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# Agronomic and Quality Performance of Three Doubled Haploid Lines Derived from a *Brassica napus/Brassica rapa* Interspecific Cross

By

Trevor Allan Miller 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science in Plant Science, Department of Agriculture Food and Nutritional Sciences

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**Date:** Dec 07/00

**In Dedication to my Parents**

"The easiest thing to pack is your education."

# Abstract

Agronomic and quality traits introgressed into doubled haploid (DH) lines from an interspecific cross of *B. napus* x *B. rapa* were investigated. The interrelationships of these traits i.e. oil, protein, seed yield, maturity, days to first flower, and duration of flowering were also examined.

Three selected DH lines were tested at three locations over two years at Michener, Ellerslie, and Kelsey. Seed yields of the three DH lines were slightly higher than the *B. rapa* parents but were significantly lower than the *B. napus* cultivar Quantum. Although yields were low for the three lines, their 1000-kernel weights were significantly higher than all other cultivars studied. The selected DH lines expressed a *B. napus* maturity pattern averaging 102.5 days. Days-to-flower was intermediate between *B. napus* and *B. rapa*, and duration of flowering showed no association with either species. Two of the three DH lines protein levels averaged 48.6 percent, while the oil content of the DH lines tended to be lower than either parent. The oil quality of these lines tended to be high in linolenic and saturated fatty acids, both of which are undesirable traits in a breeding program.

Positive associations were found between maturity and the following traits: 1000-kernel weight, seed yield, and days to first flower. A significant positive relationship was also observed between days-to-flower and yield. These positive relationships suggest that selecting for higher yielding cultivars may be obtained by selecting either for increasing days-to-flower or increasing maturity. Seed oil was negatively associated with both 1000-kernel weight and days to maturity.

A test correlating field vigor with vigor in a laboratory cold test was preformed on all genotypes in the study. A positive correlation between field vigor and the cold test vigor was observed among the genotypes, a result supported by earlier studies.

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## Abbreviations

| | |
|---|---|
| AOSA | Association of Official Seed Analysts |
| B | Bulk |
| $BC_1$ | First back-cross |
| °C | Degrees Celsius |
| cv. | Cultivar |
| C16:0 | Palmitic acid |
| C18:1 | Oleic acid |
| C18:2 | Linoleic acid |
| C18:3 | Linolenic acid |
| C22:1 | Erucic acid |
| Df | Degrees of freedom |
| DH | Doubled Haploid |
| DNA | Deoxyribonucleic acid |
| $F_1$ | First filial generation |
| $F_6$ | Sixth filial generation |
| $F_7$ | Seventh filial generation |
| FirstFlr | Days to first flower |
| FlrDur | Flowering duration |
| G | Genotype |
| g | Grams |
| GLM | General linear model |
| kg | Kilograms |

| | |
|---|---|
| L | Location |
| LS | Least Squared |
| N | Haploid chromosome number |
| NMR | Nuclear magnetic resonance |
| p | Probability |
| Seed wt | Seed weight |
| T. Gluc. | Total glucosinolates |
| TMS | Trimethysilyl |
| WCC/RRC | Western Canadian Canola/Rapeseed Recommending Committee |
| Y | Year |
| μmole/g | Micromole per gram |
| σ | Standard deviation |

# Chapter 1
# Literature Review

## 1.1 Introduction

Canola is the premier oilseed crop grown in Canada and its acreage in Western Canada has expanded rapidly over the past few decades. In 1983, prairie farmers planted 2.14 million hectares, and by 1993 the acreage of this crop had nearly doubled reaching 4.16 million hectares (Zeneca Seeds, 1995). The Canola Council of Canada reported 5.47 million hectares sown for 1998 and 5.61 million in the spring of 1999 (Statistics Canada 2000).

Canola is susceptible to a number of disease and environmental stresses common to the prairies. To manage these problems it is recommended that canola be sown in a four-year rotation (Thomas 1984). Because of the constraints (available acreage and rotation requirements), western Canada may be nearing the maximum sustainable acreage for canola. Since there is a finite land base for canola production, producers will require new varieties that offer better disease and pest resistance, greater durability to drought and heat stresses, with higher yields to supply the increasing demand for canola.

The two species of canola commonly grown on the Canadian prairies are *Brassica napus* (Argentine) and *B. rapa* (Polish). The self-compatible *B. napus* species has sustained significant yield and disease resistance improvements through extensive breeding programs. *B. rapa's* self-incompatibility, on the other hand, has greatly hindered its breeding progress (Stringam, personal communication). Although on average *B. napus* yields higher than *B. rapa*, it matures 10 to 21 days later. The later maturity predisposes *B. napus* to frost damage with subsequent losses in seed yield, grade, and oil quality. The potential higher yields of *B. napus* encourages producers to

risk these losses caused by frost damage. Since 1997, *B. napus* has occupied more acreage than *B. rapa* in the prairie provinces (Statistics Canada 1998). This shift may increase losses due to frost, as well as a decline in the overall oil quality. Prairie producers may benefit greatly from cultivars developed through interspecific hybridization that possess traits from both species, especially the elevated yields of *B. napus*, and the earlier maturity of *B. rapa*. In an interspecific crossing program, a number of quality and agronomic traits require monitoring to maintain performance standards. These are outlined in the sections that follow.

## 1.2 Canola Quality (Oil and Meal)

### 1.2.1 Oil Quality

Canola oil is considered the healthiest of all edible oils because it has the best balance of saturated, polyunsaturated and monounsaturated fatty acids of all oils, and the lowest level of saturated fats among vegetable oils (Canada Health and Welfare 1994). Saturated fats have been linked to increases in blood cholesterol levels; reduced consumption of foods containing high levels of these fats is recommended by Health and Welfare Canada (1994). The first canola quality rapeseed cultivar, Tower, was released in 1974 after its development at the University of Manitoba. For the oil to meet canola quality standards, cultivars of *B. napus* and *B. rapa* must contain no more than two percent erucic acid and less than 30 μmoles of glucosinolates in air dried oil free meal (Seeds Act 1989). Although a canola cultivar may meet the minimum standards for canola quality, quality levels may still be insufficient for cultivar registration. Recent registration standards (1999) require that erucic acid levels in submitted seeds be 0.5% or

less, and contain no more than 12 μmoles total glucosinolates/g whole seed, or of levels no higher than the average of the checks used (whichever is higher) (WCC/RCC 1999).

Canola oil consists of long chain fatty acids (the building blocks for the majority of the lipids), in either saturated or unsaturated forms. A saturated fatty acid is one with only single bonds within the carbon chain. An example of a saturated fatty acid is palmitic acid (16:0) which is composed of a 16-carbon chain with single bonds. Unsaturated fatty acids have one or more double or triple bonds within the chain (Downey and Robbelen 1989). Oleic, linoleic, and linolenic are important unsaturated fatty acids in canola oil.

Oleic acid comprises about 55 to 65 percent of the fatty acid profile for canola (Stefansson *et al.* 1961), and is desired in cooking oil because of its thermostability. Oleic acid (18:1) branches in one of two directions in the biosynthetic pathway: it either desaturates into linoleic acid (18:2) or undergoes chain elongation to form eicosenoic acid which is a precursor for erucic acid (Figure 1.1) (Bartkowiak-Broda and Krzymanski 1983).

Linoleic acid constitutes 15 to 20 % and 25 to 30 % of the fatty acids in *B. napus* and *B. rapa*, respectively (Downey 1964). Linoleic acid is a principal dietary fatty acid and the "parent" for a series of polyunsaturated acids that are essential for growth. This group of polyunsaturates arises by the desaturation and chain elongation of the linoleic acid within the human body. Linoleic acid is an essential fatty acid (vitamin F), that must be provided in the diet, as it cannot be synthesized by the body or interconverted (Holman 1981). This study also found that linoleic acid cured or prevented dermatitis in livestock and humans consuming fat deficient diets (Holman 1981).

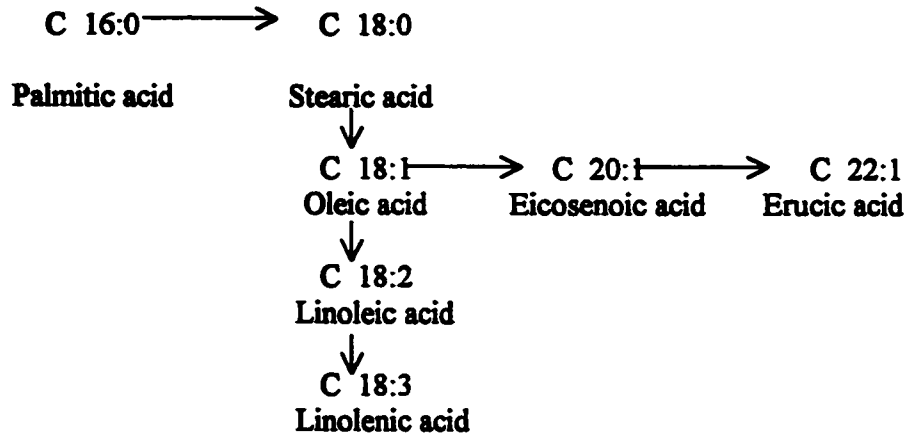


Figure 1.1 The biosynthetic pathway of fatty acids in *Brassica napus* and *B. rapa*. (Modified from Ahuja and Banga 1993)

Reduction of erucic acid within the fatty acid profile did not affect linolenic acid contents. Linolenic acid oxidizes easily and is responsible for rancidity of this oil (Labana *et al.* 1993). Typically, lower linolenic acid contents are desired to improve the storage characteristics and increase shelf-life of the oil. Rakow (1973) successfully obtained mutants of *B. napus* that were low in linolenic acid (about 5%) while not affecting the remaining profile adversely. Recently, however, this goal of reducing or eliminating linolenic acid from canola oil has been challenged. Alpha-linolenic acid (an omega-3 fatty acid) is considered to be essential for infant growth and development and protects against heart disease, thrombosis, hypertension, inflammatory and autoimmune disorders (Simopoulos 1991). Consequently, there is a consumer demand to maintain linolenic acid at its present level of about 8 to 11 percent despite the reduced shelf-life associated with it (Stringam, personal communication).

Consumption of erucic acid has been shown to depress growth, digestibility, longevity, interfere with myocardial conductance, cause cardiac lipidosis and necrosis, increase blood cholesterol, and blood coagulation (Renarid and McGregor 1976; Laryea *et al.* 1992; Sim 1986). The early varieties of rapeseed contained approximately 40 percent erucic acid. Stefansson (1961) developed the first *B. napus* line with a seed oil free of erucic acid. Downey (1964) later advanced a *B. rapa* line that was also free of erucic acid in the seed oil. This advancement in *B. napus* and *B. rapa* breeding lines led to the worldwide development of nutritionally superior, low erucic cultivars.

### 1.2.2 Meal Quality

The major component of the seed remaining after oil extraction is the canola meal. Canola meal contains about 38 to 44% protein (dry matter basis) (Downey and Robbelen 1989). This high quality protein has been used as an organic fertilizer for field crops in the Asian countries, while in the Western World it is utilized as a high protein feed for livestock and poultry (Downey and Robbelen 1989). Along with high protein levels, the meal also contains large amounts of the sulfur-containing amino acid methionine, which is used in the synthesis of protein and the conversion to the amino acid cysteine (Bell and Keith 1989). Cysteine strengthens the protective lining of the stomach and intestines which assists in an animal attaining optimal health and growth (Salim 1993). Both pea and soybean meal are low in sulfur amino acids, but high in the essential amino acid lysine and energy, while canola meal is low in lysine and energy, its sulfur amino acid levels are high (Bell and Keith 1989). This nutrient composition found in pea and soybean meal makes them good complementary feed mixes for canola meal.

One of the major factors limiting the percent protein in canola is the large amount of seed-coat or hull contained in the meal. Hulls (which are high in crude fiber and low in protein) represent 30% of the total oil-free meal weight (Bell 1995). Crude fiber content (mainly cellulose and hemicellulose) amounts to about 15% of the defatted seed meal. High crude fiber reduces feed values, and consequently reduces metabolizable energy for animals (Clandinin and Robblee 1981). Reducing hull content will reduce the crude fiber in the meal, and consequently increase the percentage of protein concentrated in the meal. This can be achieved by the development of yellow-seeded varieties. Yellow seed coat color is correlated with reduced crude fiber because of its thinner seed coat (Stringam *et al.* 1974). Producing yellow-seeded varieties provides many advantages including: (1) better meal quality due to an increase in protein; (2) a one to two percent increase in oil content (Stringam *et al.* 1974); (3) the light colored coat does not cause discoloring of the oil upon processing, even without the dehulling of the seeds, and (4) it is easier for producers to determine the degree of ripeness in yellow seeds because the chlorophyll present within the seed is not masked by the dark seed coat color (Dhillon *et al.* 1986). A shift from dark seed coat color to a yellow seed coat, would help both producers and processors achieve a higher quality end product for the consumer.

Use of traditional rapeseed varieties for food/feed (humans and commercial animals) has been limited by sulfur containing compounds called glucosinolates (Downey and Robbelen 1989). Low glucosinolates (less than 30 micromoles) are required for rapeseed to be accepted as canola quality (Thomas 1984). Glucosinolates in higher quantities adversely affect the metabolism of the thyroid in non-ruminant animals,

including humans (Bell *et al.* 1972). They also cause damage to the mucous membranes of humans and other non-ruminant animals.

Three basic meal compounds possess strong flavors and are sharp to the taste, (nitriles, thiocyanates, or isothiocyanates), reducing the palatability of the seed oil and meal. Early studies suggested that intact glucosinolates were not toxic to animals, but the nitriles, isothiocyanates and other compounds released by hydrolysis of glucosinolates caused pathological effects and reduced palatability (Appelqvist, 1972). Although it is true that those substances are toxic, it was later proven that intact glucosinolates, (without myrosinases hydrolyzing the compound) had anti-nutritional and toxic effects (Bille *et al.* 1983). To minimize the harmful health effects of glucosinolates (and derivatives), one research objective is to reduce these concentrations to an ultimate goal of zero.

## 1.3 Agronomics

The close similarities of *B. napus* and *B. rapa* in their oil and meal qualities make them excellent partners in the production of edible oils and animal feeds. However, it is their agronomic differences that make them suitable for different areas of the provinces (Table 1).

The improvement of some of these *B. napus* and *B. rapa* traits have been of particular importance for many plant breeders. Reducing the days to maturity for *B. napus* cultivars has been a major challenge for many breeders in the short season Canadian prairies. Breeding for cultivars with an earlier flowering period may reduce the maturation period and eliminate mid-summer flower blast.

