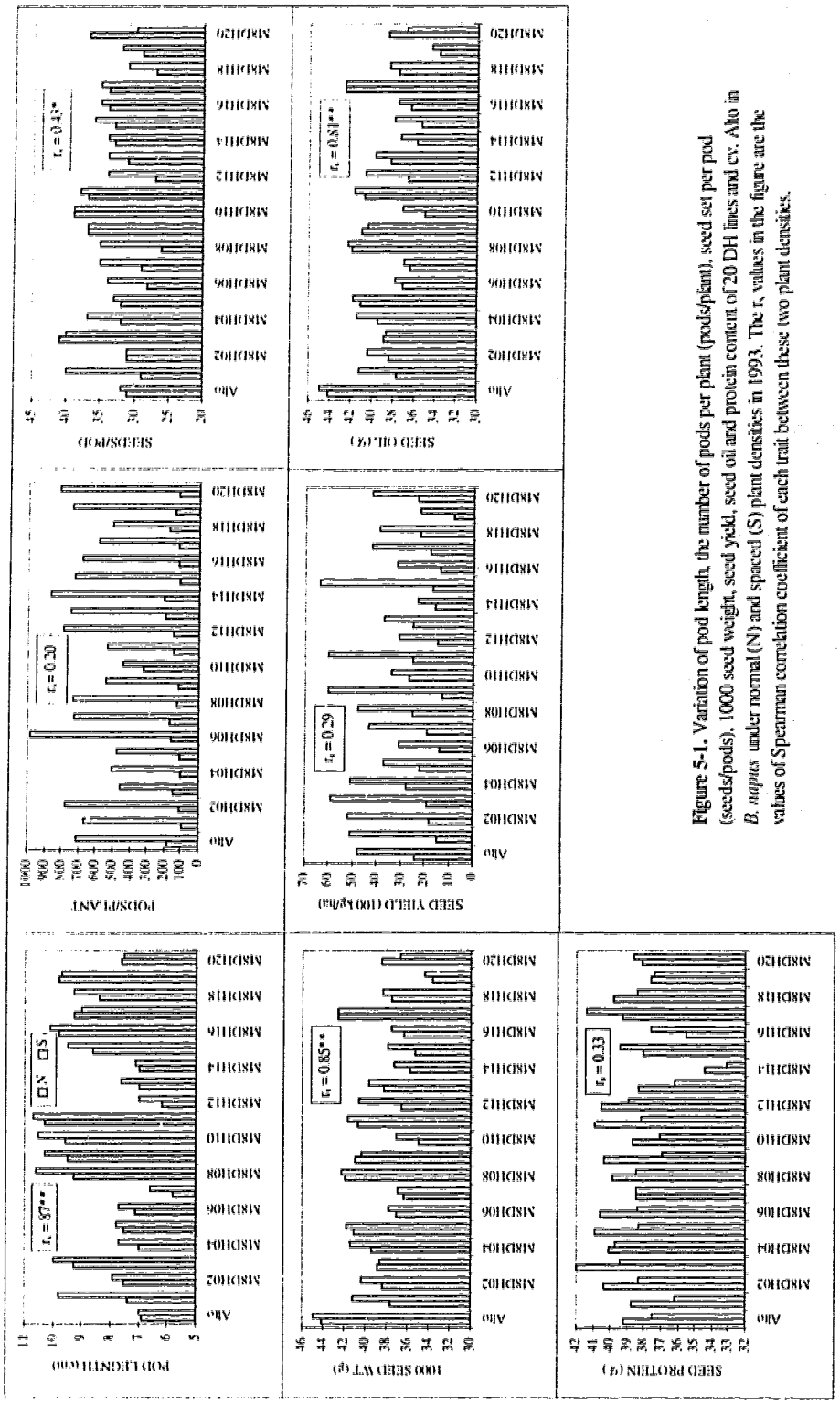

Figure 5-1. Variation of pod length, the number of pods per plant (pods/plant), seed set per pod (seeds/pods), 1000 seed weight, seed yield, seed oil and protein content of 20 DH lines and cv. Alio in *B. napus* under normal (N) and spaced (S) plant densities in 1993. The r_s values in the figure are the values of Spearman correlation coefficient of each trait between these two plant densities.

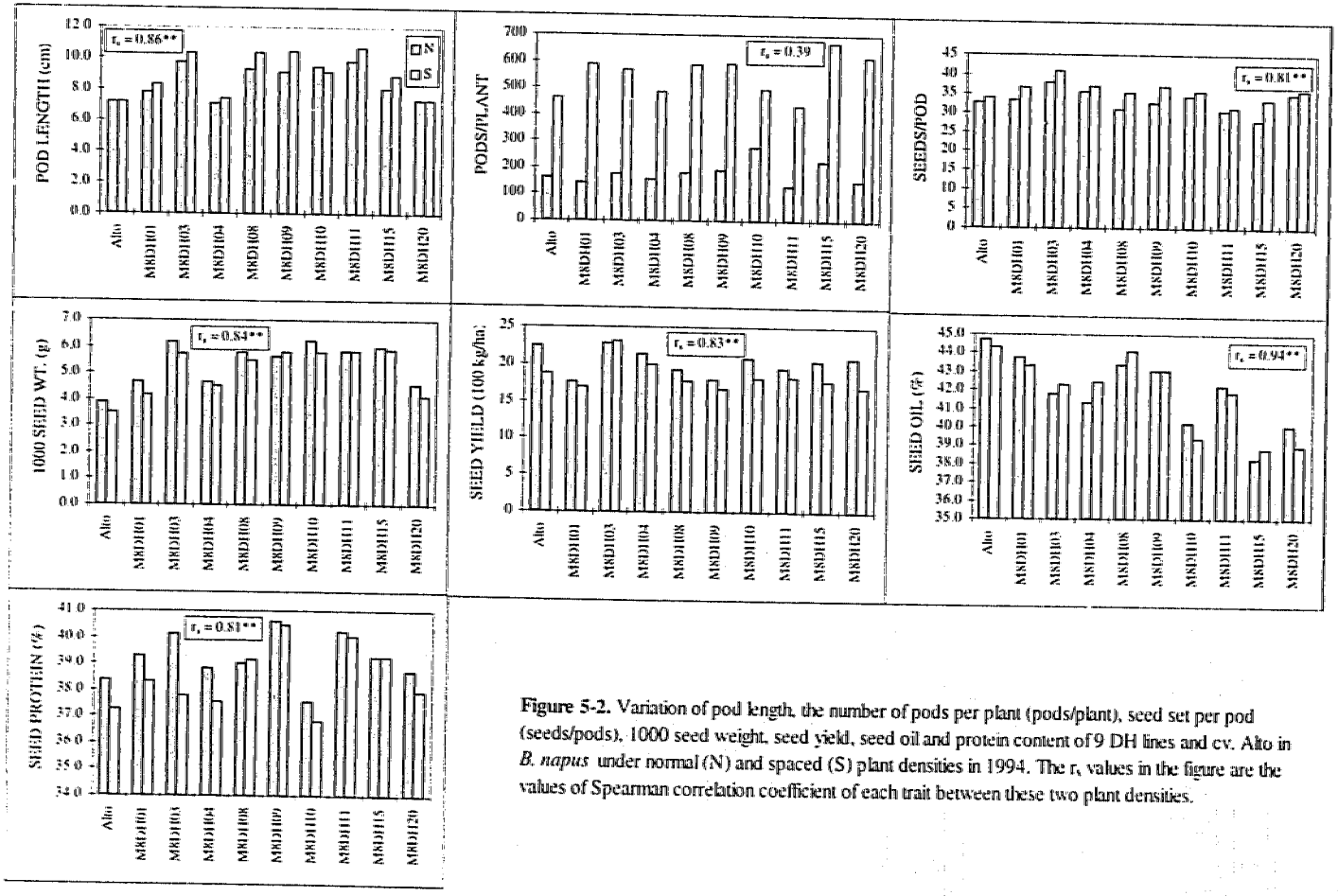

Figure 5-2. Variation of pod length, the number of pods per plant (pods/plant), seed set per pod (seeds/pods), 1000 seed weight, seed yield, seed oil and protein content of 9 DH lines and cv. Alto in *B. napus* under normal (N) and spaced (S) plant densities in 1994. The r_s values in the figure are the values of Spearman correlation coefficient of each trait between these two plant densities.

Table 5-1. Analysis of variance for pod length (PL), seed set per pod (SSP), pod number per plant (PNP), seed yield (yield), 1000 seed weight (Seed wt), seed oil (oil) and protein content of 9 DH lines and cv. Alto in *B. napus* sown in split plots in 1994

Source of variation	DF	Mean square [a]						
		PL	SSP	PNP	Yield	Seed wt	Oil	Protein
Block (B)	3	0.16	1.85	60006.68	25.86	0.16	0.19	43.10
Line/cultivar (L)	9	12.36**	54.48**	1877.11**	23.27	5.77**	31.88**	8.37**
B X L	27	0.22	4.11*	5227.03	20.68	0.62	0.58	2.19*
Plant density (D)	1	4.80**	143.11**	2767680.00**	71.82	1.24**	0.02	10.96**
L X D	9	0.48**	5.42*	12957.36*	3.80	0.09	1.01*	1.09
Error	30	0.14	1.92	5123.09	22.42	0.08	0.33	0.91

[a] The values followed by * denotes $p < 0.05$ and those followed by ** denotes $p < 0.01$.