An agronomic trait that added difficulty to the harvest of canola/rapeseed, was the height of this crop. Many canola/rapeseed crops were more than 180 centimeters in height (Buzza 1995). The introduction of shorter varieties reduced losses due to lodging even with the application of higher rates of fertilizers. Selection for cultivars that have

**Table 1.** Agronomic Characteristics of *Brassica napus* vs. *Brassica rapa*

| Characteristics | *B. napus* | *B. rapa* |
|---|---|---|
| Seed yield | Yields high under good moisture and frost-free conditions | Under good growing conditions 15 to 20% less than *B. napus* |
| Days to maturity | Can be as short as 74 days or as long as 140 days | Usually 10 days to 3 weeks earlier than *B. napus* (between 66 and 111 days) |
| Height | Variable with growing conditions (somewhere between 75 and 175 cm) | Variable with growing conditions (somewhere between 75 and 175 cm) |
| Lodging | Varies with variety; *B. napus* can be more prone to lodging due to its taller nature over *B. rapa* | Varies with variety |
| Disease tolerance | Most varieties are similar in tolerance (unless specifically bred for resistance) | Tends to be more susceptible to diseases than *B. napus* |
| Frost damage | Susceptible to late spring frosts and early fall frosts, pending upon variety maturing date | Tolerant to late spring frosts. Usually matures before fall frosts |
| Drought tolerance | More susceptible to late summer drought than *B. rapa* | Often matures early enough to escape the late summer droughts |
| Pod shatter | Shatters readily when ripe | Fairly resistant to shattering; can be straight combined without high seed loss |
| Flowering | Flowering period is usually throughout July, high mid-summer temperatures may cause some flower blast | Flowers generally throughout June or early July; usually finishes flowering before higher temperatures occur |

Modified from Thomas 1985.

increased lodging resistance is currently based on a visual rating of field plots. Although there are clear differences between cultivars for this trait, its mode of inheritance is not yet understood (Buzza 1995).

### 1.3.1 Seed Vigor Testing

The establishment of a uniform, healthy seedling stand is the first step toward a disease free, high yielding, and good quality crop. One of the ways a producer can maximize the probability of obtaining a healthy, vigorous stand is to use high quality seed. Seed lots that lack a high degree of vigor tend to succumb more easily to diseases and adverse conditions, and consequently produce erratic stands with poor crop performance (Elias and Copeland 1997). Seedling vigor has been defined in many ways, however, the Association of Official Seed Analysts (AOSA) has developed a succinct and direct definition. Seed vigor comprises those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions (ASOA 1983).

At one time, germination testing was thought to be satisfactory for interpretation of seed quality, but Delouche (1963) found that germination testing alone may produce a false interpretation of seed lot quality. He found that deterioration of a seed lot may exist although germination remains relatively high. Vigor tests should give producers more information about the physiological quality of the seed lots, providing a better estimate of potential field emergence and survival. Many vigor tests can be performed on a seed lot with a number of them providing information on physiological performance that may be expected under field conditions. Grabe (1976) and Kulik and Yaklich (1982) concluded that several tests may be required for a complete vigor evaluation.

There are approximately seven different types of vigor tests with each type having many variations on how it may be performed. The most frequently performed test is the Cold Test (Grabe 1976). Other tests include: Accelerated Aging, Conductivity, Growth

Rate, Speed of Germination, Tetrazolium, and Cool Temperature Germination test. Elias and Copeland (1997) studied the different types of vigor tests and concluded that the tests and procedures in Table 1-2, were best correlated to field emergence for canola.

Although the concept and practice of vigor testing has been known for many years, the scientific application of it for canola is still in its infancy. At present, there are no standardized vigor testing methods for canola. This is creating difficulties in the current practice of vigor testing. The experiment completed by Elias and Copeland (1997) was one of the initial attempts to apply standardized testing for canola producing data applicable to field emergence. A major problem with current testing methods is the

**Table 1-2** Commonly practiced vigor tests

| | |
|---|---|
| Accelerated Aging Test | Aging the seeds for 48 hours at 42 °C followed by germination for five days at 25 °C. |
| Conductivity Test | Soaking 200 uninjured, untreated seeds for 16 hours at 25 °C in 50 ml of distilled water. Measure the conductivity of seed leachates using a conductivity bridge apparatus. |
| Cold Test | Pre-chill the seeds for five days at 5 °C then transfer to 25 °C for five days. |
| Cold Soil Test | Place the seeds on moistened blotter paper and cover them with 350 $cm^3$ of soil followed by cold treatment for five days at 5 °C before germinating at 25 °C for five days. |

Modified from Elias and Copeland 1997

high degree of variability between tests and repetitions within the tests. If this variability can be reduced, then differentiation of vigor levels between seed lots can also be reduced (Elliot, personal communication).

Many factors affect seedling vigor: genetics, environment, seed-borne pathogens, seed size and weight, green seed count, and storage duration. With all these variables

affecting vigor, the development of standardized testing methods that are useful to a producer in predicting field emergence/vigor will prove difficult. There is obvious need for advancement in this area, and until vigor is better understood these problems will persist into the future.

## 1.4 Interspecific Hybridization (wide crosses)

The definition of a wide cross is simple, "a cross between different species or genera" but the actual practice, is not easily accomplished (Fehr and Hadley 1980). There are four main reasons for making a wide cross: (i) to transfer one or a few genes from one species to another, (ii) to obtain new character expression not found in either parent, (iii) to produce new alloploid species, and (iv) to determine the relationship of one species to another (Briggs and Knowles, 1967).

A popular reason for attempting a wide cross is to produce a new cultivated species with desirable traits from two or more existing species. Some prevalent successes of interspecific crosses are the formation of triticale (*Triticum* x *Secale*) developed from the hybridization of wheat (*Triticum durum*) and rye (*Secale cereale*) (Popov and Tsvetkov 1970) and the re-synthesis of *B. napus* through crosses involving *B. oleracea* and *B. rapa* (Harberd 1969).

According to Hadley and Openshaw (1980), one of the most important steps in the process of producing a successful cultivar from an interspecific cross is the choice of parental material. The choice of parents determines the level of difficulty encountered in the elimination of undesirable genes from the new cultivar. To minimize difficulty, the first parental choice will be elite parents and/or existing cultivars with the desirable

characteristics. If the desired genes can not be found within elite parents, then a genotype with the least undesirable characteristics should be chosen.

### 1.4.1 Achieving and Maintaining Viable Seeds

A significant barrier often encountered in the production of interspecific hybrids is the inability to obtain viable seeds or vegetative propagules in the $F_1$, and maintaining plant and/or seed vigor during later generations. To minimize these problems, plant species must be chosen that are as closely related as possible, while maintaining the desired characteristics needed for crossing (Fehr and Hadley 1980).

One major problem occurring with wide crosses is the failure of fertilization which prevents the formation of a zygote. This disharmony between the reproductive tissues can arise for a number of reasons: failure of pollen germination, insufficient pollen tube growth to traverse the style, or the inability of the male gamete to fuse with the egg. Many techniques have been developed to overcome this barrier: (i) reciprocal crosses, (ii) ploidy level modifications, (iii) pollen mixtures, (iv) pistil modification, (v) pistil or pollen chemical treatment, (vi) protoplast fusion, and (vii) large scale mating. Even when fertilization does occur, the lack of hybrid virility can be a second barrier. Following a successful fertilization, abnormal embryo or endosperm development may result in inviable $F_1$ seed. A number of reasons for inadequate post-fertilization development have been suggested: (a) incompatibility between the genomic relationships of the two parental species, (b) an incompatibly between the cytoplasm and nuclear genes, and (c) incompatibility between the embryo, endosperm and maternal tissue

(Hadley and Openshaw 1980). Various techniques have been developed to overcome these barriers.

The most common post-fertilization barrier is the abortion of the hybrid embryo. These aborted embryos sometimes can be recovered through a technique termed "embryo rescue". This is a technique that involves the dissection of the embryo from the developing seed, transfer to a nutrient medium, then transfer to a soil-based medium where it is grown to maturity. This technique has been used successfully in difficult crosses such as *S. alba* x *B. napus*. This approach evaluated the feasibility of genetic exchange between the two species (Ripley and Arnison 1990), and *B. rapa* x *B. oleracea* for resynthesis of *B. napus* (Harberd 1969). A similar technique is used in vivo/vitro embryo culture. This method is basically a modification of the embryo rescue technique. In vivo/vitro embryo culture is used if embryo abortion occurs at a very young stage of development. In this procedure, nurse tissue derived from the endosperm of the female parents is placed with the hybrid embryos on the growth medium. The immature endosperm provides crucial nutrients to the developing embryo and thus promotes the germination of the embryo on the medium. Kruse (1974) demonstrated that this technique increased the success rate of the cross between *Hordeum* x *Secale* from 1 % (obtained with the use of traditional embryo rescue), to 30 - 40 % through the use of nurse tissue. Starzycki and Krzymanski (1988) observed the same success when they used in vitro culturing to obtain better yields of hybrid plants over their traditional hand pollination techniques.

Another approach, ovary culture, is used when embryo abortion occurs in the very early stages of zygote development. If embryos abort in the very early stages, attempting

to excise them before that point can be very difficult. To overcome this problem the ovaries are cultured before abortion occurs. The ovaries are excised shortly after pollination and many of the flowering parts are removed. The pedicel is removed with the ovary and inserted with the cut end into the media. After the embryos become visible they are removed and cultured using embryo rescue techniques. This has proven to be a successful technique and many ovary cultures have produced viable interspecific hybrids in *Brassica* (Inomata 1978, Mohapatra and Bajaj 1987, Delourme *et al.* 1989, Takahata 1990). Ovule culture is also effective in overcoming post-fertilization barriers that effect the embryo in the earlier developmental stages. Ovule culture can be used in species where ovaries are large enough to facilitate excising the ovules without damage. After the fertilized ovules are excised they are placed on a nutrient medium to further develop.

A guideline used to minimize fertilization barriers when attempting a wide cross is to perform a reciprocal cross. Normally when crossing species with differing chromosome numbers, the species with the larger number of chromosomes is chosen as the female within the cross (Hadley and Openshaw 1980). Although, most of these crosses are successful, there are exceptions. Ripley and Arnison (1990) crossed *S. alba* X *B. napus*, and found that they only obtained hybrids when *S. alba* was used as the female parent. This was unexpected because *S. alba* has a lower chromosome number (n =12) than does *B. napus* (n =19) and traditionally *Brassica* interspecific hybrids are more successful when the female has the higher chromosome number (Quazi 1988, Mohapatra and Bajaj 1987). As a result, reciprocal crosses are generally completed with interspecific matings that have not been previously attempted or were unsuccessful.

If viable $F_1$ seed is obtained, there is no guarantee that this $F_1$ seed will generate plants that will produce viable seed when grown. $F_1$ sterility can frequently be attributed to genetic or chromosomal differences between the parents (Khush and Brar 1992). Amphiploids usually are produced by colchicine treatment. This chromosome doubling can overcome sterility problems. This approach addresses whether sterility is caused by genetic or chromosomal differences. If sterility is caused by genetic differences then the alloploid will most likely be sterile as well, but if it was caused by chromosomal differences then the alloploid will be at least partially fertile and of some use (Hadley and Openshaw 1980).

The final major barrier is the inadequate growth and fertility of the hybrid progeny, referred to as "hybrid breakdown". Hybrid breakdown occurs when the interspecific $F_1$ hybrid is vigorous and fertile but the $F_2$ progeny are weak and sterile. There are two hypothesis for this phenomenon (Hadley and Openshaw 1980): (a) one species contains a genotype such as AABB while the other species genotype is aabb. When crossed, the genotypes will produce a fertile and vigorous hybrid, while the progeny of the $F_1$ segregates would be phenotypically unfavorable, and (b) small structural differences (ex: minor inversions, transpositions) between the chromosomes that do not affect pairing of the $F_1$ hybrid, but for the $F_2$ progenies these differences cause sterility or lethality because of the increasing homozygosity.

Wild and distant species are a useful resource for genetic variability for disease and pest resistance as well as many important economic traits (e.g. yield, oil quality, drought and freezing tolerance). There are many pre- and post-fertilization barriers connected with interspecific crosses, and many techniques are used to overcome these

barriers. The techniques mentioned above are the most popular ones used in crosses involving Brassicaceae.

## 1.5 Brassicaceae Genomic Relationships

An aspect that can not be overlooked in the production of an interspecific hybrid, is the understanding of the genomic relationships among *Brassica* species. Brassica oilseeds are comprised of four species: *B. rapa, B. napus, B juncea,* and *B. carinata.* These four species provide approximately 12 % of the worlds edible vegetable oil (Labana and Gupta, 1991). *B. oleracea* is grown mostly as a horticultural crop, while *B. nigra* is used in the condiment industry. The first determination of chromosome numbers for these Brassica species was completed in the early 1900's. Morainaga (1934) concluded from his cytogenetic investigations that the cultivated species of Brassica could be classified into six groups. He hypothesized that three of the groups (*B. napus, B. juncea,* and *B. carinata*) were amphidiplods derived from crosses between wild forms of *B. rapa, B. oleracea,* and *B. nigra.* He assigned these groups an **A, B, C,** genome or a combination of the two genomes. *B. rapa* was assigned the **A** genome, *B. nigra* the **B** genome, and *B. oleracea* given the **C** genome. Based on his amphidiploid theory *B. juncea* would then have an **AB** genome, *B. napus* an **AC** genome, and *B. carinata* a **BC** genome. This theory was confirmed by U (1935), who completed a series of crosses between the diploid genome (A, B, C) to artificially produce the tetraploid hybrids (AB, AC, BC). From this he formed a diagrammatic representation of the interrelatedness among these species (Figure 1.2).

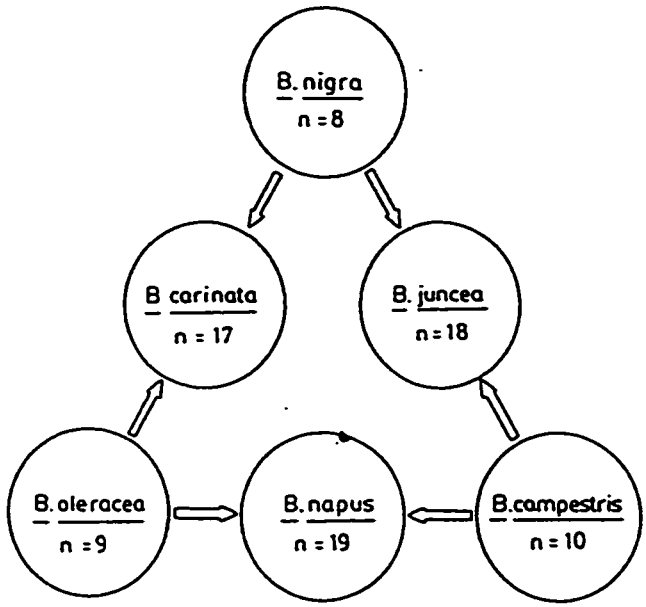


**Figure 1-2** Diagrammatic representation of the genomic relations between the species of *Brassica* with "n" indicating the haploid chromosome number and genome designations assigned by Morinaga genome (after U 1935).

Through chromosome analysis, Robbelen (1960) hypothesized that the original progenitor species that gave rise to the monogenomic species (*B. rapa*, *B. oleracea*, *B. nigra*) had a base chromosome number of six. He found that in monogenomic species, each had only six distinctly different chromosomes, with the remainder homologous to one of the six, thus suggesting that the additional chromosomes of the diploid species resulted from chromosome duplication and/or elimination. Lagercrantz and Lydiate (1996) proposed a different hypothesis. They felt the use of cytology offered limited resolution of the ancestral relationship of the Brassica genomes, since Brassica's have small and morphologically similar chromosomes. Using Brassica RFLP probes, they completed a comparative analysis of the A, B, and C genomes that revealed a conservation of genome content. This, coupled with the fact that *B. rapa*, *B. oleracea*, and *B. nigra* all had similar nuclear DNA content (Arumuganathan and Earle 1991), lead Lagercrantz and Lydiate to believe that the original progenitor species had a chromosome

number of n = 3 instead of n = 6 as suggested by Robbelen (1960). Through comparative mapping they proposed the different chromosome numbers (8, 9, and 10) among monogenomic species were the result of chromosome fission/fusion that had occurred during the divergence of the three genomes, rather than duplication and/or elimination.

## 1.6 Doubled Haploid Breeding

Generating double haploids is increasingly important as a tool in Brassica breeding programs. With this technique, the development of completely homozygous genotypes from heterozygous parents can be completed within a single generation (Stringam *et al.* 1994). This rapid "fixing" of recombinant gametes into fertile homozygous lines is exclusive to this breeding procedure. Haploid breeding can expedite the release of a new cultivar by three to four years (Stringam 1992). Stringam *et al.* (1994) used this technique to cross a source of blackleg resistance from the Australian cultivar Maluka, into their susceptible advanced *B. napus* lines from the University of Alberta. This approach was effective in developing resistant breeding lines within four years from the initial cross. The blackleg resistant cultivar, Quantum, developed by doubled haploidy, was subsequently released within 6 years of the initial cross.

Double haploid (DH) lines have been useful for determining the inheritance of important traits. One benefit is that genetic ratios are simpler because of the absence of dominance variation and within family segregation. This results in better selection efficiency, due to more distinct differences between classes that appear because of the absence of early segregating generations (Choo et al 1985). By using haploids in determining and screening for recessive traits such as yellow-seeded canola, one can

significantly reduce the number of plants that must be screened because the recessive traits are not masked (Henderson and Pauls 1992). Siebel and Pauls (1989) successfully used DH populations to study the inheritance of traits such as fatty acids and glucosinolates. Chen and Beversdorf (1990) compared two breeding populations of *B. napus* (single seed decent and DH) for seed oil fatty acid composition. They concluded that selection for altered fatty acid composition was equally efficient for both breeding systems. They inferred that progress would be greater in DH populations because the time required to obtain homozygous lines is shorter than in traditional breeding methods.

The success of the development of blackleg resistant cultivars with superior agronomics through the use of microspore culture has lead to further use of this method to develop superior lines from interspecific crosses. One of the crosses completed at the University of Alberta was with Quantum and a self-compatible line of *B. rapa*.

*B. rapa's* normal self-incompatibility, and the fact that most canola breeding programs focus on the improvement of *B. napus*, has resulted in a lag in development of superior *B. rapa* cultivars. Through the use of microspore culture following interspecific crosses, it may be possible to develop self-compatible *B. rapa* lines that can serve as a germplasm base that would also contain some of the desirable traits of *B. napus*. With this in place it should be possible to develop *B. rapa* cultivars that will surpass current yield and agronomic plateaus in this species.

## 1.7 Objectives

This project was designed to study agronomic and quality trait transfer between *B. napus* and *B. rapa* in DH lines produced from an interspecific cross of *B. napus* x *B.*

*rapa*. Specifically, the following traits to be studied were: oil, protein, glucosinolate content, fatty acid profile, seed yield, maturity, seedling vigor, lodging resistance, plant height, days to flower, and duration of flowering. These traits were studied in multi-location trials over 2 years so that genotype x environment interactions among the traits could be determined.

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# Chapter 2
# Evaluation of Agronomic and Quality Traits of Three DH Lines from an Interspecific Backcross (*Brassica napus* x *Brassica rapa* x *B. rapa*) and their Ancestral Parent Cultivars

## 2.1 Introduction

*Brassica napus* and *B. rapa* canola have dominated oilseed production across the Canadian prairies since the turn of the 20th century. The superior oil profile of canola has ensured their prominence in the world oil market. Erucic acid content less than two percent (oil-based) and glucosinolates under 30 μmoles (per gram air-dried oil-free meal) qualifies the oil as safe for human consumption, and allows the meal to be utilized as a high protein animal feed supplement (Thomas 1985).

The low percentage of saturated fats within the fatty acid profile of canola makes it desirable to consumers. Although, *B. napus* and *B. rapa* have a slightly different fatty acid profile (oleic fatty acid in *B. napus* is about 5 % higher than *B. rapa* and contains approximately 2 % more in total saturates), their blended oil is still the healthiest of all oils (Downey and Rimmer 1993).

After oil extraction, the remaining meal contains about 36 to 44 % protein. This high protein content in combination with an array of essential amino acids makes it an excellent animal feed supplement. One disadvantage of canola meal is its glucosinolate content. Glucosinolates reduce palatability and cause goiterogenic effects in non-ruminant animals (Downey and Robbelen 1989). Currently, most breeding programs are attempting to reduce glucosinolate levels so that a higher proportion of canola meal can be used in swine and poultry diets (Bell 1995).

The close similarities of the oil and meal quality between *B. napus* and *B. rapa* permits blending of the two species, but their agronomic differences make them suitable for sowing in different environments. As illustrated in Table 1.1 both species have characteristics useful to growers; *B. napus* on average yields greater than *B. rapa*, while *B. rapa* can mature up to three weeks earlier than *B. napus* thus escaping late summer droughts and early fall frosts. A suitable approach to improving the performance of these two species would be to integrate these desirable traits into a single cultivar.

Interspecific crosses have already been used to transfer traits between *Brassica* species. Blackleg resistance was transferred into *B. napus* from *B. juncea* through interspecific crosses (Roy, 1984). Love *et al.* (1990) produced a low glucosinolate *B. juncea* through an interspecific cross between *B. juncea* and *B. rapa*.

This project was developed to study trait transfer between *B. napus* and *B. rapa* in lines produced by Kubik (1999), with the objective to determine their suitability as breeding lines. It is imperative that these lines be further tested at a number of sites where they can be evaluated for superior agronomic and/or quality traits.

## 2.2 Materials and Methods

### 2.2.1 Crossing Scheme

Initial germplasm development for this project was completed at the University of Alberta, under direction of Dr. Gary Stringam. Doubled haploid embryo cultures were completed by Kubik (1999) (Figure 2.1). In Kubik's (1999) study, the DH line 94-98 was isolated from a cross between the cv Quantum (*B. napus*) and a semi-dwarf (*B. napus*) early maturing line. Line 94-98 was then crossed to a self-fertile $F_7$ *B. rapa* line,

derived from a cross between Eclipse and an University of Alberta *B. napus* breeding line 83-52692B. Line 83-52692B was an $F_6$ dwarf line developed by Dr. Brian Fowler (Crop Development Center, University of Saskatchewan) from a cross between the cv Tower and a dwarf *B. napus* line. Numerous doubled haploids with a range of fertility from completely sterile to highly fertile were obtained from the $F_1$ microspore culture. The DH line 95-376 was selected from the culture and crossed to sister lines of the self-fertile $F_7$ *B. rapa* line. This $F_7$ line (93-50025) was used in the modified backcross scheme. Buds from the progeny of each backcross were collected and subjected to microspore culture.

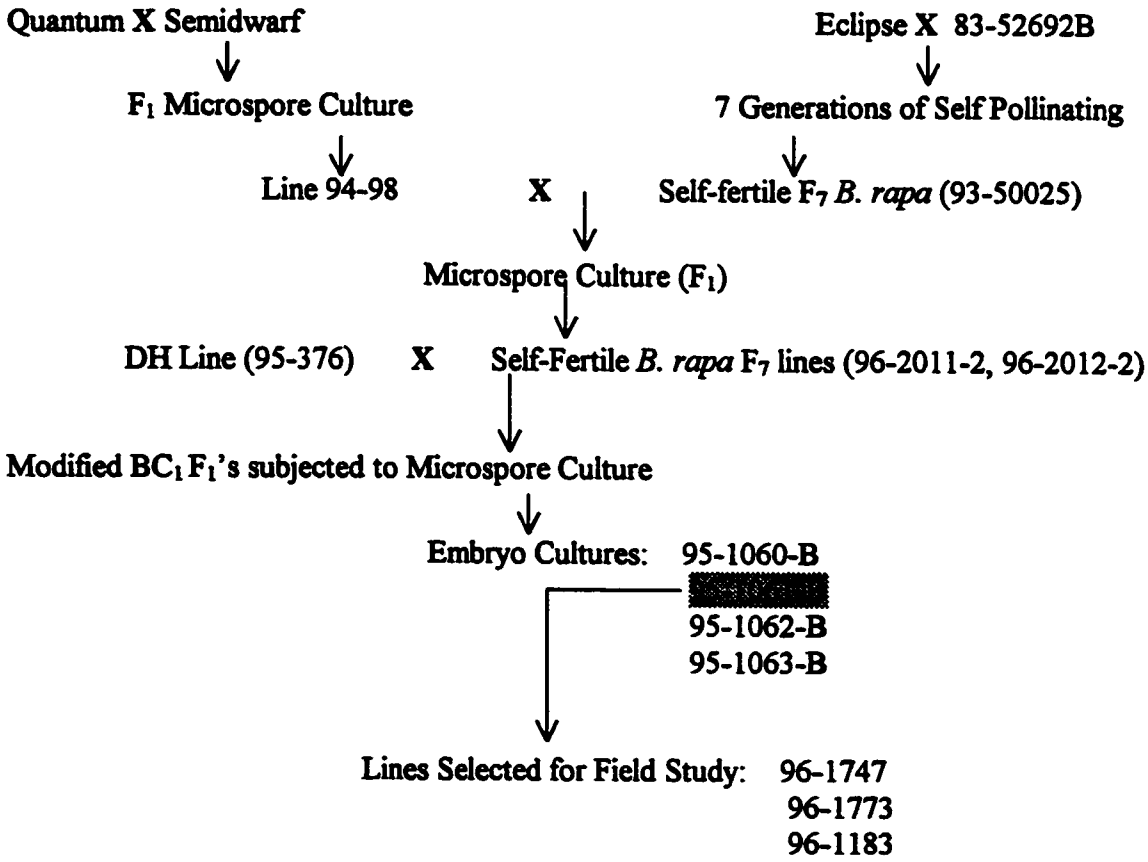


**Figure 2-1** Interspecific crossing scheme.

Three lines from the 95-1061-B embryo culture were selected for further field study based on their preliminary performance from Kubik's (1999) study. Each line selected showed superior performance for a single yield component while maintaining near average or above average performance of all other traits. Lines 96-1747, 96-1183, and 96-1773 were selected because of there superiority over other lines in the population with respect to number of pods, seeds/pod, and seed weight.

Field evaluation of the lines was conducted over two years (1998 and 1999) at three locations, Edmonton Research Station of the University of Alberta (Michener), Ellerslie, and Kelsey. Eight spring genotypes were included in this study, three DH lines from the interspecific cross *B. napus* x *B. rapa* with a backcross to *B. rapa* (96-1183, 96-1747, 96-1773), plus parents, (96-2011-2, 96-2012-2, 93-50025, cvs. Quantum, and Eclipse). All genotypes were developed from the breeding program at the University of Alberta. All locations were sown as a randomized complete block design with three replications for both years. The plots were sown with a Fabro self-propelled pre- and after- packing press drill seeder, at a seeding rate of 6.25 grams per plot. Plots were sown six meters in length (trimmed to five meters about three weeks prior to harvest) consisting of six rows with 15 centimeters between rows. All sites were dressed with 23 lbs/acre of 12-55-0 fertilizer previous to planting, and border plots of cv. Legend were included in the design to reduce edge effects. To determine time of first flower, ten plants representative of the overall plot were selected from the two central rows. When eight plants of the ten were counted with a single flower on the main raceme, the plot was entered as being in first flower on that day. End of flowering was determined similarly.

When eight out of ten plants completed flowering (no buds remaining on the main raceme), the plot was considered to be at the end of flower on that day. For maturity determinations, ten representative plants were selected from the two central rows of each plot. Two pods were removed from the bottom of the main raceme of each of the ten plants, and when 40 percent of the seeds within each pod displayed a color change (loss of chlorophyll), the plot was considered to be fully mature on that day. All plots were harvested in bulk. The following data collection procedures were completed in the same manner for both field seasons of 1998 and 1999. Seed yield of each plot was measured through the use of a Harvest-Master™ threshing system. One-thousand-kernel weight was determined in seed dried to five percent moisture. The weight of two samples of one hundred kernels from each plot was multiplied by five to estimate one-thousand-kernel weight.

### 2.2.2 Oil Content Determination

Nuclear magnetic resonance (NMR) was used to determine oil content on a whole seed basis. This was accomplished through the use of an Oxford 4000 NMR Analyzer (Oxford Analytical Instruments Ltd.). A bulk sample of approximately 1.2 grams was analyzed from each plot, and each sample was analyzed for one minute with a single repeat measurement. All raw data for oil content were multiplied by 0.95 to correct for five percent moisture present within the seed.

### 2.2.3 Protein Content Determination

Protein content was determined on a whole seed basis, through the use of a Leco FP-2000 Analyzer with a fitted nitrogen probe. A single bulk sample of 0.5 grams from

each plot was used in the analysis and the formula used to correct the data to five percent seed moisture expressed in an oil-free value was (raw protein data/0.95) / (100 – corrected oil) x 100.