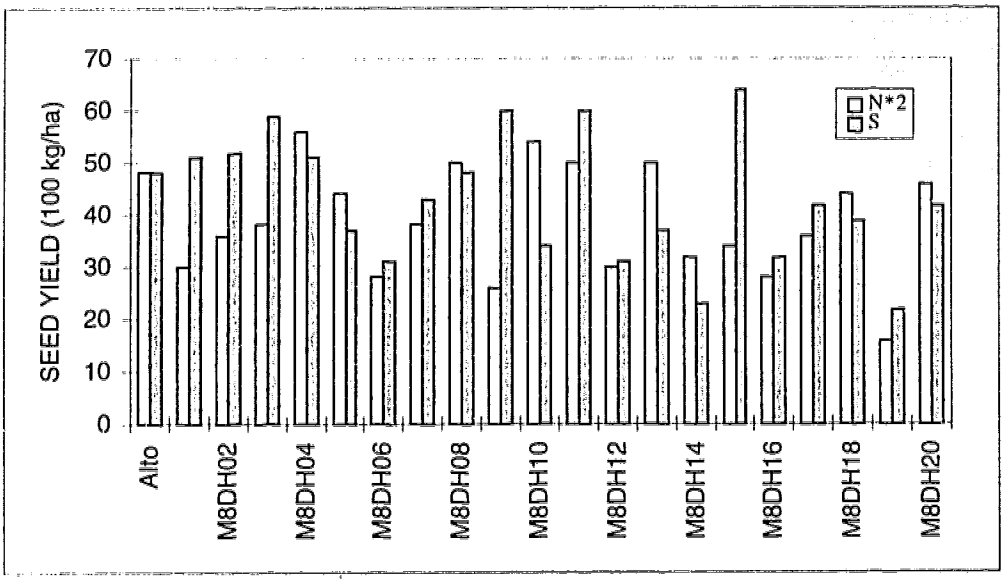

Figure 5-3. Seed yield at spaced (S) and normal (N*2) plant densities of cv. Alto and 20 DH lines of *B. napus* in an experiment with completely randomized block design in 1993.

Table 5-2. The ranks and Spearman correlation coefficients (r_s) between normal (N) and spaced (S) plant densities of pod length, seed set per pod (SSP), pod number per plant (PNP), seed yield, 1000 seed weight, seed oil and protein contents of 20 DH lines in M8 family and cv. Alto in *B. napus* sown in 1993, where the rank No. 1 has the highest value and No. 21 has the lowest.

DH lines /	Pod length (cm)		SSP		PNP		Yield (kg/ha)		1000 Seed wt (g)		Oil (%)		Protein (%)	
cultivar	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Alto	19	20	14	18	4	10	6	8	18	17	1	1	12	15
M8DH01	14	7	15	2	21	13	16	6	11	9	12	7	13	19
M8DH02	13	12	12	20	17	4	13	4	17	14	10	9	6	11
M8DH03	8	6	1	1	9	20	10	14	5	5	8	12	1	4
M8DH04	18	15	11	6	20	18	1	5	9	11	7	6	8	2
M8DH05	12	13	10	16	18	19	9	15	15	18	4	4	2	12
M8DH06	15	14	18	14	7	1	18	18	21	10	14	15	4	9
M8DH07	21	21	17	11	6	9	11	9	16	20	16	19	15	8
M8DH08	7	2	21	9	12	7	4	7	1	3	3	3	9	7
M8DH09	5	4	4	5	15	15	20	3	7	6	5	10	7	18
M8DH10	4	3	2	3	1	21	2	16	3	4	20	18	14	17
M8DH11	1	1	3	4	10	16	5	2	6	8	6	5	3	13
M8DH12	20	19	20	12	8	3	17	19	12	12	15	8	5	5
M8DH13	17	16	13	13	3	5	3	13	19	19	11	11	16	20
M8DH14	16	18	9	15	2	2	15	20	20	21	18	17	21	21
M8DH15	9	9	8	7	19	8	14	1	4	1	19	14	18	3
M8DH16	3	5	7	10	13	11	19	17	8	13	17	16	20	14
M8DH17	6	11	6	8	16	14	12	10	13	15	2	2	11	1
M8DH18	10	10	19	19	5	17	8	12	10	7	13	13	10	10
M8DH19	2	8	16	17	11	6	21	21	2	2	21	21	19	16
M8DH20	11	17	5	21	14	12	7	11	14	16	9	20	17	6
r_s	0.8701		0.4286		0.2000		0.2870		0.8533		0.8182		0.3312	
Pr > |r|	0.0001		0.0526		0.3847		0.2071		0.0001		0.0001		0.1425	

Table 5-3. The ranks and Spearman correlation coefficients (r_s) between normal (N) and spaced (S) plant densities of pod length, seed set per pod (SSP), pod number per plant (PNP), seed yield, 1000 seed weight, seed oil and protein contents of 9 DH lines in M8 family and cv. Alto in *B. napus* sown in split plots in 1994, where the rank No. 1 has the highest value and No. 10 has the lowest value.

DH lines /	Pod length (cm)		SSP		PNP		Yield (kg/ha)		1000 Seed wt (g)		Oil (%)		Protein (%)	
cultivar	N	S	N	S	N	S	N	S	N	S	N	S	N	S
Alto	9	10	6.5	8	6	9	2	3	10	10	1	1	9	9
M8DH01	7	7	5	4	9	5	10	9	8	8	2	3	4	5
M8DH03	2	4	1	1	5	6	1	1	2	5	6	6	3	7
M8DH04	10	8	2	2.5	7	8	3	2	7	7	7	5	7	8
M8DH08	4	3	8	7	4	4	8	6	5	6	3	2	6	4
M8DH09	5	2	6.5	2.5	3	3	9	10	6	4	4	4	1	1
M8DH10	3	5	4	6	1	7	4	5	1	3	8	8	10	10
M8DH11	1	1	9	10	10	10	7	4	4	2	5	7	2	2
M8DH15	6	6	10	9	2	1	6	7	3	1	10	10	5	3
M8DH20	8	9	3	5	8	2	5	8	9	9	9	9	8	6
r_s	0.855		0.814		0.394		0.830		0.842		0.939		0.818	
Pr > | r |	0.002		0.004		0.260		0.003		0.002		0.000		0.004	

Table 5-4. Pearson correlation coefficients (r) of pod length, seed set per pod (SSP), pod number per plant (PNP), 1000 seed weight (seed wt), seed yield, seed oil and protein contents calculated from the data of the normal (N) and spaced (S) plant densities of 20 DH lines and cv. Alto in *B. napus* sown in 1993 [a]

		SSP	PNP	Seed yield	Seed wt	Oil	Protein
Pod length	N	0.48*	-0.01	-0.10	0.75**	0.04	0.03
	S	0.56**	-0.50*	0.33	0.79**	-0.05	0.08
SSP	N		0.16	0.15	0.34	0.06	0.02
	S		-0.48*	0.40[b]	0.41[b]	0.11	0.03
PNP	N			0.36	-0.01	-0.29	-0.20
	S			-0.38[c]	-0.33	-0.24	-0.27
Seed yield	N				0.06	0.41*	0.25
	S				0.40[b]	0.45*	0.38[c]
Seed wt	N					-0.10	0.08
	S					-0.14	0.31
Oil	N						0.47*
	S						0.24

[a] The values followed by * or ** denote that $p < 0.05$ or $p < 0.01$, respectively. [b] $p = 0.07$. [c] $p = 0.09$.

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CHAPTER 6

GENOTYPE-ENVIRONMENT INTERACTIONS OF AGRONOMIC AND QUALITY TRAITS IN *BRASSICA NAPUS* L.

6.1 INTRODUCTION

Brassica napus L. is one of the major rapeseed species in Canada and many other areas of the world. High seed yield, and high seed oil and protein contents are major breeding objectives for this crop. Indirect selection for high seed yield through pod number, seed weight, and seed number per pod has been suggested in previous studies (Andersson and Olsson 1961, Thurling 1974 1991, Seiffert and Boeleke 1977, Shabana et al. 1990, Grosse et al. 1992) since seed yield was reported to be positively correlated with these traits. In addition, previous studies in *B. napus* have shown that seed set per pod (SSP) and seed weight are often positively correlated with pod length (Chay and Thurling 1989a,b, Thurling 1991, Chapter 4 and 5). Unfortunately, the presence of genotype x environment interactions often causes changes in the interrelationships of these traits. Consequently, such relationships have not been clearly defined and successfully applied to breeding programs. The objectives of the present study were to investigate the effects of general environmental conditions on pod length, SSP and seed weight, seed yield, seed oil and protein contents, and to study the interrelationships of these traits under various environmental conditions in the canola growing region of central Alberta, Canada.

6.2 MATERIALS AND METHODS

Seven spring type *B. napus* genotypes, cv. Alto, 88-1426K (G3), 92-20501 (G4), 91-54165NK (LPHIPR), 91-22521 (GD), 92-21213 (HIOIL), 92-20504 (HIYLD), were grown at three locations, Edmonton Research Station of the University of Alberta (Michener), Ellerslie and Kelsey, Alberta, in 1993 and 1994. Of these genotypes, Alto was an established cultivar in these areas. G4, GD, LPHIPR and HIYLD were doubled haploid lines derived from microspore culture. HIYLD was registered as cv. Quantum in 1995 (Stringam et al. 1995). G3 was an advanced inbred line developed from pedigree selection. All genotypes were developed from the breeding program at the University of Alberta. The field plot layout was a randomized complete block design with three replications at each location each year. Each plot consisted of four 6-meter rows with 23 cm spacing between rows. The plots were seeded with a Fabro drill seeder (Fabro Ltd. Swift Current, Sask., Canada) at a seeding rate of 1.25 g of seeds per row. To determine pod length and SSP, five plants were randomly sampled from the two central rows of each plot. Pod length and SSP were determined from the first five pods formed on the main raceme of each plant when seeds contained therein began to turn brown in color. At maturity, all four rows of each plot were harvested in bulk. Seed yield, 1000 seed weight, seed oil and protein contents of each line/cultivar were determined from the seed bulk, using the methods described in section 3.2.2, with the exception that 26.2 g of seeds per sample were used in the determination of seed oil content.

The data were analyzed by analysis of variance. Variance and covariance components, and heritability of each trait were estimated using the principles of Comstock and Moll (1963) (see Appendix D for details). Phenotypic correlation of the traits investigated were calculated using the same formulae presented in section 5.2.2. Genotypic correlations of the traits investigated were calculated from the variance and covariance components, also using the same principles for estimation of heritability (see Appendix D for details). Most of the computations were done with SAS/STAT 6.0 (SAS Institute Inc. 1989).