### 2.2.4 Fatty Acid Profile Establishment

A single sample from each plot was examined for its fatty acid profile using a Gas Liquid Chromatograph (HP model 5890, series 1) with auto-sampler. Fatty acid content was determined by the ISO 5508 (1990) procedure for animal and vegetable oils.

### 2.2.5 Analysis of Glucosinolate Content

Glucosinolate determination was completed on the defatted meal obtained after oil was extracted according to the triemthysilyl (TMS) derivatization of desulphated glucosinolate procedure (Raney and McGregor 1990). Analysis was accomplished by a Gas Liquid Chromatograph (HP model 5890, series 1) with auto-sampler. Total glucosinolates were reported on a whole seed basis (μmole/g).

### 2.2.6 Data Analysis

All data computations were completed through the use of SAS/STAT 7.0 (SAS Institute Inc. 1998). The data were first checked for normality and homogeneity of variances before other analyses were completed. The General Liner Model (GLM) procedure was used to calculate significance for all field and most laboratory data. Tukeys test was preformed in this procedure on all measured characteristics. Least-squares means (lsmeans) were calculated for glucosinolates and fatty acids, and cluster analysis was completed with SPSS Base 10.0 (SPSS Inc. 1999) using three types of

clustering methods (Wards, Single, and Complete linkage) under a hierarchical based clustering procedure.

## 2.3 Results and Discussion

All data were confirmed to be normally, identically (i.e. homogeneous) and independently distributed by SAS/STAT 7.0 procedures (SAS Institute Inc. 1998). None of the data needed to be transformed to conform to the assumptions of normality. All breeding lines included in this study were selected from the thirty-five backcross DH lines included in Kubik's (1999) preliminary greenhouse and field evaluations (Figure 2.1). The lines selected for the field study were analyzed to estimate the introgression of agronomic and quality traits of interest in the *B. napus* x *B. rapa* interspecific crosses.

To estimate the agronomic and quality relationships between the genotypes, cluster analysis was completed on all of the measured traits (excluding height). The distant parents, Quantum and Eclipse (Figure 2.1) were used in the analysis as a reference to *B. napus* and *B. rapa* commercial cultivar performance. Dendrograms were included in the analysis to search for diversity patterns between the three breeding lines and the parental cultivars, since introgression from either *B. napus* or *B. rapa* could be more clearly identified through this type of analysis.

Three types of clustering methods were used in this study (Wards, Single and Complete linkage) to compare the results using multiple methods, and reduce the likelihood of erroneous conclusions resulting from a reliance on a single procedure. If natural groupings existed within the genotypes studied, then different methods of classification should provide similar results. Thus, the consistency of clustering based on

the different methods of grouping, provides good evidence that natural clusters were present.

### 2.3.1 Agronomics

Seed yields were collected from all plots, for all genotypes. Significant differences in yields were found among the genotypes at the 99 percent confidence interval. The highest yields were recorded from the *B. napus* parent Quantum. Quantum consistently ranked highest over all locations and years, thus not only showing superiority in seed yield but also in cultivar stability (Table 2-1).

For seed yield, nearly identical results were obtained from all clustering methods. All three hierarchical methods assigned the eight lines into two clusters with one outlier (Figure 2.2). Quantum was consistently separated as the outlier as it did not group with any other lines in all methods. Cluster analysis revealed that the interspecific DH lines were most closely associated to the *B. rapa* parent in relation to seed yield (Figure 2.2). The absence of the interspecific DH lines clustering with the *B. napus* line Quantum, suggests that increased seed yield that is characteristic of *B. napus* was not successfully transferred to any of the DH lines.

Seed weight was measured on all plots over both years. The three breeding lines produced significantly higher seed weights (96-1773-1 being the highest) than the other genotypes within the study (Table 2-2). Quantum was the closest ancestral parent for this trait, but was still significantly lighter than any of the breeding lines. Although the selected breeding lines displayed superior seed weights over all other lines tested, an

outstanding increase in this single yield component was not effective in producing a higher yielding cultivar (Table 2-1, 2-2).

In relation to seed yield, the remaining two components were not recorded in the present study, but were measured in Kubiks (1999) study. These were, number of pods per plant (from main raceme), and number of seeds per pod. Of the 35 lines evaluated in his study, 96-1747-2 and 96-1183-1 displayed a higher number of pods/plant, and seeds/pod respectfully, and thus were selected for further field evaluation in this study. Although all of the breeding lines selected excelled in individual yield components, none of the progeny displayed outstanding yield performances, suggesting that successful selection for yield improvement is not simply selecting for the sum of yield components. Chay and Thurling (1989a, 1989b) examined the possibility of yield enhancement of *B. napus* through the selection of increased pod length. They concluded that yield improvement could be achieved through introgression of long pod genes, provided this process was accompanied with an improvement of other yield components.

Early Chinese breeders utilized *B. napus* x *B. rapa* interspecific crosses in an effort to reduce the maturation period in *B. napus* (U 1935). It was of interest in this study to determine if a reduction was transferred into the breeding lines from these interspecific crosses. In the present study, a *B. napus* maturation pattern was displayed by the three interspecific DH lines. Average days to maturity for these lines was 102.5 with a standard deviation of 0.32, closely resembling Quantum (103.7 days). Under all three clustering methods two distinct clusters were formed, grouping the breeding lines with Quantum, and Eclipse with the *B. rapa* types (Figure 2.3). The data showed that a *B. napus* type of maturity pattern was likely inherited by all three breeding lines.

Days to flower and flowering duration were also recorded in this study. The breeding lines displayed an intermediate flowering pattern between *B. rapa* and *B. napus* (i.e. first flower started after Eclipse and ended before Quantum). The lines more closely resembled the *B. napus* parent, with the mean number of days to flowering for the breeding lines at 49.4 (standard deviation 1.82). The distant *B. napus* parent Quantum, averaged 53.3 days and the *B. rapa* parent averaged 44.6 days to first flower; thus relative to these cultivars the breeding lines only slightly favored a *B. napus* flowering pattern (Table 2-3). Although it appears that the DH lines showed significant improvement in the number of days required to reach first flower, the anticipated improvement in maturity was not observed (Tables 2-3 and 2-4). For flowering duration, cluster analysis failed to separate the genotypes into distinct groupings as the clustering results were inconsistent.

To increase straw strength in the interspecific crosses, a semi-dwarf *B. napus* line was crossed with Quantum (Figure 2-1). Height was previously recorded for these genotypes in an earlier study (Kubik 1999) to detemine if the shorter stature was maintained in the interspecific population, but because of low plant numbers in the plots, the data were inconclusive. Further investigation from this study revealed that the heights of the three DH lines were closely associated to the *B. rapa* parent (Table 2-5).

Lodging data were not collected in the first year of this study. As a result any conclusions would have been based solely on the second year data, likely resulting in erroneous conclusions. Collection of these data would have complemented the height data in determining the success in the transfer of stiff straw strength in this interspecific cross.

Another trait that could not be measured in the first field season was blackleg (*Leptosphaeria maculans*) resistance, and could not be included in the data for interpretation. Pathogen infection throughout the field test plots was too low for adequate scoring. The lack of poor infection throughout the plots may have been due to low precipitation coinciding with the disease sporulating cycle, or simply from a lack of the infectious propagules at the site.

### 2.3.2 Quality

Canola oil is primarily used for edible purposes and is the most valuable commercial component of the seed; consequently, breeding for cultivars with high oil contents is an important objective. However, an increase in the seed oil content must not occur at the expense of yield. Generally, an effective method for increasing oil content is to develop cultivars possessing yellow seeded hulls. Yellow-seeded varieties normally produce about a two percent increase in oil content over black-hulled varieties (on a whole seed basis) (Stringam *et al.* 1974). A thinner seed coat is characteristic of yellow seeded varieties, resulting in a larger portion of the seed containing an oil rich embryo (Stringam *et al.* 1974). Results from this study support the conclusions of Stringam *et al.* (1974). Relative to oil, all four yellow-seeded genotypes exceeded the black-seeded genotypes (Table 2-6). The mean total oil content for the yellow-hulled genotypes was 47.1 percent while the mean oil content for the black hulled genotypes was significantly lower at 42.6 percent. The three breeding lines were significantly lower than all other genotypes in the study (Table 2-6). In general, total oil contents were found to be higher. These results support earlier conclusions (Kubik 1999) that in the breeding population,

total oil content appears to be more variable and have an overall lower trend than in the parental genotypes.

For all lines, the complete fatty acid profile was determined. In the fatty acid profile, C18's, C22's and total saturates were the only fatty acids observed to display significant differences from typical *B. napus* or *B. rapa* profiles. The fatty acid content of canola is comprised mostly of oleic (18:1), linoleic (18:2), and linolenic (18:3) acids. Quantum and Eclipse displayed typical *B. napus* and *B. rapa* profiles, for oleic, linoleic and linolenic (Table 2-7) (Downey 1983). The three breeding lines exhibited linolenic fatty acid levels characteristic of *B. rapa* profiles, but differed from *B. rapa* by possessing higher saturate levels commonly associated with *B. napus*, and thus not fitting either profile (Table 2-7). Since two important breeding objectives for canola are, (1) to reduce the total amount of saturated fatty acids, and (2) to reduce the level of linolenic acid (Ahuja *et al.* 1993), this interspecific cross did not appear to be effective in reaching either of these goals.

Feeding experiments with animals involving erucic acid (C22:1) have demonstrated many deleterious effects as being the cause of a plethora of health problems. Health problems such as depressed growth, digestibility, longevity, interference with myocardial conductance, cardiac lipidosis and necrosis, increased blood cholesterol and coagulation all have been documented in association with erucic acid (Renaud and McGregor 1976; Sim 1986; Laryea *et al.* 1992; Ahuja *et al.* 1993). Because of these findings, the amount of erucic acid allowed in *B. napus* and *B. rapa* oil used for human consumption is strictly regulated. One requirement is that it must contain less than two percent erucic acid (C22:1). Results of this study indicated an acceptable level

of erucic acid in the tested genotypes. All genotypes were significantly less than the two percent threshold required for canola standards (Table 2-7).

A second requirement necessary for canola quality is that they possess no more than 30 μmoles of glucosinolate per gram of air-dried oil-free meal. The mean glucosinolate content for the breeding lines was 9.4 μmoles/gram which was lower than expected. Mean glucosinolate contents found from the same population in a previous study (Kubik 1999) was 15.5 μmoles/gram. These concentrations were lower than the typical concentrations of 17 and 21μmoles/gram for *B. napus* and *B. rapa* respectively (Uppström 1995). These lower concentrations of glucosinolates may have been caused by environmental conditions. High/low amounts of precipitation, low temperatures, frosts and reduced radiation have all been shown to affect glucosinolate levels (Sang et al. 1986, Ishii and Saijo 1987, Rosa 1992). None of these variables were measured in this study, thus making conclusions on their effects influencing the glucosinolate levels speculative.

Adequate protein levels were maintained in this cross for two of the three DH interspecific lines. The mean protein level for genotypes 96-1773, and 96-1747 was 48.6% which is comparable to the 48.5% average recorded for the cultivar Eclipse. The DH line 96-1183 was significantly lower than the other lines in the study by approximately two percent. One technique to improving protein quality is to reduce the crude fibre content of the seed. This can be achieved through the development of yellow-seeded varieties which possess thinner seed coats. Since much of the seed crude fiber is located in the seed coat, a thinner hull results in less crude fiber content of the seed overall (Appelqvist 1972). Since none of the breeding lines expressed a yellow seedcoat

it is a reasonable hypothesis that a lower crude fiber content was not captured from this interspecific cross. This hypothesis is coupled by the assumption that none of the black-hulled species used in this study possessed an uncharacteristically thin seed coat, thus suggesting crude fiber content would be consistent with regular black-hulled varieties.

The potential of these specific DH lines for further use for the purposes of breeding stock or varietal release do not seem to suit what is needed in a breeding program. Outstanding agronomic/quality traits were not observed for these DH lines, with the exception of seed weight. The DH line 96-1773 was significantly heavier than all other genotypes in the study (Table 2-2). Although this did not result in increased yields, the transfer of this trait into other breeding lines may still be of interest since it is a component of yield.

To determine the usefulness of this cross for the production of lines possessing favorable agronomic and/or quality characteristics, further studies would have to be conducted.

Table 2-1 Tukey Grouping for yield (kgs) for three DH lines (**bold**) (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

| Mean | Genotype | Tukey Grouping |
|---|---|---|
| 32.39 | Quantum | A |
| **26.24** | **96-1773** | **B** |
| | | **B** |
| **24.5** | **96-1747** | **B** |
| | | **B** |
| **24.3** | **96-1183** | **B** |
| | | B C |
| 23.01 | 93-50025 | C |
| | | D C |
| 20.81 | 96-2011 | D C |
| | | D C |
| 20.21 | Eclipse | D |
| | | D |
| 18.74 | 96-2012 | D |

Table 2-2 Tukey Grouping for kernel weight (g) for three DH lines (**bold**) (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

| Mean | Genotype | Tukey Grouping |
|---|---|---|
| **4.47** | **96-1773** | **A** |
| **4.21** | **96-1183** | **B** |
| | | **B** |
| **4.16** | **96-1747** | **B** |
| 3.66 | Quantum | C |
| 3.19 | 96-2011 | D |
| | | D |
| 3.14 | 93-50025 | D |
| | | D |
| 3.06 | 96-2012 | D E |
| | | E |
| 2.94 | Eclipse | E |

Table 2-3 Tukey Grouping for days to first flower for three DH lines (**bold**) (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

| Mean | Genotype | Tukey Grouping |
|---|---|---|
| 53.3 | Quantum | A |
| **50.3** | **96-1183** | **B** |
| | | **B** |
| **50.3** | **96-1747** | **B** |
| **47.2** | **96-1773** | **C** |
| | | C |
| 46.8 | Eclipse | C |
| 45.2 | 96-2012 | D |
| | | D |
| 44.7 | 96-2011 | D |
| | | D |
| 44.6 | 93-50025 | D |

Table 2-4 Tukey Grouping for days to maturity for three DH lines (**bold**) (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

| Mean | Genotype | Tukey Grouping |
|---|---|---|
| 103.7 | Quantum | A |
| **102.6** | **96-1747** | **B** |
| | | **B** |
| **102.4** | **96-1183** | **B** |
| | | **B** |
| **102.4** | **96-1773** | **B** |
| 93.4 | 96-2011 | C |
| 92.6 | Eclipse | D |
| | | D |
| 92.5 | 96-2012 | D |
| | | D |
| 92.3 | 93-50025 | D |

Table 2-5 Tukey Grouping for height in the ancestral parents and DH lines at three locations over two years.

| Mean | Genotype | Tukey Grouping |
|---|---|---|
| 104.6 | Quantum | A |
| **97.5** | **96-1747** | **B** |
| | | **B** |
| 95.5 | 96-2011 | B |
| | | B |
| 95.2 | Eclipse | B |
| | | B |
| 94.4 | 93-50025 | B |
| | | B |
| 93.7 | 96-2012 | B C |
| | | B C |
| **93.2** | **96-1183** | **B C** |
| | | C |
| **88.3** | **96-1773** | C |

Table 2-6 Tukey Grouping for oil (%) and seed color for three DH lines (**bold**) (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

| Mean | Genotype | Tukey Grouping | Seed Color |
|---|---|---|---|
| 47.79 | 93-50025 | A | Yellow |
| | | A | |
| 47.0 | Eclipse | A B | Yellow |
| | | B | |
| 46.85 | 96-2012 | B | Yellow |
| | | B | |
| 46.67 | 96-2011 | B | Yellow |
| | | B | |
| 46.4 | Quantum | B | Black |
| **42.71** | **96-1747** | **C** | **Black** |
| | | C | |
| **41.96** | **96-1773** | **C** | **Black** |
| **39.26** | **96-1183** | **D** | **Black** |

Table 2-7 Ls means for the dominant fatty acids (% Total Profile) and total glucosinolate content of air dried oil free meal (μmole/gram) for three DH lines (**bold**) (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

| Genotype | Saturates | Oleic C18:1 | Linoleic C18:2 | Linolenic C18:3 | Erucic C22:1 | T. Gluc |
|---|---|---|---|---|---|---|
| 1) Quantum (*B. napus*) | 6.59 | 59.91 (60) | 19.86 (20) | 11.39 (11) | 0.17 | 13.0 |
| 2) Eclipse (*B. rapa*) | 5.81 | 57.36 (55) | 21.95 (25) | 12.92 (13) | 0.12 | 9.8 |
| 3) 96-50025 | 5.90 | 59.19 | 20.5 | 12.33 | 0.17 | 10.5 |
| 4) 96-2011 | 5.75 | 58.64 | 21.03 | 12.4 | 0.1 | 11.1 |
| 5) 96-2012 | 5.82 | 58.13 | 22.12 | 12.61 | 0.04 | 10.8 |
| **6) 96-1747** | **6.70** | **58.27** | **20.0** | **13.05** | **0.1** | **10.9** |
| **7) 96-1773** | **6.68** | **56.89** | **21.36** | **13.14** | **0.1** | **9.8** |
| **8) 96-1183** | **6.77** | **56.92** | **21.04** | **13.32** | **0.09** | **7.4** |

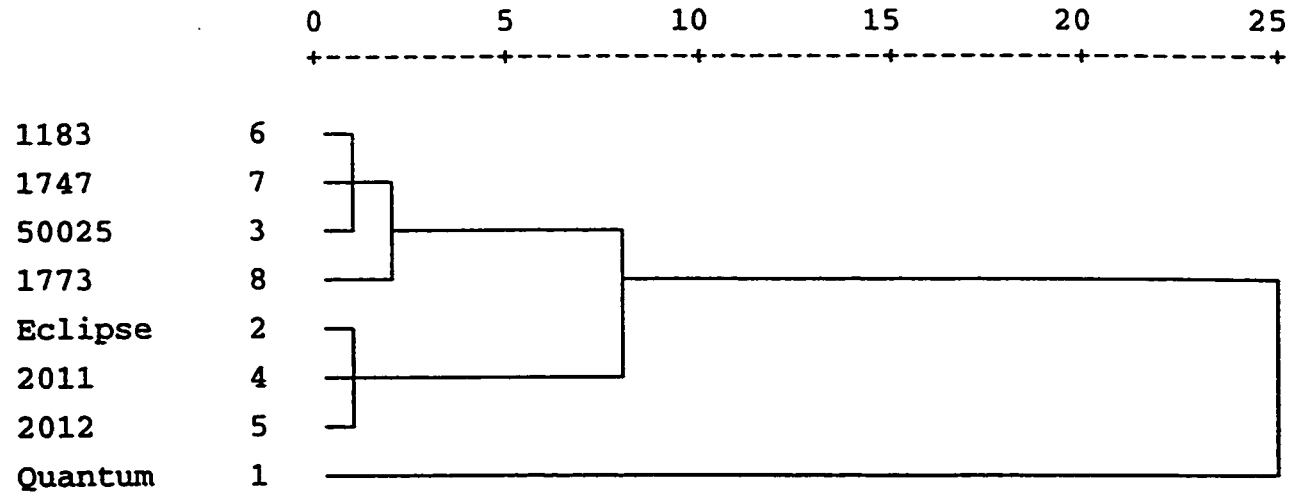


Figure 2.2 Dendrogram of yield (kg) using Ward Method for three DH lines (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

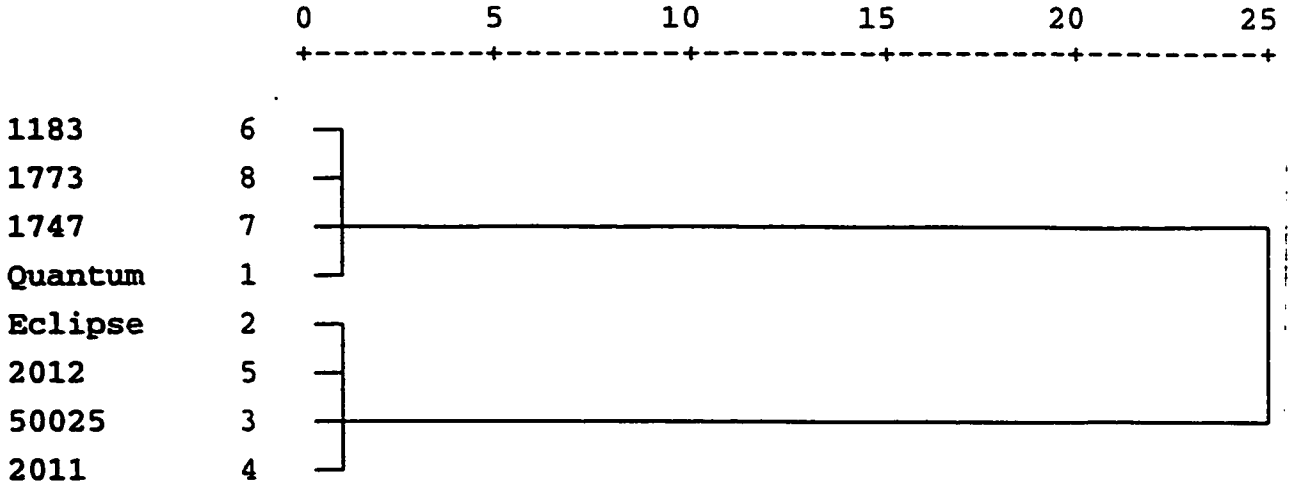


Figure 2.3 Dendrogram for days to maturity using Ward Method for three DH lines (*B. napus* x *B. rapa*) and their ancestral parents at three locations over two years.

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# Chapter 3
# Agronomic and Quality Trait Interactions (Genotype x Environment) of Doubled Haploid Progeny from an Interspecific Cross between *B. napus* and *B. rapa*.

## 3.1 Introduction

Selection of a canola cultivar with high seed yield accompanied by high oil and protein content have always been major breeding objectives for canola. Separating genotypic from environmental effects for these traits has posed an enduring dilemma for plant breeders. Isolating genetic influences apart from environmental influences that inevitably occur within field trials is crucial, so that the true genetic gains of the selected lines may be predicted. Environmental influences such as different years, locations, climate, pathogens, and fertility, are inevitably associated with the genetic influences in trait expression. The presence of genotype x environment interactions unfortunately causes changes in the interrelationships of these traits. In part, the objective of this study was to investigate the effects of environmental influences (years and locations) on yield, oil, protein, maturity, flowering duration, and seed weight, as well as the interrelationships between these traits in the DH progeny of the interspecific cross *B. napus* x *B. rapa*, (backcrossed to *B. rapa*) evaluated in the central Alberta canola region.

## 3.2 Materials and Methods

Field evaluation of plant material was conducted over two years (1998 and 1999) at three locations, Edmonton Research Station of the University of Alberta (Michener), Ellerslie, and Kelsey. Eight spring genotypes were used for this study, three DH lines

derived from an interspecific cross, 96-1183, 96-1747, 96-1773, and the parents, 94-50025, 96-2011, 96-2012 and cvs. Quantum, and Eclipse. All genotypes were developed from the canola breeding program at the University of Alberta. All locations were sown as a randomized complete block design with three replications for both years. The plots were sown with a Fabro self-propelled pre- and after packing press drill seeder, at a seeding rate of 6.25 grams per plot. Plots were sown six meters in length (trimmed to five meters about three weeks prior to harvest) consisting of six rows with 15 cm between rows. All sites were dressed with 23 lbs/acre of 12-55-0 fertilizer prior to planting, and border plots of cv. Legend were included in the design to reduce edge effects. To determine time of first flower, ten plants representative of the overall plot were selected from the two central rows of each plot. When eight plants of the ten with a single flower on the main raceme were counted, the plot was entered as being in first flower on that day. End of flowering was determined similarly. When eight of the ten plants were devoid of buds on the main raceme, the plot was considered to be at the end of flowering on that day. For maturity, ten representative plants were selected from the two central rows of the plots. Two pods were removed from the bottom of the main raceme of each of the ten plants. When 40 percent of the seeds within each pod displayed a color change (loss of chlorophyll) the plot was considered to be fully matured. All plots were harvested in bulk. Yield, 1000-kernel weight, oil, and protein contents of each cultivar were determined from a bulk of the harvested seed, using the methods described in section 2.2.

Statistical computations were completed through the use of SAS/STAT 7.0 (SAS Institute Inc. 1999). The data for this section were analyzed by general linear models

(GLM). Variance and covariance components of each trait were estimated by the MANOVA procedure in SAS/STAT 7.0. Phenotypic correlations of traits were calculated using the formula $r_{ij} = \sigma_{ij}/(\sigma_i\sigma_j)^{0.5}$ where $\sigma_{ij}$ is the covariance of traits i and j, and $\sigma_i$ and $\sigma_j$ are the standard deviations of i and j, respectively (Baker 1986). Computations for phenotypic correlations were performed on the complete data set.

## 3.3 Results and Discussion

### 3.3.1 Genotype x environment interactions

Environmental interactions commonly confound the performance of a genotype. In this study, significant environmental interactions were observed for all traits (Table 3-1). Despite these interactions some traits displayed consistent outstanding results. For instance, 1000-kernel weight was highest for line 96-1773 across both years (Figure 3-1) and at all locations (Figure 3-2). Similarly for yield, Quantum was the highest in all environments over both years. These results suggest that outstanding traits, both qualitative (i.e.: disease resistance) and quantitative (i.e.: yield) can be identified in as few as two years. The data further suggest that superior traits from these lines evaluated, would maintain their superiority if tested in additional years.

Significant environmental interactions between years were observed among all measured traits (Table 3-1 and Figure 3-1). In the first year certain traits (yield, flowering duration) had minimal variability, and all traits showed lower mean values relative to the second year (with the exception of first flower, which remained relatively constant). This phenomenon may have been due to the below average rainfall in the first

year of the study (Environment Canada, communication). Although there were substantial differences between mean values in the two years, the increase and decrease in each value was consistent among all genotypes. The large differences of the mean values of the traits between the two years may have resulted from significant year interactions (Table 3-1). F-values for year interactions were larger than F-values for year x genotype interactions indicating differences in trait expression between years were due to environmental changes. Therefore, these results indicate that all traits were strongly influenced by environmental conditions over both years, demonstrating that an evaluation of these traits only within a single field season may result in an inaccurate estimation of trait performance.

The relationship among locations was as complicated as years. The location of each trial significantly influenced these traits, and displayed a much larger effect on the traits than did genotype x location interactions (Table 3-1). In this situation notable differences in the interactions among individual traits were observed. For example, seed yield and flowering duration were considerably different between locations (Figure 3-3 and 3-4). This result suggests that a specific genotype may be better in one location than another. To confirm these results, an independent testing and selection program would be needed. Costs associated with the establishment of independent programs for different geographical areas can be substantial, therefore generally, the benefits gained from developing a cultivar for a specific environment may be uneconomical as well as impractical.

With respect to days to maturity all three DH lines from the interspecific cross averaged 102.5 days, closely resembling Quantum's (*B. napus*) pattern of 103.7 days.

Eclipse's (*B. rapa*) maturity was significantly shorter than that of the interspecific DH lines averaging 92.6 days (Table 3-4). Although a high degree of environmental interaction can be seen with this trait (Table 3-1), the large differences in maturity displayed between *B. napus* and *B. rapa* along with the DH lines so closely resembling a *B. napus* pattern, have reduced the chance of misinterpretation of the data due to environmental influences.

### 3.3.2 Interrelationships between oil, protein, seed yield, 1000-kernel weight, flowering duration, first flower, and maturity

Analysis of phenotypic correlations across all three locations and both years revealed significant positive associations between maturity and yield, and a highly significant association between maturity and kernel weight (Table 3-2). There were no significant relationships observed between maturity and protein content, nor between maturity and flowering duration. These correlations suggest it would be possible to simultaneously select for high kernel weight and yield through the selection of later maturing cultivars. Although selection for these traits is possible, this approach would not be desired in a breeding program designed for the Canadian prairies where growing seasons are relatively short. The provincial average for days to maturity in *B. napus* cultivars is approximately 106 days (Agricultural Canada Website 2000), and in Western Canada, the average number of frost-free days in a growing season is less than 100 (Downey and Rakow 1987). Thus, current cultivars are already utilizing the full length of the growing season leaving limited opportunities for cultivar improvement by lengthening maturity.

The data showed no significant correlations at the 5% level between 1000-kernel weight and yield (Table 3-2), but this may have been due to the limited number of genotypes and data points observed in the study. Nevertheless, the data indicated that high kernel weights and high yields both had positive trends. This is shown in Figures 3-1, 3-2, and in the LS means (Table 3-3) where 96-1773, 96-1747, 96-1183, and Quantum consistently had the highest 1000-kernel weights across all three locations in both years, and also had the highest yields under the same conditions. These results are consistent with Thurling (1974) and Chay and Thurling (1989) who both stated that seed yield can be increased through the selection of high seed weights. Thus, selecting for high seed weight should be an effective approach to seed yield improvement.