6.3 RESULTS AND DISCUSSION

6.3.1 Genotype x environment interactions and estimation of heritabilities

Significant genotype x year interactions were observed in all traits (Table 6-1). This was similar to the finding in Chapter 4, where significant genotype x year interactions were observed for all traits except the number of pods per plant, when 25 DH lines and cv. Alto were tested over two years at one location. The presence of significant genotype x year interaction suggested that it would be difficult to accurately

evaluate the performance of these traits with limited years. This difficulty arises from the lack of understanding of the real nature of genotype x year interaction and the lack of control in factors associated with this interaction. Nevertheless, the outstanding genotypes with a specific trait could still be identified. For instance, GD had the highest values in pod length, 1000 seed weight and protein contents over years (Fig. 6-1). Similarly, HIYLD had the highest values in SSP and seed yield, and HIOIL consistently had the highest oil content over years. These observations suggest that genotypes with outstanding performance of a specific trait, including multigenic traits such as seed yield or seed oil content, in one year would still likely be superior to other genotypes if tested for additional years. In a breeding program, usually large numbers of genotypes have to be evaluated. Unfortunately, the presence of genotype x environment interactions makes it very difficult to differentiate one genotype from others without extensive testing. Constraints in resources frequently limit the scale of testing, and consequently, the discovery of superior genotypes. To resolve this dilemma increasing the genetic variability of traits of interest appears to be the most effective approach because outstanding genotypes would appear only in the presence of a large genetic variation for the trait of interest.

In contrast to the complicated relationship between genotype and year, no significant interaction of genotype x location was observed on any trait except protein content (Table 6-1). This was also clearly shown in Fig. 6-2. When one genotype had an increased or a decreased value at a specific location, the values of this trait for other genotypes were also increased or decreased. However, the mean values of each of the six traits were different at the three locations, clearly indicating that the performance of all traits were dependent on environmental conditions at each location. This observation implies that performances of genotypes for a specific trait should be compared within locations. If comparisons are made across locations, the genotypes should be tested at the same locations in the same years.

There were clear differences in response to the variation in years and locations among the six traits. Pod length was the least variable trait over years and across locations. The analysis of variance components and the estimation of heritabilities (Table 6-2) revealed that most of the variation in pod length came from genotypes with small variation from genotype x year, whereas genotype x location and genotype x year x location interactions were minimum. Thus pod length is a highly heritable trait, having the highest heritability (0.92) among the six traits.

Seed weight was also a very stable trait as indicated by its very small values of variance components in genotype x year (0.16), genotype x location (0.01), and genotype x year x location (0.05). Since there was only very limited genetic variation (0.18) in the population, seed weight had only a heritability of 0.65. Since seed weight is stable over years and locations, extensive evaluation is not required for this trait. Thus it appeared that increasing genetic variation in a population is the most important element to improve seed weight.

SSP was very similar to pod length in its responses to variation in location and year x location interaction, but SSP was obviously more sensitive to year variation than pod length as indicated by the high proportion of genotype x year component (1.63).

Seed oil content varied very little over locations but there was a small genotype x year x location interaction in addition to the variation from genotype x year interaction. Because of the large genetic variation indicated by the high value of the genotype component (3.21), seed oil had the second highest heritability (0.81) among the traits investigated. Of the six traits investigated, seed yield and protein contents were most sensitive to environmental variations indicated by the presence of variance components in genotype x year, genotype x location, and genotype x year x location.

The generally higher sensitivity of these traits to variation in year than to variation in location indicated that increasing the number of years was more important than increasing the number of locations for accurate evaluation of these traits. The differences in heritability among these traits suggested that, when there was a large genetic variation available, quick improvement could be made in breeding for pod length, SSP, seed weight, and seed oil content with testing of few years and locations, whereas more years and locations would be needed for evaluation of seed yield and protein content. Consequently, improvement for these two traits would be more difficult and progress would be much slower.

6.3.2 Interrelationships of pod length, seed set per pod, 1000 seed weight, seed yield, seed oil and protein content

Data analysis across three locations and two years revealed high positive associations of pod length with SSP, seed weight and seed protein content (Table 6-3). No relationship was observed between pod

length and seed yield, nor between pod length and oil content. These results suggested that it should be possible to select for high SSP, large seeds and high seed protein content through selection for long pods. On the other hand, long pods appeared to be an undesirable trait for seed yield and seed oil content (also see following discussion) within the genotypes investigated, which was demonstrated by the genotype GD. This genotype had the longest pod length and the highest value in seed weight among the seven genotypes across three locations over two years (Fig. 6-1 and Fig. 6-2.), but produced the lowest seed yield, the second lowest seed oil content following G4 at each location (Fig. 6-2).

No significant relationship between SSP and seed yield was observed in the present study either from the combined data from three locations over two years (Table 6-3), or at individual locations (Table 6-4). This might be due to the limited genotypes investigated, where both traits showed no statistically significant difference at the 5% level. Nevertheless, there was an indication that high SSP may be a desirable character of a high seed-yielding genotype. This was clearly demonstrated in Fig. 6-1 and Fig. 6-2, where HIYLD, which consistently had the highest value of SSP, produced the highest seed yield across three locations and over two years. Indeed, HIYLD had an outstanding yield performance even in the Western Canadian Canola/Rapeseed Recommending Committee performance trials, and was registered as cv. Quantum in 1995 (Stringam et al. 1995). Thus, increasing SSP should be an effective approach for seed yield improvement. A similar conclusion was reached in Chapter 4, where SSP was shown to be the only trait with a consistent positive contribution to seed yield.

A negative correlation between seed yield and seed weight (Table 6-3) was unexpected, considering that seed weight was one of the seed yield components. Data analysis at individual locations revealed a pronounced negative correlation between seed yield and seed weight at Kelsey ($r = -0.80$) (Table 6-4). For most genotypes, all traits except seed oil content had the poorest performance at this location (Fig. 6-2). Obviously, the general environmental conditions at Kelsey in those years were unfavorable for the production of these genotypes. Under those conditions, plants would tend to produce smaller but more seeds per pod to produce a maximum seed yield. Consequently, the genotypes with smaller but more seeds per pod would have higher seed yield than those with larger seeds, resulting in a negative correlation between seed yield and seed weight. Furthermore, only seven genotypes were investigated, and some of the genotypes had extreme values in one trait or/and the other, such as pod length of GD, and SSP and seed yield of HIYLD. The results obtained from these extreme genotypes might be different from what would be obtained from the vast majority of genotypes which a breeder usually deals with in a breeding program. In fact, no relationship between seed yield and seed weight was obtained in the studies reported in Chapter 4. Moreover, previous studies have shown that seed yield can be improved by selection for high seed weight (Morice 1960, Musnicki 1975). Thus, this highly significant negative correlation should be viewed as a special case, rather than the typical relationship of these two traits that one would expect in a breeding program.

Seed yield was also positively correlated with seed oil content (Table 6-3). The same relationship of these two traits was found in previous studies from 20 DH lines tested at two row spacings and plant densities (Chapter 5). It is known that high seed yield and high seed oil content are two essential characteristics for a rapeseed cultivar. Seed oil content also has a higher heritability, and is easier to measure accurately than seed yield. A positive correlation between these two traits can be used to advantage in rapeseed breeding. Genotypes should be evaluated for seed oil content first, and only genotypes which have a satisfactory oil content, and would also likely have high seed yield, should be advanced for additional evaluation on seed yield. This approach would be more efficient and economical than selection for both traits simultaneously, or selection for seed yield first.

In addition to SSP and seed weight, the number of pods per plant should also be considered in discussion on seed yield in rapeseed breeding, since the number of pods per plant is also an important factor determining the seed yield of a rapeseed cultivar. Although the number of pods per plant was not investigated in the studies in this chapter, a positive correlation between seed yield and the number of pods per plant has been reported in previous studies (Andersson and Olsson 1961, Seiffert and Boelcke 1977). Shabana et al. (1990) found through path coefficient analysis that pod number per plant had the highest direct effect on seed yield per plant. However, earlier studies (Chapter 4 and 5) found that the number of pods per plant was mainly dependent on the nutritional resources available, and this trait is very difficult to be accurately evaluated. Thus, in terms of selection criteria, it seems that the number of pods per plant should not become a major concern.

Similar to previous reports (Grami and Stefansson 1977a b, Grami et al. 1977), a significant negative correlation was found between seed oil and seed protein contents in the present study, when the data were analyzed from the averages across three locations over two years (Table 6-3). At individual locations, these two traits also appeared to be negatively correlated, although the negative association was more pronounced at Ellerslie than at the other two locations (Table 6-4). It appeared that small seeds were favorable to high seed oil content but unfavorable to high protein content, whereas large seeds had just the opposite effect. For example, genotype HIOIL, which had the lowest value in 1000 seed weight over years and across locations among the seven genotypes (except G3 at Michener), produced the highest seed oil content but the lowest protein content (Fig. 6-1 and Fig. 6-2). In contrast, genotype GD, which had the highest value in seed weight, produced the highest seed protein content but the second lowest seed oil content at each individual location. Thus, simultaneously improving seed oil and protein content under environmental stressful conditions seems a dilemma and remains a challenge to rapeseed breeders.

Table 6-1. Analysis of variance for pod length (PL), seed set per pod (SSP), seed yield (yield), 1000 seed weight (Seed wt), seed oil and protein contents in *B. napus*.

Source of variation	DF	Mean square [a]					
		PL	SSP	Yield	Seed wt	Oil	Protein
Year (Y)	1	0.12	1.25	149.85	10.30 *	57.67	4.42
Location (L)	2	3.82	162.06	2115.72 *	1.63	500.01*	918.73
Y X L	2	0.85*	27.88	106.96	0.33	19.10	118.67 **
Replication/(Y X L)	12	0.20	8.67	37.50	0.07	7.05 **	11.98 **
Genotype (G)	6	16.78**	89.16 [b]	291.38 [c]	5.08 [d]	72.05 *	27.05 [e]
Y X G	6	1.35 **	21.50 *	82.73 *	1.67 **	13.76 **	7.59 **
L X G	12	0.14	4.61	34.79	0.28	2.89	3.90 *
Y X L X G	12	0.13	6.80	22.08 **	0.19 **	2.33 **	1.33
Residual	72	0.15	6.91	21.33	0.04	0.76	1.02

[a] The values followed by * denotes $p < 0.05$ and those followed by ** denotes $p < 0.01$.

[b] $p = 0.10$. [c] $p = 0.08$. [d] $p = 0.08$ [e] $p = 0.09$.

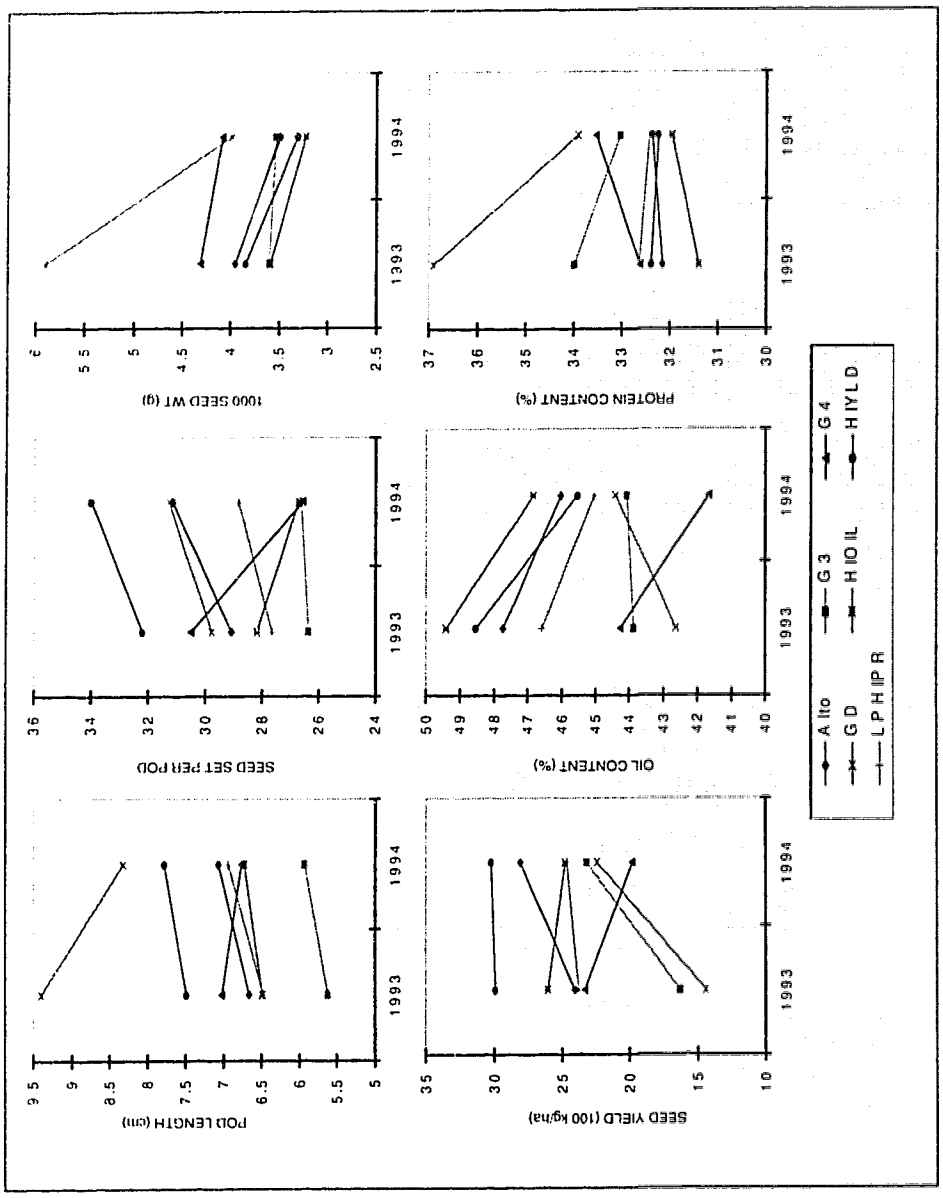

Figure 6-1. Genotype x year interaction for pod length, seed set per pod, 1000 seed weight, seed yield, seed oil and protein content of seven genotypes in *B. napus* tested at three locations over two years, 1993-94.

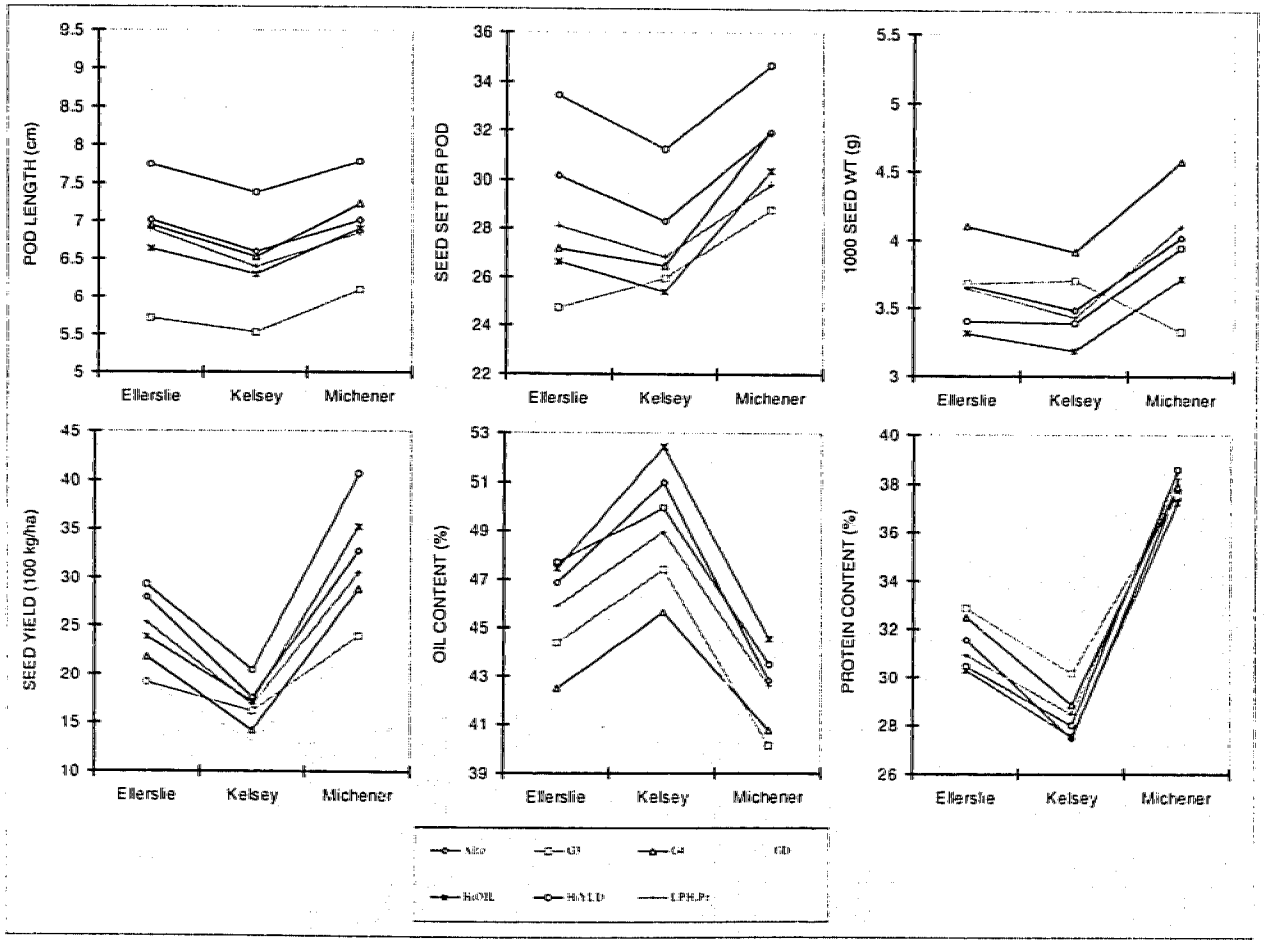

Figure 6-2. Genotype x location interaction for pod length, seed set per pod, 1000 seed weight, seed yield, seed oil and protein content of seven genotypes in *B. napus* tested at three locations over two years, 1993-94.

Table 6-2. Variance components and the heritabilities of pod length, seed set per pod (SSP), seed yield (yield), 1000 seed weight (Seed wt), seed oil and protein content estimated from field trials with seven genotypes (g) in *B. napus* tested at three locations (l) over two years (y) with three replications (r) at each location.[a]

		Estimated variance components (VC) and their standard deviations (SVMS)											
	DF	Pod length		SSP		Yield		Seed wt		Oil		Protein	
		VC	SVMS	VC	SVMS	VC	SVMS	VC	SVMS	VC	SVMS	VC	SVMS
S_g^2	6	0.86	0.47	3.88	2.55	10.89	8.46	0.18	0.15	3.21	2.04	0.94	0.79
S_{yg}^2	6	0.14	0.08	1.63	1.23	6.74	4.69	0.16	0.09	1.27	0.77	0.70	0.43
S_{lg}^2	12	0	0	0	0	2.12	2.60	0.01	0.02	0.09	0.23	0.43	0.26
S_{ylg}^2	12	0	0	0	0	0.25	3.02	0.05	0.02	0.52	0.30	0.11	0.18
S_e^2	72	0.15	0.06	6.90	0.43	21.33	0.76	0.04	0.03	0.76	0.14	1.02	0.17
S_p^2		0.93		5.08		16.19		0.28		4.00		1.50	
h^2		0.92		0.76		0.67		0.65		0.81		0.62	

[a] S_g^2 = genotype, S_{yg}^2 = year x genotype. S_{lg}^2 = location x genotype, S_{ylg}^2 = year x location x genotype, S_e^2 = residual, S_p^2 = phenotypic variance, and h^2 = heritability.

Table 6-3. Phenotypic [$r(x,y)_p$] and genotypic [$r(x,y)_g$] correlations of pod length, seed set per pod (SSP), 1000 seed weight (seed wt), seed oil and protein content in *B. napus* calculated from the data of seven genotypes tested at three locations over two years.[1]

	SSP		Yield		Seed wt		Oil		Protein	
	$r(x,y)_p$	$r(x,y)_g$	$r(x,y)_p$	$r(x,y)_g$	$r(x,y)_p$	$r(x,y)_g$	$r(x,y)_p$	$r(x,y)_g$	$r(x,y)_p$	$r(x,y)_g$
Pod length	0.71*	0.71[a]	-0.08	-0.01	0.77*	0.77[a]	-0.16	-0.09	0.58	0.58[a]
SSP			0.53	0.53	0.24	0.24[a]	0.21	0.18	0.07	0.32
Yield					-0.64	-0.64[a]	0.82*	0.82[a]	-0.79*	-0.79[a]
Seed wt							-0.69	-0.66	0.88**	0.88[a]
Oil									-0.78*	-0.78[a]

[1] The values followed by * or ** denote $p < 0.05$ and $p < 0.01$, respectively; The values followed by [a] indicate that estimated $r(x,y)_g$ is greater than the $r(x,y)_p$, and then set to be equal to the $r(x,y)_p$.