Two positive correlations were found between days to first flower, and for yield and maturity at the $p = 0.05$ and $p = 0.01$ significance levels respectively (Table 3-2). Both correlations were anticipated as both yield and maturity increase with an extension of anthesis development. Thurling and Vijendra Das (1979) observed the same positive correlation in a study focusing on seed yield of *B. napus* and pre-anthesis development.

Significant negative correlations were observed between oil content and maturity, and oil content and 1000-kernel weight (Table 3-2). The negative correlation between oil and maturity was unanticipated, and contradicts the source-sink relationship one would expect to see between these two traits (i.e. a longer period to provide nourishment equaling an increase in oil). Gambhir *et al.* (1979) tested this theory with a study on the effects of maturity and oil yield using *B. juncea*. They found significant increases in seed oil content up to 55 days after flowering (the beginning of pod maturation), but no significant changes were observed after the onset of maturity (55 days) to full maturity

(75 days). In contrast, data from this study suggest that shorter maturity is associated with increased oil production (although these correlations do not take into account seed color, which has been shown to affect seed oil levels). Yellow-hulled genotypes produce up to 2 % higher oil contents than brown- or black-hulled genotypes (Stringam *et. al.* 1974). The data set in this study included four yellow-seeded genotypes and four black-seeded genotypes. The lines with yellow hulls were *B. rapa* genotypes, and as such were also earlier maturing. Because all of the earlier maturing varieties in this study also expressed yellow hulls, (Table 3-4) this in effect, resulted in a negative correlation between maturity and oil (Table 3-2).

One-thousand-kernel weight was also negatively correlated with oil content (Table 3-2). Bing (1996) and Tang *et al.* (1997) found the same relationship among these two traits. Tang *et al.* (1997) separated total oil content into two fractions (embryo oil and seed coat oil) and further ascribed this negative association specifically to embryo oil content. A significant negative correlation was found in this study between seed weight and embryo oil content, but no significant difference was found between seed-coat oil content and seed weight. This specific association between seed weight and seed oil is supported by Bing (1996), who reported a strong positive association between seed weight and protein content. This would be expected if the negative correlation from seed weight and seed oil was attributed to the embryo of the seed. Since the major fractions of both seed oil, and seed protein are found within the seed embryo, and since these two traits are commonly found negatively correlated to one other, one would expect to see the positive association that Bing (1996) reported between seed weight and seed protein. With seed weight having a strong negative correlation with oil, and since it is one of the

seed yield components, simultaneously breeding for high yielding, high oil cultivars through the use of parents with high seed weights, will likely prove to be a challenge for canola breeders.

There was a significant negative correlation between flowering duration and yield (Table 3-2). This was unexpected because these two traits historically are known to be independent of each other (Stringam, personal communication). Separate phenotypic correlations from both field seasons were completed on these traits. The results showed that the correlation from 1998 was not significantly different from zero (0.23), although in 1999 a strong negative correlation was observed (-0.82). Consequently, it appears that the significant correlation found within the total data set was caused solely by environmental effects of the second year of the study. Without consistent results over both years, no definitive conclusions may be made from the data set. Since only eight genotypes were investigated in this study, and the data somewhat limited, the possibility of random associations appearing among traits is greater than would be likely seen in a vast collection of genotypes that would normally be available in a breeding program.

**Table 3-1.** Analysis of variance for oil, protein, yield, 1000-kernel weight (Seed wt), flowering duration (FlrDur), days to maturity (Mature), and days to first flower (FirstFlr) for three interspecific DH lines from the cross (*B. napus* x *B. rapa*) and their parents at three locations over two years.

| Factors and interactions | Df | F Values | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Oil | Protein | Yield | Seed wt | FlrDur | Mature | FirstFlr |
| Location (L) | 2 | 33.82** | 89.94** | 9.02** | 350.73** | 59.78** | 445.19** | 139.59** |
| Replication | 3 | 0.22 | 0.94 | 1.39 | 11.04 | 0.27 | 1.66 | 1.22 |
| Year (Y) | 1 | 1.17 | 180.83** | 529.00** | 446.26** | 489.81** | 9437.57** | 2.37 |
| Genotype (G) | 7 | 290.85** | 57.86** | 35.25** | 425.38** | 13.20** | 960.44** | 337.48** |
| L X Y | 2 | 105.66** | 233.97** | 106.60** | 107.37** | 54.88** | 11.93** | 0.81 |
| L X G | 14 | 3.41** | 2.45** | 2.24** | 8.05** | 6.41** | 17.60** | 2.66** |
| Y X G | 7 | 5.18** | 9.44** | 13.87** | 2.99** | 18.38** | 23.06** | 22.02** |
| L X G X Y | 14 | 2.71** | 2.36** | 2.17* | 4.73** | 4.28** | 9.51** | 4.01** |

[1] Values followed by * or ** denote $p < 0.05$ or $p < 0.01$, respectively

**Table 3-2.** Phenotypic correlation coefficients of oil content (Oil), protein content (Protein), yield, 1000-kernel weight (Seed wt), flowering duration (FlrDur), days to maturity (Mature), and days to first flower (FirstFlr) for three interspecific DH lines from the cross (*B. napus* x *B. rapa*) and their parents at three locations over two years.

| | Oil | Protein | Yield | Seed wt | FlrDur | Mature |
|---|---|---|---|---|---|---|
| Oil | | | | | | |
| Protein | 0.60 | | | | | |
| Yield | -0.16 | 0.30 | | | | |
| Seed wt | -0.82** | - 0.27 | 0.44 | | | |
| FlrDur | 0.28 | 0.09 | -0.67* | -0.42 | | |
| Mature | -0.71* | -0.08 | 0.68* | 0.81** | -0.47 | |
| FirstFlr | -0.41 | 0.10 | 0.65* | 0.45 | -0.28 | 0.74** |

[1] Values followed by * or ** denote $p < 0.05$ or $p < 0.01$, respectively

**Table 3-3.** Ls means for yields (kg) and 1000-kernel weights (g) for three interspecific DH lines (**bold**) from the cross (*B. napus* x *B. rapa*) and their parents at three locations over two years.

| Yield | | 1000 kernel weight | |
|---|---|---|---|
| Quantum | 32.39 | **4.47** | **1773** |
| **1773** | **26.24** | **4.21** | **1183** |
| **1747** | **24.48** | **4.16** | **1747** |
| **1183** | **24.30** | 3.66 | Quantum |
| 50025 | 23.10 | 3.19 | 2011 |
| 2011 | 20.81 | 3.14 | 50025 |
| Eclipse | 20.21 | 3.06 | 2012 |
| 2012 | 18.74 | 2.94 | Eclipse |

**Table 3-4.** Ls means for days to maturity and seed color for three interspecific DH lines (**bold**) from the cross (*B. napus* x *B. rapa*), and their parents at three locations over two years.

| | Days to Maturity | Seed color |
|---|---|---|
| Quantum | 103.7 | Black |
| **1747** | **102.6** | **Black** |
| **1183** | **102.4** | **Black** |
| **1773** | **102.3** | **Black** |
| 2011 | 93.4 | Yellow |
| Eclipse | 92.6 | Yellow |
| 2012 | 92.5 | Yellow |
| 50025 | 92.3 | Yellow |

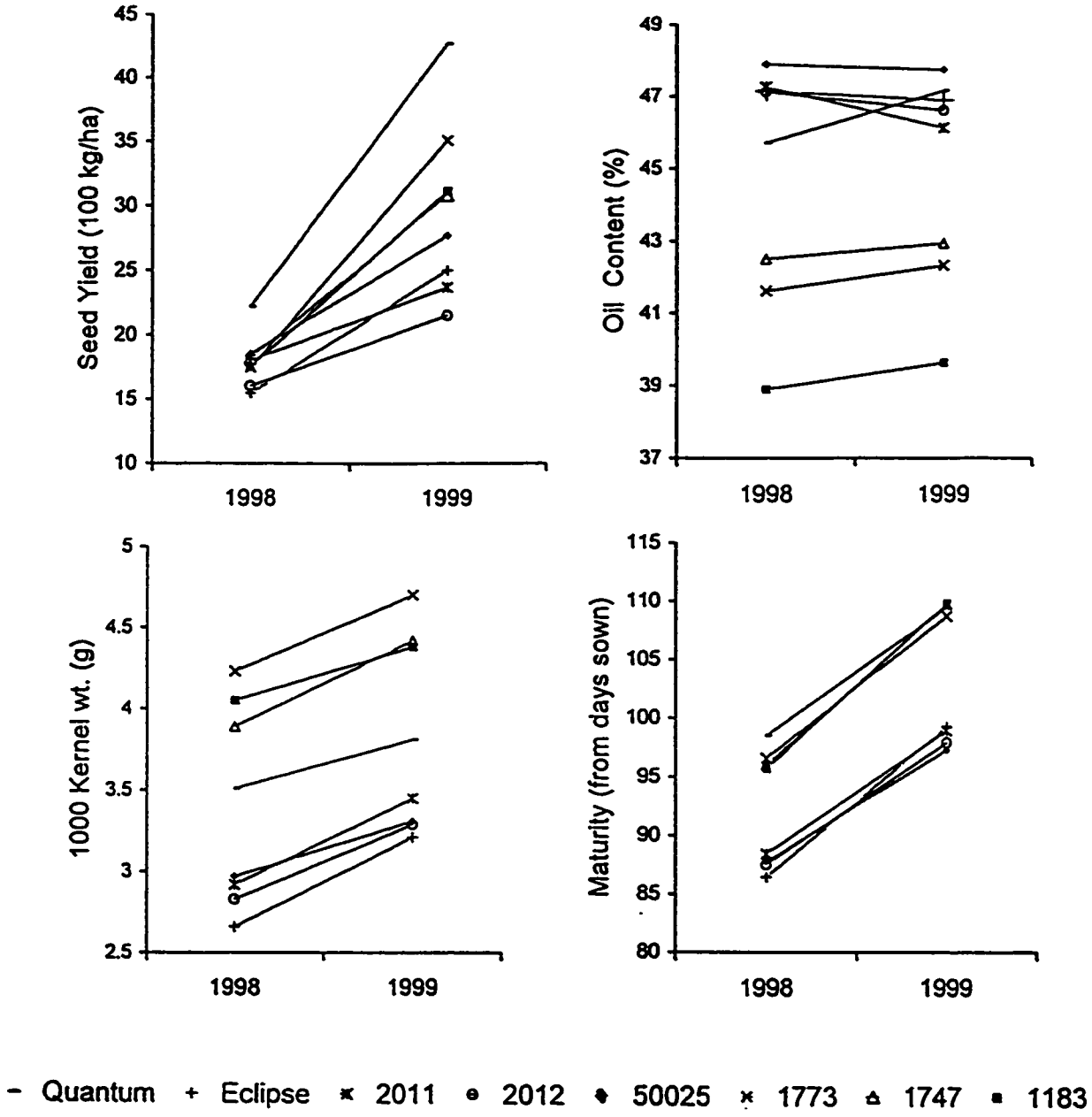


**Figure 3-1** Genotype x year interactions for seed yield, oil content, 1000-kernel weight, and days to maturity for three DH lines from the cross *B. napus* x *B. rapa*, and ancestral parents at three locations over two years (1998 and 1999).

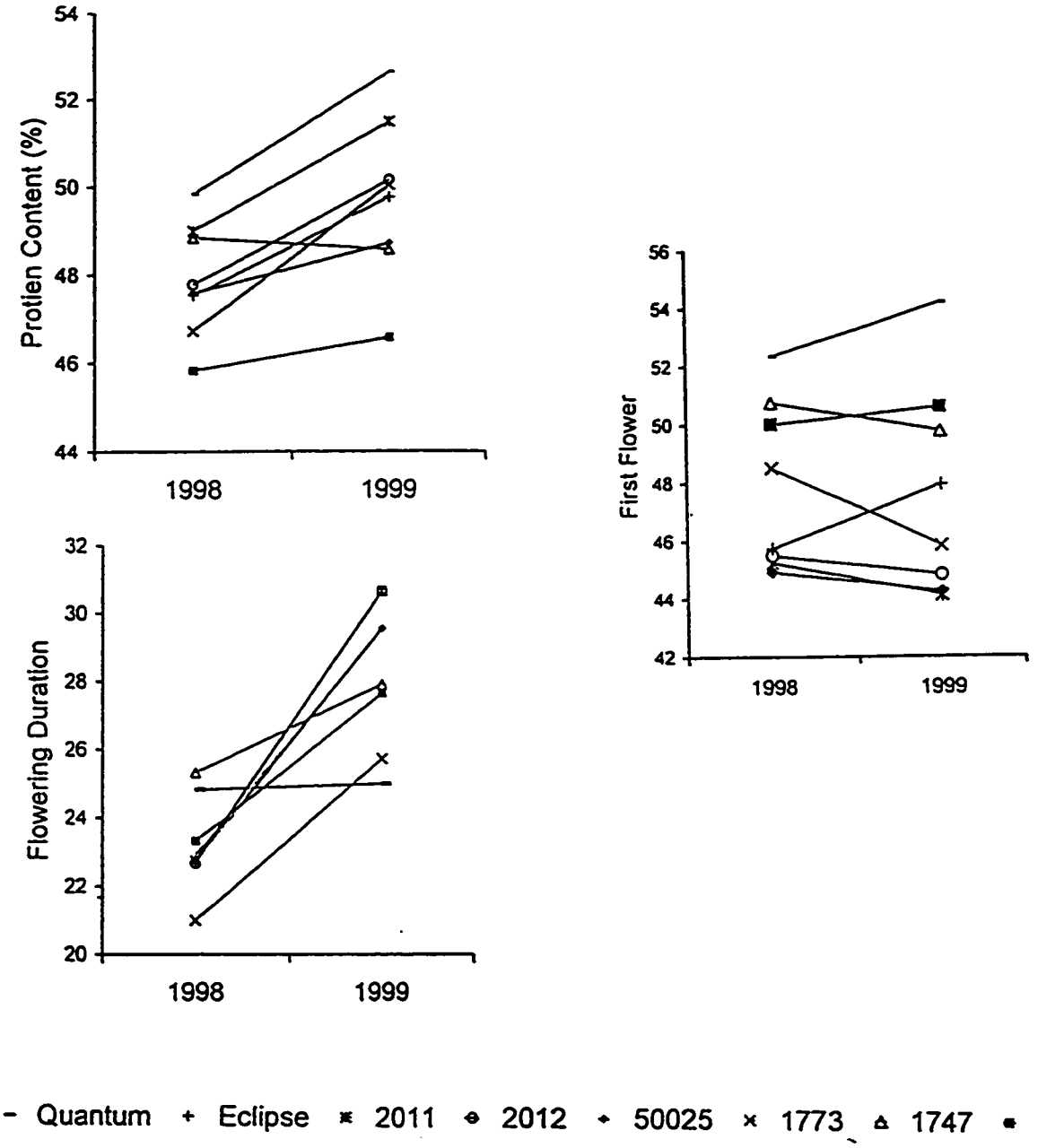


**Figure 3-2** Genotype x year interactions for protein content, flowering duration, and days to first flower for three DH lines from the cross *B. napus* x *B. rapa*, and ancestral parents at three locations over two years (1998 and 1999).