Table 6-4. Phenotypic correlation coefficients of pod length, seeds set per pod (SSP), seed yield (yield), 1000 seed weight (Seed wt), seed oil and protein contents in *B. napus* at three individual locations over two years[a]

	Location	SSP	Seed wt	Yield	Oil	Protein
Pod length	Ellerslie	0.80*	0.66	0.10	-0.05	0.38
	Kelsey	0.68	0.65	-0.22	-0.14	0.44
	Michener	0.59	0.83*	-0.15	-0.28	0.89**
SSP	Ellerslie		0.17	0.61	0.38	-0.06
	Kelsey		0.15	0.45	0.06	0.01
	Michener		0.45	0.55	0.20	0.52
Seed wt	Ellerslie			-0.61	-0.71*	0.92**
	Kelsey			-0.80*	-0.69	0.91**
	Michener			-0.29	-0.39	0.75*
Seed yield	Ellerslie				0.79*	-0.77*
	Kelsey				0.67	-0.72*
	Michener				0.89**	-0.23
Oil	Ellerslie					-0.78*
	Kelsey					-0.69
	Michener					-0.42

[a] The values followed by * or ** denote that $p < 0.05$ or $p < 0.01$, respectively.

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CHAPTER 7
GENERAL DISCUSSION AND SUMMARY

7.1 INTRODUCTION

The present studies can be generally classified into two areas: 1) inheritance of seed yield and its related traits, and seed oil and protein contents in spring *B. napus*, with the emphasis on the inheritance of the long-podded trait; 2) interrelationships of these traits. Different results for a specific trait or a relationship were occasionally observed, depending on the specific study. In this chapter results obtained from all studies will be summarized and discussed in relation to the improvement of rapeseed yield, seed oil and protein contents.

7.2 INHERITANCE OF POD LENGTH, SEED YIELD, THE NUMBER OF PODS PER PLANT, SEED SET PER POD, SEED WEIGHT, SEED OIL AND SEED PROTEIN CONTENTS *IN B. NAPUS*

7.2.1 Inheritance of pod length

It has been reported in the previous studies that pod length in spring *B. napus* was determined by two dominant genes acting in a complimentary fashion (Chay and Thurling 1989a,b). The data from both DH line populations and conventional genetic analysis in the present studies, however, have shown that the inheritance of the long-podded trait best fits a model of three independent nuclear genes with additive gene action. This difference may be a result of the different genotypes investigated. Moreover, it has been shown in the present study that pod length is affected by environmental conditions. For example, reduced plant density can result in a slight increase in pod length. However, pod length is a highly heritable trait, having a broad sense heritability of 0.7 on the basis of individual plants and 0.9 on the basis of lines tested with multiple years and locations. The rank correlation in pod length from DH lines between two years, or between two plant densities reached approximately 0.9. Thus, pod length can be selected easily and reliably. Since only three nuclear genes are involved in the long-podded trait, it should not be difficult to incorporate this trait into other genotypes with other desirable traits through conventional breeding approaches.

7.2.2 Inheritance of seed yield and seed yield components

Seed yield is the most important, but also the most elusive trait in rapeseed breeding. It is generally recognized that seed yield has a large genotype x environment component and controlled by multiple genes. The limited information on seed yield in the present study also showed that seed yield can be greatly influenced by environmental factors. For instance, seed yield of DH lines was poorly correlated (r_s = 0.22-0.29) between two years, or between two plant densities, when more than 20 genotypes were evaluated. However, the accuracy of seed yield estimation can be greatly increased with proper experimental designs. The data from a split plot in RCB design, for example, showed that seed yield of different genotypes was highly correlated (r_s = 0.83) between two plant densities, although it appeared that seed yield at the reduced plant density was higher. This suggested that the effect of plant density on seed yield in split plot design was more accurately estimated than that in RCB design. It should be noted, however, that only 10 genotypes were investigated in one year in this experiment. In a breeding program, usually a large number of genotypes have to be evaluated against designated check(s). Using a split plot design to evaluate every genotype against check(s) would be impractical. Thus, effective seed yield evaluation for both accuracy and efficiency remains a challenge to rapeseed breeders. A medium level of heritability (h^2 = 0.67) of seed yield was obtained from tests of seven genotypes over three locations and two years. This value was mainly a result of a relatively large genotypic variance against a small variance in genotype x location and genotype x year x location. Again, the experimental scale was small. In addition, large variation in the estimated variance components were observed, as indicated by a large standard deviation for all components. Thus, one needs to properly interpret a heritability value in order to use it in a practical breeding program. Discussion in this area is beyond the scope of this study.

Although a larger number of studies have been conducted to elucidate the relationship between seed yield and its components, there is little information on the inheritance of seed yield components in the literature. The present study showed that seed weight was the most stable trait among the three seed yield

components, having a broad sense heritability of approximately 0.6 based on either single plants or DH lines. The highly positive rank correlation (approx. 0.85) of seed weight between years and between plant densities also indicated that seed weight had a relatively high heritability. SSP was a typical quantitative character, and was less stable than seed weight, which is indicated by its low rank correlation (approx. 0.44) between years, or between plant densities. Although SSP had a higher heritability (0.76) than seed weight (0.65) when estimated from the tests in multiple locations and years in Chapter 6, this higher value mainly resulted from the higher genetic variance among the genotypes investigated, in comparison to the lower genetic variance of seed weight. The number of pods per plant was also a typical quantitative trait, and it is more responsive to environmental variation than SSP. For instance, reduced plant density greatly increased the number of pods per plant. Thus, selection for seed weight should be easier and more effective than for SSP, whereas selection for the number of pods per plant would be more difficult.

7.2.3 Inheritance of seed oil and protein content

Grami and Stefansson (1977a,b) found that oil, protein and the sum percentage of oil and protein were determined by the maternal genotype in *B. napus*, and that gene action was mainly additive with insignificant dominance and absence of epistasis. The heritability of the sum of oil and protein was higher than that of individual traits. From the studies on the differences in protein, oil and the sum of protein and oil between two *B. napus* cultivars, Midas and Tower, where the latter with an elevated oil and protein content was developed from the former by a joint selection for the oil and protein content (Stefansson and Kondra 1975), Grami et al. (1977) found that at least five to seven genes were involved for protein, one for oil, and two for the sum of protein and oil. Thus, the inheritance of oil content should be simpler than protein content. However, studies have also shown that the correlation between oil content of individual plants and their progenies was poor (Olsson 1960, Thompson 1983). Oil and protein contents were strongly modified by environmental factors (Rakow 1979, Zhao et al. 1993).

The data from the present study showed that seed oil was a highly heritable trait. When estimated from seven genotypes tested at three locations over two years, a heritability of 0.81 was obtained. Highly positive rank correlation (approx. 0.8 to 0.9) of seed oil content of DH lines also supported this conclusion. In comparison, seed protein content was less stable than seed oil content, which was indicated by its low heritability (0.6), and highly variable rank correlation coefficients (0.3-0.8) between years, or between plant densities. Thus, selection for oil content should be more effective than for protein content . .apeseed breeding.

7.3 Interrelationships of pod length, the number of pods per plant, seed set per pod, 1000 seed weight, seed yield, seed oil and protein contents in *B. napus*

Interrelationships of pod length, the number of pods per plant, seed set per pod, 1000 seed weight, seed yield, seed oil and protein content investigated in the present study are summarized in Fig. 7-1. These interrelationships and their implications in rapeseed breeding will be discussed with reference to the previous reports by other researchers.

7.3.1 Relationships between pod length and seed yield/seed yield components

Pod length, seed yield and seed yield components are interrelated traits. Most of these traits can be strongly influenced by environmental conditions as indicated in the present and many previous studies. No relationship between any two traits can be defined without consideration of the others and environmental conditions which, unfortunately, can not be clearly defined in many cases. Therefore, discussion on this subject is very difficult. In the present study, there was a general compensating relationship between pod length and the number of pods per plant, but other factors, such as plant density, influenced the magnitude of this relationship. A reduced plant density increased the magnitude of this relationship. Chay and Thurling (1989b) reported a similar negative correlation between these two traits, but the negative correlation was observed only at normal plant density. Since the number of pods per plant is an important factor influencing seed yield of a cultivar, the question is how much this compensating relationship would affect seed yield. The results of the present study indicated that this compensating relationship should not become a major concern in rapeseed breeding. This conclusion was based on the following facts: (1) At normal plant density, the negative association of these two traits was either not obvious or very weak. (2) At spaced plant

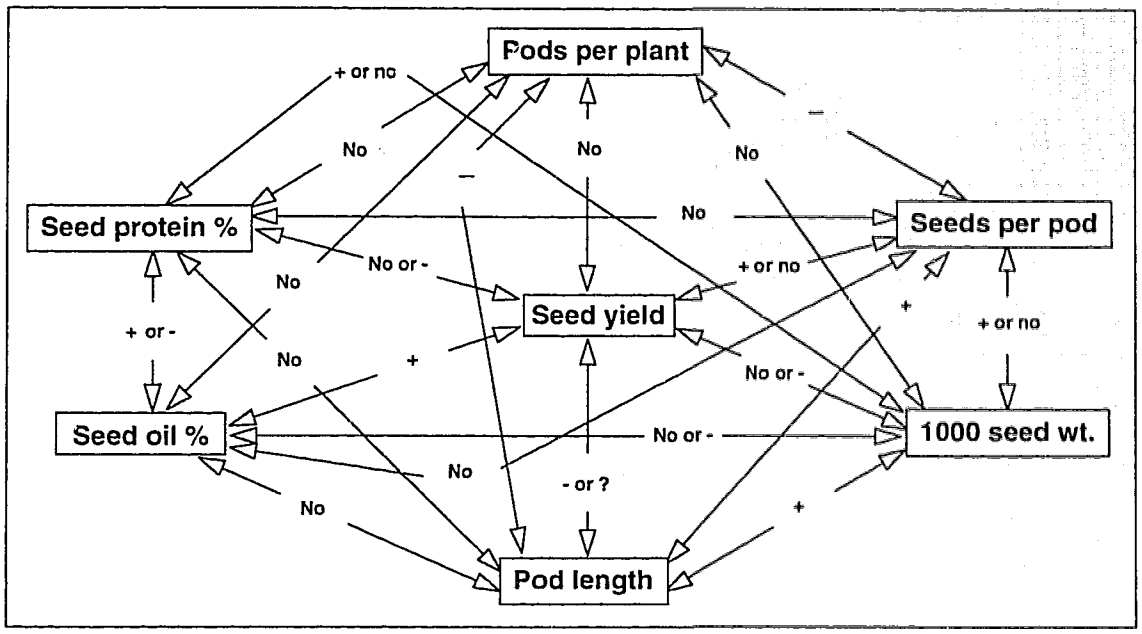

Figure 7-1. Interrelationships of pod length, the number of pods per plant (pods per plant), seed set per pod (seeds per pod), 1000 seed weight, seed yield, seed oil and protein content in *B. napus*, where the sign(s) within a specific double headed line represents the general relationship of the two traits linked by that line, with '+' representing a positive correlation, '-' a negative correlation, 'No' no relationship, and '?' unclear, respectively.

density, there was a remarkable increase in the number of pods per plant. Therefore, the gain in seed yield from the increased number of pods per plant would be much greater than the amount of seed yield loss due to reduced pod length. In addition, the absolute values of both pod length and the number of pods per plant increased at spaced plant density. Furthermore, the number of pods per plant can be changed through agronomic measures such as seeding rate or row spacing, even if a genotype has long-podded characteristic. (3) Pod length had a positive association with SSP and seed weight (see discussion later). Thus, indirectly, long pods would have a favorable effect on seed yield. It follows that selection for long pods is unlikely to significantly reduce the number of pods per plant of a genotype, and consequently, its seed yield potential.

In general, there was a positive association between pod length and SSP, and 1000 seed weight. This was in agreement with previous studies (Chay and Thurling 1979a,b). Large variation in this relationship was also observed in the present study, where a genotype with a pod length of 7 - 8 cm could have more than 40 seeds per pod, suggesting there was huge potential to improve the SSP without significantly increasing pod length.

Given these interrelationships, a more interesting consideration is the potential value of the long-podded genotypes in seed yield improvement. Within the genotypes investigated in this study, there was no obvious advantage of long-podded genotypes for seed yield over some of the genotypes with normal pod length. Path coefficient analysis revealed a direct negative effect of pod length on seed yield. However, no significant positive or negative relationship between these two traits was determined using correlation

analysis, presumably resulting from the effects of other components. Thus, it is difficult to clearly define the relationship between pod length and seed yield. This difficulty arises from a number of factors. Firstly, pod length and the number of pods per plant had a compensating relationship, but there were positive associations between pod length and SSP, and seed weight. These traits, individually or collectively, can influence seed yield. Secondly, all these traits are subject to variation. The degree of variation depends on genotypes, environmental conditions, and genotype x environment interactions. These genetic and environmental factors can complicate the interrelationships of these traits, and their relationships with seed yield. Thirdly, although the parental genotypes used for producing the DH lines, from which most of data were obtained, had large differences in their pod length, they might not necessarily have large differences in SSP and seed weight, and seed yield (no detailed data collected). Consequently, the DH lines produced had large differences in their pod lengths, but their differences in SSP, seed weight, and seed yield were small compared with the differences in their pod lengths. This would also influence the relationships between pod length and SSP, seed weight, and seed yield. Lastly, it is not clear whether there is any potential to combine the long-podded trait with high SSP. As mentioned earlier, considerable variation in SSP was observed. A genotype having a pod length of 7 - 8 cm could contain approximately 40 or more seeds per pod. At the same time, genotypes with a pod length of 12 - 13 cm were also observed. If the long pod and high SSP could be combined into one genotype, giving no significant adverse effect on other agronomic traits, it should be possible to produce high-yielding cultivars by increasing pod length. Therefore, there are still unanswered questions about the potential value of the long-podded trait in seed yield improvement of rapeseed.

7.3.2 Relationship between pod length and seed oil and seed protein contents

No studies have been reported previously regarding the relationship between pod length and seed oil or protein content. In the present study, a negative correlation ($r = -0.26 \sim -0.52$) was observed between pod length and seed oil content on a single plant basis, but no relationship between these two traits was observed based on lines in any other studies in any years, or at any plant densities, or at any locations. It is known that both pod length and seed oil content are subject to small variation. Their relationship estimated from single plants might be less accurate than that from lines. Similarly, no relationship was observed between pod length and seed protein content on either a single plant basis, or a line basis, or at any plant densities, except a positive genetic correlation ($r_g = 0.58$) determined from seven genotypes grown at three locations over two years. This positive correlation was mainly due to a highly positive association at the Edmonton Research Station (Michener). Thus, in general, there was no significant relationship between pod length and seed oil, or seed protein content, but with certain genotypes, longer pods could have higher protein content. Thus, it should be possible to develop long-podded genotypes with high seed oil and seed protein content.

7.3.3 Interrelationships of seed yield and seed yield components

7.3.3.1 Seed yield vs. the number of pods per plant

No relationship between seed yield and the number of pods per plant was found in the present study. Since both traits are quantitative and have a low heritability, it is expected that they would have no consistent relationship. Thus, it is impractical to select for high seed yield through selection for the number of pods per plant. This conclusion differs from the reports in previous studies (Andersson and Olsson 1961, Allen et al. 1971, Allen and Morgan 1972, Richards and Thurling 1979, Shabana et al. 1990). Some of these previous studies dealt with individual plants, such as the study by Shabana et al. (1990), where a positive correlation between the number of pods per plant and seed yield should be expected. It was unclear in other reports whether the positive correlation referred to the number of pods per plant, or the number of pods per unit area. It was shown in the present study that the number of pods per plant was greatly affected by row spacing and plant density, which in turn greatly influenced seed yield of a genotype. Thus, it is important to have a uniform plant density when seed yields of different genotypes are compared. Rapeseed breeders tend to predict the yield potential of a genotype in their breeding nurseries by looking at its pod canopy since this trait can be easily visualized in fields, whereas evaluation of SSP and seed weight is more time-consuming. The uniformity of plant density, or possibly a genotype's sowing dates and maturity as well, should also be considered when one is using this approach.

7.3.3.2 *Seed yield* vs. *seed set per pod*

Positive correlation between seed yield and SSP has been reported in previous studies (Andersson and Olsson 1961, Allen et al. 1971, Allen and Morgan 1972, Rawat and Anand 1977, Kler et al. 1992). In the present study, the relationship between seed yield and SSP varied with different genotypes and experimental conditions, but path coefficient analysis revealed that SSP had a direct positive contribution to seed yield. Comparison of SSP and seed yield of seven genotypes tested at three locations over two years also showed that SSP was a distinctive character of the genotype HIYLD which consistently produced the highest seed yield at every location and in each year. Thus, it appears that high SSP is a desirable characteristic of high seed-yielding genotypes.

7.3.3.3 *Seed yield* vs. *seed weight*

Morice (1960) suggested that the best character of selection for seed yield was seed weight per pod in *B. napus*. A higher seed weight was shown to be the distinguishing character of a high-yielding cultivar over others (Musnicki 1975). However, Andersson and Olsson (1961) found little or no correlation, or even a negative correlation, between seed weight and seed yield. It seems from these studies that the relationship between seed weight and seed yield has not been clearly defined. Similarly, no relationship between these two traits was found in the present study, except a negative genetic correlation (r_g = -0.64) determined from seven genotypes grown at three locations over two years. As has been discussed in 6.3.2, this negative correlation was a special instance that occurred under environmental stress conditions. Two, or more possibilities, may account for the difference observed between the present and some of the previous studies. Firstly, seed weight in the present study was on a line basis, whereas in Morice's study, it was on a basis of individual pods. Secondly, the majority of genotypes investigated in the present study were DH lines which were not selected for any traits, whereas the genotypes in Musnicki's study were released cultivars. It is likely that in a released cultivar, high seed weight, and high seed yield could have already been intentionally selected together in the course of a cultivar development. Based on the present study, it appears that in general seed yield and seed weight have no significant relationship, but they might become negatively associated under environmental stressful conditions. Nevertheless, such a special relationship is interesting. If the targeted growing area of a cultivar frequently has unfavorable environmental conditions for rapeseed production, the desirable seed size of this cultivar would be small seeds. It should be remembered that this indication came from an experiment with seven genotypes tested at three locations over two years. It would be interesting to study the relationship between seed weight and seed yield over a wide range of genotypes and environments.

7.3.3.4 *The number of pods per plant* vs. *seed set per pod*

There is little information available in the literature on the relationship between pods per plant and SSP. Shabana et al. (1990) observed no relationship between the number of pods per plant and SSP on a basis of individual plants. In the present study, no significant relationship between these two traits was found from DH lines of the M7 family, but there was a negative correlation ($r = -0.48$, $p < 0.05$) between them in DH lines of the M8 family only at spaced plant density. Thus, SSP and the number of pods per plant seem to have a compensating relationship only at a reduced plant density. However, this negative correlation should not become a major concern in rapeseed breeding, because (1) the negative correlation occurs only at reduced plant density which could be changed through agronomic measures, and (2) at the reduced plant density, there was a remarkable increase in the number of pods per plant. Accordingly, the gain in seed yield through increased number of pods per plant would be much greater than the quantity of yield loss through decrease in SSP. Indeed, studies in Chapter 4 and 5 have shown that a reduced plant density was beneficial to high seed yield.

7.3.3.5 *The number of pods per plant* vs. *seed weight*

No information is available in the literature on the direct relationship between the number of pods per plant and seed weight. McGregor (1987) observed an increase in both the number of pods per plant and seed weight from two *B. napus* cultivars in the field which had a reduced plant density from hail injury. However, the relationship between these two traits was not conducted. In the present study, no relationship was found between these two traits, except a weak negative correlation ($r = -0.39$, $p < 0.05$) determined from DH lines of the M7 family in 1994. Apparently, genetic contribution to this negative correlation should be very small. Thus, in general there was no relationship between these two traits. Even if they had a

slight compensating relationship, this relation would not significantly affect the seed yield potential of a rapeseed cultivar. Similar reasons discussed in 7.3.3.4 can be applied to this conclusion.

7.3.3.6 Seed set per pod vs. *seed weight*

Again, there is no information available in the literature on the relationship between SSP and seed weight. In the present study, a positive correlation ($r = 0.54$, $p < 0.01$) was determined from the DH lines of the M7 family in 1993, but no significant relationship was found from the same lines in 1994, or from any other studies. It appears that the relationship of these two traits depends on the genetic and environmental factors and/or their interactions. In any case, no compensating relationship was observed. Since both traits are important components of seed yield, a lack of compensating relationship suggests that both traits could be improved without sacrificing one or the other.