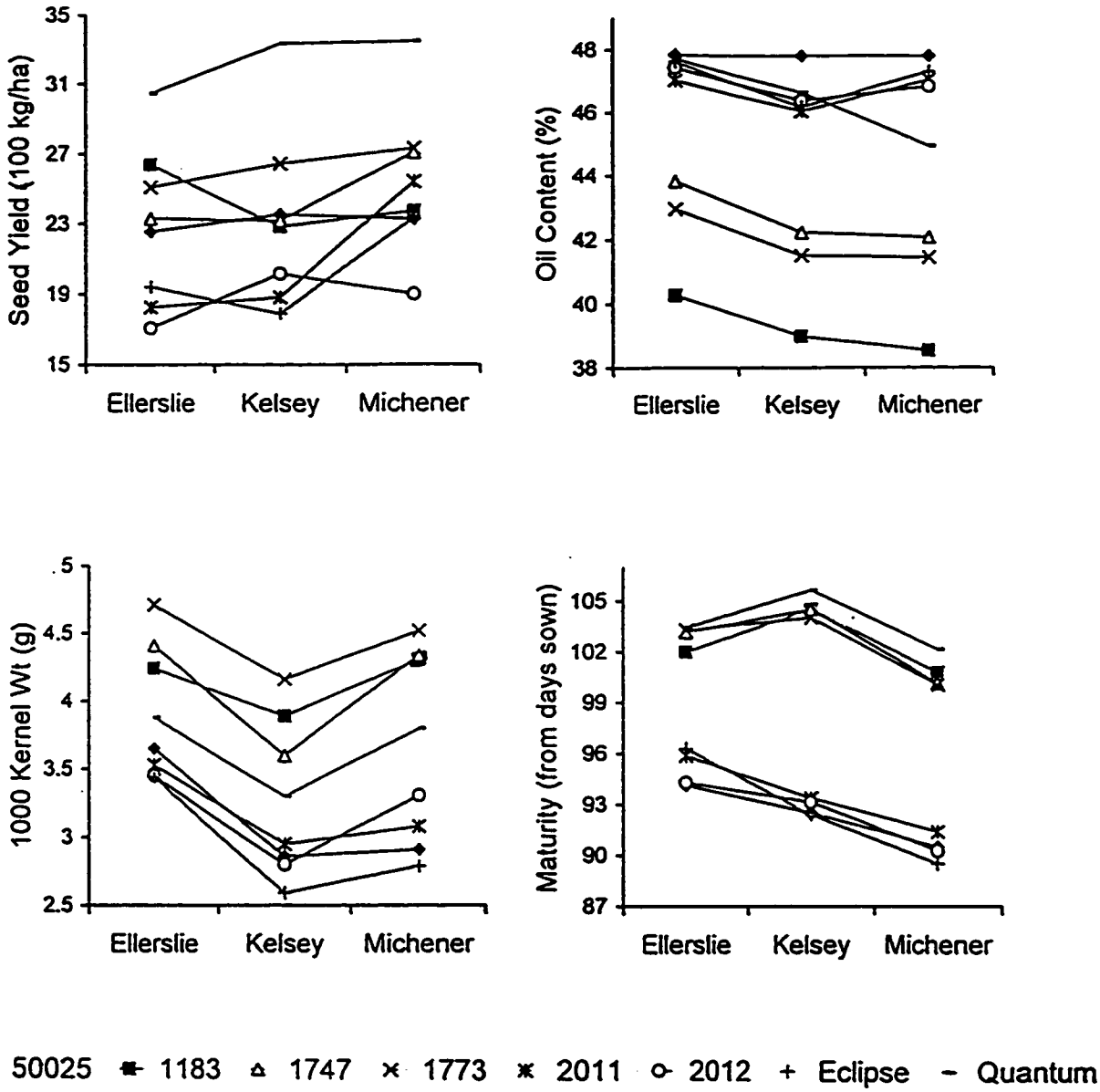


**Figure 3-3** Genotype x location interactions for seed yield, oil content, 1000-kernel weight, and days to maturity for three DH lines from the cross *B. napus* x *B. rapa*, and ancestral parents at three locations over two years (1998 and 1999).

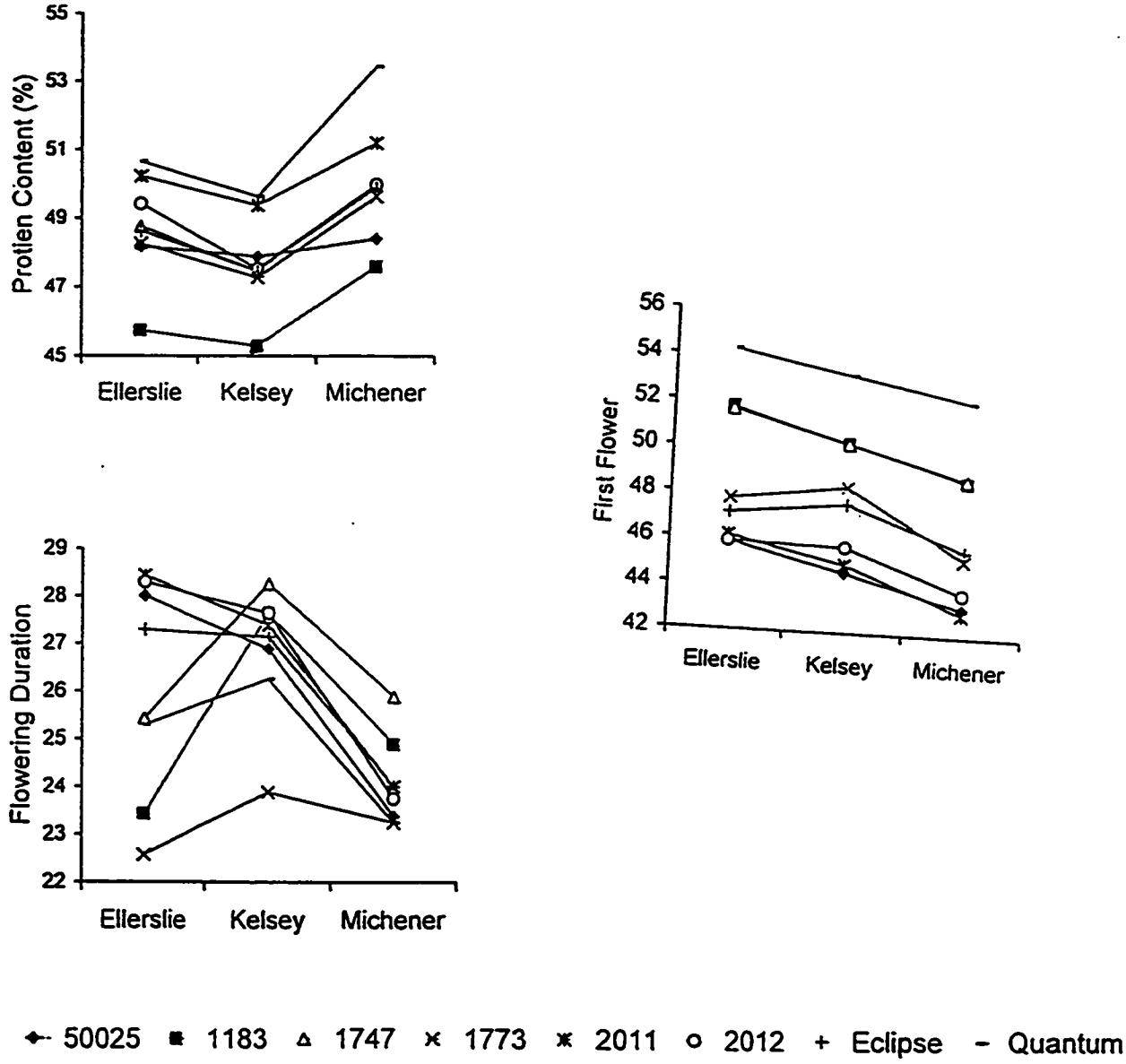


**Figure 3-4** Genotype x location interactions for protein content, flowering duration, and days to first flower for three DH lines from the cross *B. napus* x *B. rapa*, and ancestral parents at three locations over two years (1998 and 1999).

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# Chapter 4
# Seedling Vigor of DH Progeny from the Interspecific Cross (*Brassica napus* x *Brassica rapa*) and Ancestral Parents

## 4.1 Introduction

The use of high quality seed is the first step toward successful crop production. Choosing a seed source with a high level of vigor is an important contributor to seed quality. Seedling vigor may influence yield, time to maturity, and crop uniformity. Seedling vigor has been described as the seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions (AOSA 1983). Although, germination testing has been the accepted practice for determining seed quality, germination levels can remain fairly high even after physical deterioration of the seed (Elias and Copeland 1994). Thus, vigor testing is needed to provide additional information about the physiological quality of the seed.

Seed size is considered to be a significant factor in the determination of seedling vigor with several crops (Kaufmann and McFadden 1960, Kaufmann and Guitard 1967). The chemical composition of crops such as wheat, and bean have been found to play a significant role in vigor establishment (Lowe and Ries 1971, Bulisani and Warner 1980, Ries 1971). For example, seed protein levels were found to be positively correlated with vigor in crops such as wheat, bean, barley and oats (Ries *et al.* 1970, Ries 1971, Lopez and Grabe 1971, Schweizer and Ries 1969). To date, this characteristic associated with vigor has not been tested in an oilseed crop. In oilseeds, two major forms of readily available energy are accessible to the seed during germination: (1) protein and (2) oil. As

such, oil content cannot be ruled out as a major contributor to seedling vigor along with protein.

Another important aspect of vigor is the correlation between vigor testing and field emergence. Several types of vigor tests have been developed to measure the various components of vigor. For example, the Electrical Conductivity Test was developed to indicate the amount of mechanical damage sustained by the seed. A further aspect of vigor is how well the seed germ endures over time, commonly referred as the "storage potential" of the seed. Tests such as Accelerated Aging were developed to help predict this aspect of vigor. However, the most frequently performed test is the Cold Test (20/20 Seed Labs, personal communication). This test was devised to simulate the adverse conditions often encountered in spring field planting. The cold test represents the lowest emergence that one would expect to occur when planting under reasonably satisfactory conditions. This test indirectly gives additional information on mechanical damage, physiological condition, and effects of chemical treatment on seed, rendering it one of the most comprehensive vigor tests available (Grabe 1976, AOSA 1983). Despite the availability of these tests, research on their correlation to canola/rapeseed field emergence has been limited; one such test was conducted by Elias and Copeland (1994).

The objectives of the present study were to: 1) determine the relationship of seed protein and/or oil content to the seedling vigor of the eight genotypes used in the field study as described in chapter two, and 2) evaluate the relationship between the Cold Test results and field seeding establishment with the use of the same eight genotypes.

## 4.2 Materials and Methods

The seed source for this study was obtained from the eight genotypes used in the field study (96-1747, 96-1773, 96-1183, 96-2011, 96-2012, 93-50025, Quantum, and Eclipse). The experiment was completed in four stages, (1) laboratory Cold Test, (2) seed analysis, (3) field vigor evaluation, and 4) statistical correlations.

### 4.2.1 Cold Test

For this study a modified tray method was used. Test units consisted of a standard plastic tray measuring 52 x 27 x 5 cm in length, width, and depth. Each tray accommodated 8 x 100 seed treatments. Two trays were prepared containing eight seed samples with two replicates of each genotype. A 50/50 sand/soil mix was evenly spread within the tray to a depth of 2.5 cm. Since the seed was not treated with any fungicide a sterilized sand/soil mix was used. After planting, seeds were covered with a few millimeters of the sand/soil mixture, followed by moderate compaction of the soil substrate with a small wood block. Each tray was then soaked with 900 ml of water pre-chilled to 5 ° C and covered with an airtight top to maintain moisture. Trays were then placed in a non-illuminated chamber with a constant ambient temperature of 5 ° C for 12 days. Following this period, they were moved to a CMP4030 Conviron growth chamber, with a photoperiod of 16/8 hr day/night, and a 20/15 ° C day/night temperature regime for a two day grow out period. The seedlings were evaluated according to the protocol of 20/20 Seed Labs Inc.. A seedling was scored as vigorous if it possessed all primary seedling structures and if these structures were not deemed "abnormal" as described in the AOSA seedling evaluation handbook (see appendix A).

### 4.2.2 Seed Analysis

#### 4.2.2.1 Oil Content Determination

Nuclear magnetic resonance (NMR) was used to determine oil content on a whole seed basis. This was completed through the use of an Oxford 4000 NMR Analyzer (Oxford Analytical Instruments Ltd.). A bulk sample of approximately 1.2 grams was analyzed from each test sample. Each sample was analyzed for one minute with a single repeat measurement. All raw oil content data were multiplied by 0.95 to correct for five percent moisture present within the seed. For each sample an estimate on the absolute amount of oil within each seed was determined. One-thousand-kernel weights were recorded on each sample, then divided by 1000 to estimate the average individual seed weight. The individual seed weight was then multiplied by the percent oil found within that sample providing an estimation of the absolute oil content.

$$\frac{\text{1000-Kernel wt.}}{1000} \times (0.95 \times \text{Oil Content}) = \text{Absolute Oil Content per Seed}$$

#### 4.2.2.2 Protein Content Determination

Protein content was determined on a whole seed basis by combustion, through the use of a Leco FP-2000 Analyzer with a fitted nitrogen probe. A single bulk sample of 0.5 grams from each plot was used in the analysis. All samples were estimated for absolute protein and corrected for five percent moisture present within the seed in the same manner as described under oil content determination.