When all interrelationships of seed yield and seed yield components were considered, it seems that seed yield can not simply be selected on the basis of any one of these components alone. In addition, seed yield is influenced by a number of other genetic and environmental factors such as a cultivar's maturity, resistance to pests, tolerance to shattering, drought, frost damage, lodging, soil fertility and moisture, different cultivation practices etc. Each of these factors may influence one or more of the other traits measured in this study. Most of these factors can not be easily and accurately estimated, predicted or controlled by plant breeders who, unfortunately, have to breed cultivars to meet all challenges from these factors. Therefore, replicated trials with multiple locations and years would remain to be the most reliable method for accurate estimation of seed yield.

7.3.4 Relationships between seed oil content and the number of pods per plant, seed set per pod, seed weight or seed yield

The information on the relationship between seed oil and seed yield, seed yield components is limited in the literature, although it was suggested that larger seeds might have a higher seed oil content because the proportion of seed coat declines with the increase of seed size (Hutcheson, 1984). Degenhardt and Kondra (1984) reported that there was no direct relationship between seed yield and seed oil or protein in *B. napus* from the data of five genotypes tested at two locations over two years. More recently, Daun et al. (1995) demonstrated, using the data from surveys conducted from 1956 to 1994 on oil and protein values of No. 1 Canada canola, that both seed yield and oil content had increased during this period, and that these two traits were positively correlated ($r = 0.69$).

No relationship between seed oil and the number of pods per plant was found in the present study. Except for a weak positive correlation ($r = 0.33$, $p < 0.05$) determined from DH lines of the M7 family in 1993, no relationship between seed oil content and SSP was obtained from any other experiments. Thus, there was virtually no relationship between seed oil and the number of pods per plant, or SSP. Therefore, it should be possible to incorporate high seed oil, large number of pods per plant and high SSP into one genotype.

There were differences in the relationship between seed oil content and seed weight. When DH lines of the M7 and M8 families were tested at Edmonton Research Station (Michener), no relationship was found between these two traits. However, the means of these two traits were negatively correlated ($r_g = -0.66$) when seven genotypes were tested at three locations over two years. At individual locations, the negative association was particularly more pronounced at Ellerslie ($r = -0.71$) and Kelsey ($r = -0.69$) than at Michener ($r = -0.39$). As has been discussed in Chapter 6, it seemed that the general conditions for the production of the genotypes investigated were the best at Michener and the poorest at Kelsey. These differing environmental conditions were indicated by the relative values of six traits, i.e. pod length, SSP, 1000 seed weight, seed yield, seed oil and protein content, at these three locations. For most of the genotypes, all these six traits except seed oil content had the highest values at Michener, and the lowest values at Kelsey, while the values at Ellerslie were intermediate (see Fig. 6-2). Thus, this negative relationship between seed oil and seed weight should be viewed as a special case that occurred under stressful conditions, rather than a typical relationship of these two traits. Nevertheless, this special relationship is of importance in rapeseed breeding. If the a breeding program was to develop cultivars for environmental stressful conditions, an ideal cultivar would have small seeds for high seed oil content. A similar conclusion was reached regarding the relationship between seed yield and seed weight in 7.3.3.3. Therefore, for genotypes grown under unfavorable conditions, small seeds appears to be a desirable characteristic to produce high seed yield and seed oil content.

A significant positive correlation between seed oil and seed yield was also obtained in studies presented from Chapter 4 to Chapter 6. The majority of these genotypes were DH lines which had not been selected for any of these two traits, as compared with the genotypes in the early report (Daun et al. 1995) which had already been selected for both high seed yield and high seed oil. Thus, the results from the present study suggested that a positive association between seed oil and seed yield may not be uncommon. If so, it could be used advantageously in rapeseed breeding, considering that a high value for both traits is required for a modern rapeseed cultivar.

7.3.5 Relationship between seed protein content and the number of pods per plant, seed set per pod, seed weight or seed yield

There is little information available in the literature on the relationship between seed protein content and seed yield or seed yield components. Although there is no statistical data showing the relationship between seed yield and seed protein content, it is still very interesting to note from the report by Daun et al. (1995) that for the top grade canola/rapeseed cultivars at harvest, seed protein content has been declining although both seed yield and seed oil content have been increasing. It is unclear what exactly caused this trend. In the present study, no relationship was found between seed protein content and the number of pods per plant, SSP or seed weight in majority of genotypes investigated. With certain genotypes, however, large seeds had a positive association with high seed protein content, particularly under unfavorable environmental conditions which has been discussed in Chapter 6. The relationship between seed protein content and seed yield differed with the environmental conditions under which the relationship was investigated. When seven genotypes were tested at three locations over two years, there was a significant negative correlation at Ellerslie ($r = -0.77$) and Kelsey ($r = -0.72$), but not at the Edmonton Research Station (Michener). As mentioned earlier, general conditions were better at Michener than at Kelsey or Ellerslie for the production of the genotypes tested. Apparently, the relationship of these two traits was influenced by the environmental conditions at these locations. Under favorable conditions there was no relationship between these two traits, whereas there was a compensating relationship under stress conditions. This argument was supported by the studies using DH lines. In these studies, DH lines were grown at Michener, and no significant relationship was observed between seed yield and seed protein content in any year, or at any plant density. Such a negative association would therefore occur only when the genotypes under evaluation were subject to a stress environment. Thus, any simultaneous comparison of seed yield and protein content of different genotypes or investigation of their relationship should be based on the results from the same locations and/or years.

7.3.6 Relationship of seed oil and protein content

Grami et al. (1977) found that seed oil and protein are negatively correlated. A recent report by Daun et al. (1995) also demonstrated that there was a high negative correlation between seed oil and protein content ($r = -0.76$) among the top canola/rapeseed cultivars at harvest in Canada from 1956 to 1994. The surveys on individual cultivars grown from 1992 to 1994 in Canada also revealed a significant negative correlation between oil and protein content for both *B. napus* ($r = -0.93$) and *B. rapa* ($r = -0.89$). It should be noted that these negative correlation values were obtained from the cultivars grown in true production situations rather than from breeding lines.

Various, sometimes contradictory, results were obtained regarding the relationship of seed oil and seed protein contents in the present study. Based on single plants, a negative correlation (-0.45) was determined from one BC_1 population, but not from the other BC_1 population, or from the F_2 generation. When the relationship was determined from DH lines, no relationship was found from the M7 family, but a positive correlation (0.47) was obtained from the M8 family only at the normal plant density. When seven genotypes were tested over two years at three locations, a general negative correlation ($r = -0.78$) between these two traits was found. Analysis at individual locations revealed that the negative association was more pronounced at Ellerslie ($r = -0.78$) and Kelsey ($r = -0.69$) than at Michener ($r = -0.42$). Thus, the relationship of seed oil and protein contents appears to depend on the genotypes and environmental conditions. It is difficult to clearly define the relationship of these two traits.

Apart from the interrelationships discussed earlier, row spacing and plant density significantly affected the expression of the traits investigated in the present study except seed oil content, and sometimes their interrelationships. Since this area has been extensively discussed in Chapter 5 and there are no overlapping studies in different chapters, it will not be discussed here again.

7.4 SUMMARY

Inheritance of pod length, the number of pods per plant, seed set per pod, seed weight, seed oil and seed protein contents, and seed yield in *B. napus* were investigated, using DH lines and/or conventional genetic analysis. The long-podded trait (11-13 cm) was found to be controlled by three independent nuclear genes with additive gene action, and pod length had a broad sense heritability of 0.7 - 0.9. Seed weight was the most stable trait among the number of pods per plant, seed set per pod and seed weight. Seed set per pod was less stable than seed weight, while the number of pods per plant was most responsive to environmental variations. Significant genotype x year x location interaction existed on seed yield, and the influence of any individual yield component on seed yield was small.

Of the two seed quality traits oil and protein, seed oil content generally had a higher heritability, and consequently was more stable, than seed protein content.

Thus, in rapeseed breeding, selection for pod length, seed weight and seed oil content would be easier and more effective than selection for seed set per pod and seed protein content, whereas selection for the number of pods per plant would be difficult. Selection for seed yield remains the most difficult.

Plant densities influenced the expression of individual traits. At spaced plant density, there was a great increase in the number of pods per plant, and slight increases in pod length and seed set per pod, but slight decreases in 1000 seed weight and seed protein content. Seed oil content was insensitive to changes in plant density.

Interrelationships of pod length, the number of pods per plant, seed set per pod, 1000 seed weight, seed yield, seed oil, and protein content varied with specific traits, and in some cases also varied with genotypes and environmental conditions under which a specific relationship was estimated. In general, no significant association was found between seed yield and pod length, the number of pods per plant, seed set per pod, and 1000 seed weight. However, path coefficient analysis revealed a direct negative effect of pod length on seed yield, whereas seed set per pod had a direct positive contribution to seed yield. An experiment with three locations in two years also indicated that a high value of seed set per pod was a distinctive characteristic of a high-yielding genotype. Under unfavorable environmental conditions, genotypes with small seeds appeared to have higher seed yield. Positive association between seed yield and seed oil content was frequently observed, suggesting that these two traits could be improved simultaneously. Under favorable environmental conditions, seed yield had no relationship with seed protein content, but these two traits had a negative association under unfavorable environmental conditions.

Pod length had a positive association with seed set per pod, and seed weight, but a negative association with the number of pods per plant. No relationship was found between pod length and seed oil content, nor between pod length and seed protein content.

No relationship between seed size and seed oil, or protein content was found under favorable environmental conditions. Under unfavorable environmental conditions, however, seed size was positively associated with high protein content, but negatively associated with seed oil content. It appeared that small-seeded genotypes would have higher seed oil content than large-seeded genotypes under unfavorable environmental conditions.

Seed oil and protein content were negatively correlated only when environmental conditions were unfavorable, suggesting that these two traits could be improved simultaneously under favorable environmental conditions.

7.5 References

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APPENDICES

APPENDIX A. B5 MEDIA

A.A.1 B5 Wash Medium (1 L)

- B5 x 10 stock (below)	100 mL
- sucrose	130 g
- ddH_2O added to a final volume of 1 L	
- stir contents until dissolved	
- pH adjusted to 6.0	
- sterilize in autoclave	

A.A.2 B5x 10 (frozen stock) (1 L)

- KNO_3	12.50 g
- $MgSO_4$-$7H_2O$	1.25 g
- $CaCl_2$-$2H_2O$	3.75 g
- $(NH_4)_2SO_4$	0.67 g
- NaH_2PO_4-H_2O	0.75 g
- Fe 330	0.20 g
- B5x 10 vitamin stock (below)	50.00 mL
- B5x 100 micronutrient stock (below)	50.00 mL
- KI stock (below)	5.00 mL
- ddH_2O added to a final volume of 1 L	
- stir contents until dissolved	
- store in freezer as 100 mL samples	

A.A.3 B5x 10 (frozen vitamin stock) (1 L)

- myoinositol	10.00 g
- nicotinic acid	0.10 g
- pyridoxine HCl	0.10 g
- thiamine HCl	1.00 g
- ddH_2O added to a final volume of 1 L	
- stir contents until dissolved	
- store in freezer as 100 mL samples	

A.A.4 B5 x 100 (frozen micronutrient stock)

- $MnSO_4$-$7H_2O$	1.00 g
- H_3BO_3	0.30 g
- $ZnSO_4$-$7H_2O$	0.20 g
- Na_2MnO_4-$2H_2O$	0.025 g
- $CuSO_4$-$5H_2O$	0.0025 g
- $CoCl_2$-$6H_2O$	0.0025 g
- ddH_2O added to a final volume of 1 L	
- stir contents until dissolved	
- store in freezer as 100 mL samples	

A.A.5 KI Stock

- KI	0.83 g
- ddH_2O added to a final volume of 1 L	
- stir until contents dissolved	
- refrigerate (2 - 5 °C)	

APPENDIX B. NLN CULTURE MEDIUM (1 L)

- ddH_2O	500 mL
- KNO_3	0.125 g
- $MgSO_4$-$7H_2O$	0.125 g
- $Ca(NO_3)_2$-$4H_2O$	0.50 g
- KH_2PO_4	0.125 g
- Fe 300	0.04 g
- B5x 100 vitamin stock (Appendix A)	10.00 g
- B5x 100 micronutrient stock (Appendix A)	10.00 g
- glutathione	0.03 g
- L-glutamine	0.80 g
- L-serine	0.10 g
- sucrose	130 g
- ddH_2O added to a final volume is 1 L	
- stir contents until dissolved.	
- pH adjusted to 6.0	
- filter sterilize and store in fridge (2 - 5 °C)	

APPENDIX C. B5 SOLID CULTURE MEDIUM (1L)

- B5 x 10 stock (appendix A)	200 mL
- sucrose	20 g
- GA_3 (0.15 mg/l)	1 mL
- ddH_2O added to a final volume of 1 L	
- stir contents until dissolved.	

- pH adjusted to 5.7
- agar 8 g
- sterilize in autoclave
- pour 10 ml into petri plates (100x 15 mm)
- approximately 100 plates can be poured.
- allow samples to solidify
- seal in plastic bags until used

APPENDIX D. FORMULAE USED FOR THE CALCULATION OF VARIANCE COMPONENTS AND THEIR STANDARD DEVIATIONS, AND FOR THE ESTIMATION OF THE HERITABILITIES.

A.D.1. Table of analysis of variance for the experiment with seven genotypes (g=7) tested over two years (y=2) at three locations (l=3) with three replications (r=3) in an RCB design, where all factors have random effects.

SOURCE	DF	EXPECTED MEAN SQUARE	ABBR
YEAR	1	Var(Error)+3Var(YEAR*LOCAT*GENOTYPE) +9Var(YEAR*GENOTYPE)+7Var(REP(YEAR*LOCAT)) +21Var(YEAR*LOCAT)+63Var(YEAR)	
LOCATION	2	Var(Error)+3Var(YEAR*LOCAT*GENOTYPE) +6Var(LOCAT*GENOTYPE)+7Var(REP(YEAR*LOCAT)) +21Var(YEAR*LOCAT)+42Var(LOCAT)	
YEAR*LOCAT	2	Var(Error)+3Var(YEAR*LOCAT*GENOTYPE) +7Var(REP(YEAR*LOCAT))+21Var(YEAR*LOCAT)	
REPLICATION (YEAR*LOCAT)	12	Var(Error)+7Var(REP(YEAR*LOCAT))	
GENOTYPE	6	Var(Error)+3Var(YEAR*LOCAT*GENOTYPE)+6Var(LOCAT*GENOTYPE) +9Var(YEAR*GENOTYPE)+18Var(GENOTYPE)	M1
YEAR*GENOTYPE	6	Var(Error)+3Var(YEAR*LOCAT*GENOTYPE) +9Var(YEAR*GENOTYPE)	M2
LOCAT*GENOTYPE	12	Var(Error)+3Var(YEAR*LOCAT*GENOTYPE)+6Var(LOCAT*GENOTYPE)	M3
YEAR*LOCAT*GENOTYPE	12	Var(Error)+3Var(YEAR*LOCAT*GENOTYPE)	M4
Error	72	Var(Error)	M5

A.D.2. Variance components and heritability

A.D.2.1. Variance components of genotype $\left(S_g^2\right)$, year x genotype $\left(S_{yg}^2\right)$, location x genotype $\left(S_{lg}^2\right)$, year x location x genotype $\left(S_{ylg}^2\right)$, and residual $\left(S_e^2\right)$:

$$S_g^2 = \frac{M1 + M4 - M2 - M3}{ylr} \qquad S_{yg}^2 = \frac{M2 - M4}{lr} \qquad S_{lg}^2 = \frac{M3 - M4}{yr}$$

$$S^2_{ylg} = \frac{M4 - M5}{r} \qquad S^2_e = M5$$

A.D.2.2. Phenotypic variance $\left(S^2_p\right)$: $S^2_p = S^2_g + \frac{S^2_{yg}}{y} + \frac{S^2_{lg}}{l} + \frac{S^2_{ylg}}{yl} + \frac{S^2_e}{ylr}$.

A.D..2.3. Heritability $\left(h^2\right)$: $h^2 = \frac{S^2_g}{S^2_p}$

A.D.3. Variance of mean square (VMS) and standard divination of variance components (SVMS)

A.D..3.1. Variance of MS for genotype (VMS_g) and the standard divination of S^2_g ($SVMS_g$):

$$VMS_g = \left(\frac{1}{ylr}\right)^2 \left(\frac{2MS_g^2}{df_g + 2} + \frac{2MS_{yg}^2}{df_{yg} + 2} + \frac{2MS_{lg}^2}{df_{lg} + 2} + \frac{2MS_{ylg}^2}{df_{ylg} + 2}\right).$$

$$SVMS_g = \sqrt{VMS_g}.$$

A.D..3.2. Variance of MS for year x genotype (VMS_{yg}) and the standard divination of S^2_{yg} ($SVMS_{yg}$):

$$VMS_{yg} = \left(\frac{1}{lr}\right)^2 \left(\frac{2MS_{yg}^2}{df_{yg} + 2} + \frac{2MS_{ylg}^2}{df_{ylg} + 2}\right). \quad SVMS_{yg} = \sqrt{VMS_{yg}}.$$

A.D..3.3. Variance of MS for location x genotype (VMS_{lg}) and the standard divination of S^2_{lg}:

$$VMS_{lg} = \left(\frac{1}{yr}\right)^2 \left(\frac{2MS_{lg}^2}{df_{lg} + 2} + \frac{2MS_{ylg}^2}{df_{ylg} + 2}\right). \quad SVMS_{lg} = \sqrt{VMS_{lg}}.$$

A.D..3.4. Variance of MS for year x location x genotype is ($SVMS_{ylg}$) and the standard divination of S^2_{ylg} ($SVMS_{ylg}$):

$$VMS_{ylg} = \left(\frac{1}{r}\right)^2 \left(\frac{2MS_{ylg}^2}{df_{ylg} + 2} + \frac{2MS_e^2}{df_e + 2}\right) \quad SVMS_{ylg} = \sqrt{VMS_{ylg}}.$$

A.D..3.5. Variance of MS for residual ($SVMS_e$) and the standard divination of S^2_e ($SVMS_e$):

$$VMS_e = \frac{2MS_e^2}{df_e + 2}. \quad SVMS_e = \sqrt{VMS_e}.$$

A.D.4. Covariance components, phenotypic and genotypic correlations

A.D.4.1. Symbols for covariances, variance and covariance components, phenotypic variance and covariance components between trait X and trait Y of seven genotypes (g=7) tested over two years (y=2) at three locations (l=3) with three replications (r=3) in an RCB design, where all factors have random effects.

SOURCE	VARIANCE COMPONENT OF X	COVARIANCE OF X AND Y	VARIANCE COMPONENT OF Y	COVARIANCE COMPONENT OF X AND Y
GENOTYPE	$S(x)_g^2$	COV1	$S(y)_g^2$	$COVCOMP(x,y)_g$
YEAR*GENOTYPE		COV2		$COVCOMP(x,y)_{yg}$
LOCAT*GENOTYPE		COV3		$COVCOMP(x,y)_{lg}$
YEAR*LOCAT*GENOTYPE		COV4		$COVCOMP(x,y)_{ylg}$
ERROR		COV5		$COVCOMP(x,y)_e$
PHENOTYPIC VARIANCE/COVARIANCE COMPONENT	$S(x)_p^2$		$S(y)_p^2$	$COVCOMP(x,y)_p$

$$COVCOMP(x,y)_g = \frac{COV1 + COV4 - COV2 - COV3}{ylr} \quad (A)$$

$$COVCOMP(X,Y)_{yg} = \frac{COV2 - COV4}{lr} \quad (B)$$

$$COVCOMP(x,y)_{lg} = \frac{COV3 - COV4}{yr} \quad (C)$$

$$COVCOMP(x,y)_{ylg} = \frac{COV4 - COV5}{r} \quad (D)$$

$$COVCOMP(x,y)_e = COV5 \quad (E)$$

$$COVCOMP(x,y)_p = A + \frac{B}{y} + \frac{C}{l} + \frac{D}{yl} + \frac{E}{ylr}$$

A.D.4.2. Phenotypic correlation:

$$r(x,y)_p = \frac{COVCOMP_p}{\sqrt{S(x)_p^2 S(y)_p^2}}$$

A.D.4.3. Genotypic correlation:

$$r(x,y)_g = \frac{COVCOMP_g}{\sqrt{S(x)_g^2 S(y)_g^2}}$$