### 4.2.3 Field Vigor Evaluation

Details of plot preparation are given in Materials and Methods in chapter two.

The average number of seeds each plot received was approximately 1750; the seed number per plot varied with seed size due to drilling procedure (see Chapter 2 Materials and Methods). Twenty days after planting a vigor measurement was recorded for each plot. An arbitrary rating system was devised to measure relative vigor levels between plots. The plots were ranked visually on a five point scale with 1 to 5 representing poor to excellent vigor respectively. Seedlings with a fully developed first true leaf were considered established and included as part of the ranking.

### 4.2.4 Statistical Analysis

Statistical computations were completed through the use of SAS/STAT 7.0 (SAS Institute Inc. 1999). All correlation analysis were completed using Pearsons correlations with the PROC CORR command. The data were checked for normality and homogeneity of variance using the GLM model statement and found to conform to the parameters.

## 4.3 Results and Discussion

The genotypes were split into two groups based on their phenotypic differences (*B. napus* and *B. rapa*). The *B. napus* group consisted of Quantum, 96-1773, 96-1747, and 96-1183 and the *B. rapa* grouping was Eclipse, 93-50025, 96-2011, and 96-2012. The three interspecific DH lines were grouped with the *B. napus* cultivar Quantum because they all displayed predominately *B. napus* characteristics (seed color, seed weight, seed quality). Seed quality data (oil and protein) for the genotypes in the *B. rapa* grouping, were similar for these parameters (less than 1% for oil and 3% for protein) (Table 4.1) making any conclusions about vigor based on these differences among these genotypes suspect.

### 4.3.1 Correlation of Cold Vigor Test to Protein and Oil Contents

In the *B. napus* trial, no significant correlation was observed between seed protein content and the cold vigor test (Table 4.2). These results contradict the results of previous studies examining the relationship between protein and vigor in cereals and pulses (Lowe and Ries 1971, Ries 1971, Lowe et al. 1972, Bulisani and Warner 1980, Torres and Paulsen 1982). Thus, it is assumed that other factors affect the vigor response for canola. The correlation coefficient for oil content on a whole seed basis in relation to the cold vigor test was 0.71 (Table 4.2) but was only statistically significant up to the 70% confidence level. Although this correlation was below the 95% confidence level, the relationship suggests that further study into this vigor response is needed.

### 4.3.2 Relationship of Field Vigor to the Cold Test, Seed Proteins and Seed Oil

With the *B. rapa* group there was a significant correlation between the Cold Vigor Test and the observed field vigor (Table 4.2). This agrees with Elias and Copelands (1994) who reported a high correlation between a canola Cold Test results and field emergence. Johnson and Wax (1978) reported similar results for soybean using the same Cold Test under comparable conditions. The *B. napus* grouping did not show a significant correlation between the Cold Test and field vigor at the $p = 0.05$ level but a sufficiently positive correlation was observed (Table 4.2). A reason for this weak significance among the *B. napus* grouping may have been the result of the limited number of genotypes used in this study.

A high positive correlation was apparent between field vigor and oil content within the *B. napus* group, accompanied by a weak association with protein content

(Table 4.2). These field observations are consistent to the results reported in the laboratory Cold Test further suggesting that oil content may influence vigor. The evidence presented here is preliminary and further studies are essential. Subsequent studies should also include a larger number of genotypes from each species so that more reliable statistical comparisons can be made.

**Table 4.1** Average protein and oil contents of the eight genotypes collected at Michener in 1999.

| Grouping | Genotype | Protein | Oil |
|---|---|---|---|
| *Brassica napus* | Quantum | 53.98 | 45.95 |
| | 96-1773 | 50.28 | 41.89 |
| | 96-1747 | 48.72 | 42.61 |
| | 96-1183 | 46.99 | 39.46 |
| *Brassica rapa* | Eclipse | 50.81 | 47.03 |
| | 93-50025 | 48.41 | 47.64 |
| | 96-2011 | 51.44 | 46.98 |
| | 96-2012 | 50.98 | 46.51 |

**Table 4.2** Pearson correlation coefficients for protein, oil (whole seed basis) and comparisons between Cold Testing and field vigor evaluations.

| Grouping vigor | Protein | | Oil | | Cold Test & Field Correlations |
|---|---|---|---|---|---|
| | Cold Test | Field Vigor | Cold Test | Field Vigor | |
| B. napus | 0.37 | 0.59 | 0.71 | 0.80 | 0.68 |
| B. rapa | -0.13 | 0.17 | 0.01 | 0.30 | 0.95* |

The values followed by * denote that $p < 0.05$.

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# Chapter 5
# General Discussion

## 5.1 Introduction

This project was developed to study the potential of the introgression of agronomically important traits between *B. napus* and *B. rapa* through an interspecific cross. From these crosses, there was the potential to produce an economically viable genotype, or germplasm base useful for further breeding purposes. In this chapter pertinent results obtained from all studies will be discussed and summarized in relation to beneficial or unexpected traits exhibited by the DH lines from the *B. napus* x *B. rapa* x *B. rapa* interspecific backcross.

## 5.2 Summary

### 5.2.1 Agronomics

Seed yield is undoubtedly the most important trait in canola breeding, and as such was of high interest in this DH interspecific study. Average yield data from the Tukey grouping showed that the overall yields for the DH lines were significantly lower than the ancestral parent Quantum (Table 2-1). The highest yields recorded for the interspecific DH lines was by 96-1773 at 26.24 kg/plot which averaged 6.15 kg/plot less than Quantum. Although there were significant G x L interactions in relation to yield (Table 3-1), showing that the genotypes reacted independently in the different environments, the three DH interspecific genotypes did not capitalize on these differing environments to produce outstanding yields in any of the locations. This may suggest that the interspecific

DH lines in this study are not only poorly adapted to producing high yields in a wide range of environments but also poorly adapted to producing high yields in specific environments.

In relation to yield components, the data suggested that a correlation existed between 1000-kernel weight and seed yield. The DH line 96-1773 was significantly heavier than the other lines (96-1183, and 96-1747), and also yielded the highest of the other two DH lines. From two successive experiments, Chay and Thurling (1989a, 1989b) found that the selection of increased seed weight can positively impact seed yields if an appropriate genetic background is chosen to avoid the reduction which may occur in other seed yield components. The results from this correlation suggest that the heavier kernel weights achieved through this cross may prove to be beneficial in further interspecific breeding programs.

For maturity, all of the interspecific DH lines closely resembled the later *B. napus* pattern. No intermediary or *B. rapa* maturity patterns midway between the two species were observed. This may result from the fact that all DH interspecific progeny may have maintained the full complement of the *B. napus* genome, containing 19 bivalents, (10 A + 9 C) as stated by Kubik (1999). Kubik (1999) hypothesized that having less than a complete haploid set of the *B. napus* genome in a *B. napus* cytoplasm resulted in the collapse of the developing plant embryo. This suggests that maturity may be influenced by additive genes carried by the nine bivalents from the C genome and thus enabled the DH progeny to resemble the maturity of *B. napus*.

In the interspecific DH lines, days to flower was significantly reduced over that of the *B. napus* parent (Table 2-3). As this trait is strongly correlated with maturity (Table

3-2), it would suggest that maturity, (although not successfully improved in this cross) may be indirectly improved through further study aimed at reducing days to flowering.

Through this data, environmental influences regarding days to flower suggests that this trait is more strongly influenced by dependent location specific conditions (day length, soil composition, nutrient availability) rather than broader independent conditions (rainfall, heat units) (Table 3-1). The highly significant location F-value for days to flower indicates there were large differences in values between the different locations relative to each year, but the non-significant location x year F-value shows that differences among the sites between the years were minimal. Thus between years at the same location, random factors such as rainfall and temperature had little effect on influencing the initiation of first flower. This leads to the conclusion that days to flower can be influenced by factors such as nutrient regimes, nutrient availability and photoperiod (location) that can be directly under the control of a producer. This conclusion suggests that if a producer can influence the onset of first flower and as shown in this study can be correlated to maturity, then it may be postulated that the producer may indirectly influence the onset of maturity as well. It is noted that discussion in this area is beyond the scope of this study, and due to limited data, highly speculative.

Since vigor has been extensively discussed in Chapter 4 and there are no overlapping studies related to vigor in different chapters, only a single point will be summarized here. If vigor does positively correlate with seed oil content, then as a result vigor will incidentally increase in a breeding program, because increasing oil content in this crop is an important and common objective.

### 5.2.2 Quality

The fatty acid profile of the three DH interspecific crosses displayed two prominent deleterious traits (high saturates and high linolenic acid). The high saturates found in this cross may make it an undesirable bridge for transfer of the characteristically low saturates found in a *B. rapa* profile into *B. napus*. Because, these high saturates were found in all the DH interspecific genotypes, and since these were derived from a single cross, (Figure 2-1) inferences about the segregation of the fatty acid profile (i.e.: high saturates) may prove inaccurate when progeny from additional crosses are analyzed. The high linolenic acid that is characteristic of *B. rapa* was expressed in the DH interspecific crosses. In the earlier study by Kubik (1999), it was hypothesized that the high linolenic fatty acids found in the profile was the result of environmental influences. In the present study significant interactions were observed for oil in Y x G, L x G, and L x Y x G. Although these interactions were significant, and the environment (particularly temperature) has been documented as affecting linolenic acid levels (McGregor 1974), linolenic acid contents in this study were relatively unaffected. Conclusions from the data in this study indicates that this cross was not successful in the transfer of the low linolenic trait characteristic of *B. napus*. It should be noted that these conclusions have been drawn from only a single cross, and may not apply in every situation.

Seed oil content levels for the DH interspecific lines were significantly lower than all other genotypes (Table 2-6), but were the most stable of all genotypes expressing minimal Y x G, and L x G interactions (Figures 3-2, and 3-3). The crosses may have displayed desirable stability in relation to oil, but the primary goal of producing lines with acceptable levels of oil was not achieved. The average oil content for the three

crosses was 41.3 percent, which is significantly lower than the *B. napus* and *B. rapa* cultivars (Quantum and Eclipse ) by approximately 5% (Table 2-6).

Protein contents for the interspecific crosses relative to Quantum and Eclipse displayed adequate stability in that fluctuations due to environmental influences and/or interactions were similar in both species (Figures 3-2, and 3-3). Protein levels for the two DH lines 96-1773, and 96-1747 were acceptable at 48.4%, and 48.7%, respectively. Although protein contents for the DH line 96-1183 was significantly lower than the other interspecific lines, this line overall, had an acceptable level of protein.

## 5.3 Conclusions

Agronomic and quality analysis of traits pertinent to a breeding program were completed on the three DH lines from an *B. napus* x *B. rapa* interspecific cross. The inability to isolate *B. rapa* germplasm from the study with a high yield potential, has refocused breeding efforts to develop an early maturing *B. napus* germplasm (Stringam, personal communication). The DH lines were primarily *B. napus* phenotypes introgressed with some *B. rapa* traits. Unfortunately, the combination of a high yielding early maturing phenotype was not achieved through this approach. Other undesirable combinations were observed as well. High linolenic acid and high saturates were associated together in the fatty acid profile of the interspecific DH lines. Although there were many undesired traits resulting from this cross, desirable traits were also observed, the most prominent was increased seed weight in all three DH lines. Although no outstanding agronomic lines were obtained for potential use in a germplasm base, this cross was successful in producing stable traits introgressed from both species. This study

suggests that the successful introgression of a number of desirable traits in a single cross is problematic. The possibility that success may be achieved through additional crosses can not be ruled out.

## 5.4 References


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# Appendix A

## ABNORMAL SEEDLING DESCRIPTION

**Cotyledons**

- decayed at point of attachment.
- less than half of the original cotyledon tissue remaining attached.
- less than half of the original cotyledon tissue free of necrosis or decay.

**Epicotyl**

- missing (may be assumed to be present if the cotyledons are intact).

**Hypocotyl**

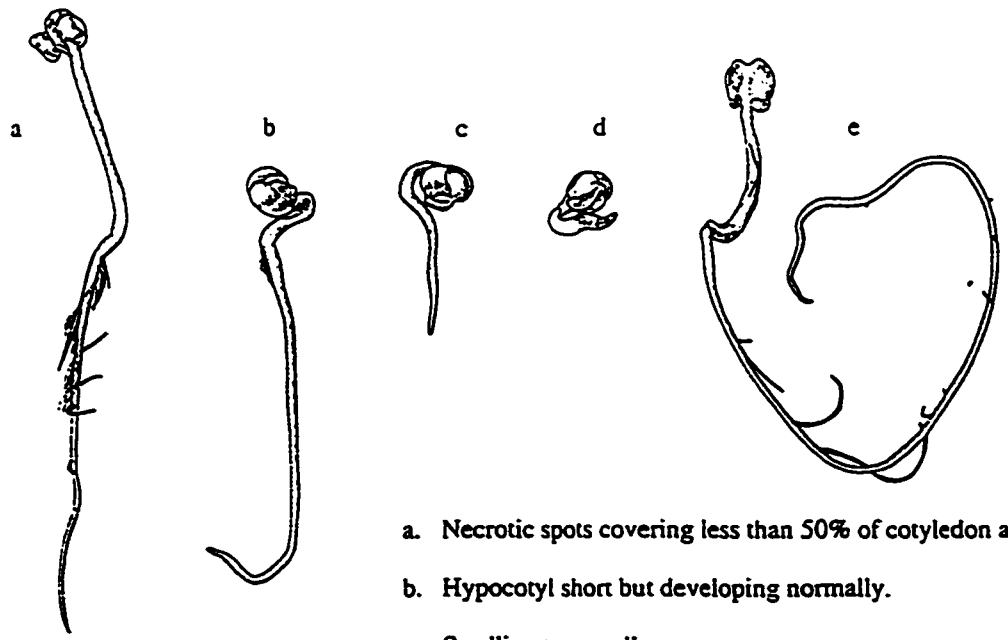
- deep open cracks extending into the conducting tissue.
- malformed, such as markedly shortened, curled or thickened.
- watery.

**Root**

- weak, stubby or missing primary root (secondary roots will not compensate for a defective primary root).

**Seedling**

- one or more essential structures impaired as a result of decay from primary infection
- albino




a. Necrotic spots covering less than 50% of cotyledon area.

b. Hypocotyl short but developing normally.

c. Seedling too small.

d. Stubby primary root, poor hypocotyl development.

e. Hypocotyl lesions.

**Illustrations "a" and "b" denote a normal seedling while "c, d, and e" denote an abnormal seedling [(ASOA 1983) see page 20 for full reference].**