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University of Alberta

Evaluation of Doubled Haploid Lines Derived from Interspecific Crosses between *Brassica napus* and *Brassica rapa*.

by

Thomas James Kubik ©

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 Agricultural, Food and Nutritional Science

Edmonton, Alberta

Spring 1999

University of Alberta

Library Release Form

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Title of Thesis: Evaluation of Doubled Haploid Lines Derived from Interspecific Crosses between *Brassica napus* and *Brassica rapa*.

Degree: Master of Science

Year this Degree Granted: 1999

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Dr. Gary R. Stringam

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Date: 26 Jan 1999

Abstract

The introgression of traits between *Brassica napus* and *B. rapa* during interspecific crosses was studied using the doubled haploid method to "fix" desirable genotypes and eliminate fertility problems caused by unpaired chromosomes. Cytological evaluation showed that all interspecific doubled haploid lines contained 38 chromosomes during meiosis. Anomalies in chromosome pairing raised questions about the cytological stability of some interspecific DH lines. Morphologically, the interspecific DH lines appeared more like the *B. napus* parent, with some *B. rapa* influence. White rust resistance was observed in all progeny, while a continuous range of blackleg resistance was recorded in the population. Analysis of oil quality indicated that fatty acid profiles did not fit either a typical *B. napus* or *B. rapa* profile. Glucosinolate levels in the interspecific lines were reduced below those seen in either parent species. Agronomic assessment showed the potential of interspecific crosses for the improvement of these species. RAPD markers revealed polymorphic differences that demonstrated an introgression of genetic material occurred from *B. rapa* to *B. napus*.

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Abbreviations

BC_1F_1. First backcross, first filial generation

BUT Butanyl glucosinolate

cm. Centimeters

cv. Cultivar

DBS. Distance between seeds

°C. Degrees Celsius

C16:0 Palmitic acid

C18:0 Stearic acid

C18:1 Oleic acid

C18:2 Linoleic acid

C18:3 Linolenic acid

C22:1 Erucic acid

Days Flw. Days to Flower

DH. Doubled haploid

DH_1. Doubled haploid-first generation.

DNA. Deoxyribonucleic acid

dNTP. Deoxyribonucleotide triphosphate

DSI. Disease severity index

F_1. First filial generation

F_6. Sixth generation offspring

F_7. Seventh generation offspring

g. Grams

GC. Gas chromatograph

L. Beak Length of the Beak

L. Carpel Length of the Carpel

lsmean. Least-squares mean

ml. Milliliter

mm. Millimeters

mM. Millimolar

N. Haploid chromosome number

ng/µl. Nanograms per microleter

NMR. Nuclear magnetic resonance

NT. Not tested

OHButanyl Hydroxy-Butanyl glucosinolate

OHIndoyl Hydroxy-Indoyl glucosinolate

PCR. Polymerase chain reaction

RFLP. Restriction fragment length polymorphism

PG. Pathogenicity groups

PMC Pollen Mother Cell

SE. Standard error

Seedwt 1000-Seed weight

Stdev. Standard deviation

T. Alkenyl Total Alkenyl glucosinolates

Taq. Thermus aquatus

T. Gluc. Total glucosinolates (Sum Total Alkenyl and Total Indoyl)

T. Indoyl Total Indoyl glucosinolates

U. Units

µM. Micromolar

µmol/g. Micromoles per gram

µmol $m^{-2}s^{-1}$. Light intensity in micromoles per meter squared second

UV. Ultraviolet

WCC/RRC. Western Canadian Canola/ Rapeseed Recommendation Committee

w/v. Weight per volume

Chapter 1

Literature Review.

1.1. Introduction:

Canola production occupies a significant proportion of the agricultural land base in Canada. Canola and barley production compete for second place with respect to seeded area, while wheat production continues to dominate the majority of the seeded area. The number of hectares utilized for canola production in Canada has steadily increased over the last ten years from 2.6 million hectares in 1986 to 6.0 million hectares in 1994. An increase in the price of wheat in 1996 caused a drop in canola production to 3.4 million hectares, but the Canola Council of Canada reports that canola production has rebounded and 5.2 million hectares are expected for 1998 (Statistics Canada, 1998). The potential for disease and insect outbreaks limits the recommended utilization of the area for canola production to a recommended once in a 4-year rotation. Current farming practices favour crop diversity to minimize risk. If increasing demands for oil and meal are to be met, productivity must continually increase.

On average, 47% of the canola production occurs in Saskatchewan, 33% in Alberta, 19% in Manitoba and the remaining 1% in Ontario and British Columbia. Saskatchewan and Manitoba are classified as having mid to long season zones and therefore have a long enough growing season for Argentine canola (*Brassica*

napus L.) varieties to reach maturity (Statistics Canada, 1998). In Alberta, prior to 1996, Polish canola (*B. rapa* L., formerly *B. campestris* L.) varieties dominated the canola acreage because of the short growing season in a major portion of the canola producing areas (Thomas P, personal communication). Agronomic advances in *B. rapa* have been hindered by the difficulties of breeding a self-incompatible species, while in *B. napus*, a self-compatible species, yield performance and disease resistance have been improved dramatically over the past ten years through breeding. The potential yield benefit of *B. napus* has attracted more Alberta farmers to growing cultivars of this species, although there is a risk that the crop may be damaged by early fall frost (Stringam GR, personal communication). Alberta farmers, and the canola industry could greatly benefit from new cultivars of *B. rapa* containing a number of the superior agronomic traits found in *B. napus*, or *B. napus* cultivars with early maturity.

1.2. Canola Quality Oil and Meal:

Varieties of *B. napus* and *B. rapa* with less than 2% erucic acid and less than 30 micromoles of glucosinolates per gram of air-dried oil-free meal are designated as canola quality to distinguish them from rapeseed (Thomas, 1984).

The oil obtained from canola seed is highly desirable. It contains the lowest level of saturated fat and the highest level of unsaturated fat of any edible oil, with moderate levels of polyunsaturates. The fatty acid profile of canola oil contains a

high proportion of linoleic and alpha-linoleic acid, two fatty acids that are essential to the human diet (cannot be synthesized in the human body). One characteristic that sets canola apart from rapeseed is the low levels of eicosenoic and erucic fatty acids in the fatty acid profile of canola. Through feeding experiments with laboratory animals, these fatty acids were found to have anti-nutritional properties and therefore have been selected against in breeding programs (Uppström, 1995).

The fatty acid profiles of *B. napus* and *B. rapa* are slightly different. *B. napus* canola varieties tend to have approximately 5% more oleic fatty acid (18:1) in their profiles but have a higher total saturate content than *B. rapa* varieties, which tend to have a higher proportion of linolenic fatty acid (18:3) and approximately 2% less total saturates (Downey and Rimmer, 1993).

An increased level of oleic acid improves the cooking quality of the oil (Dhillon et al., 1993), while linoleic acid (18:2) or vitamin F is an important building block for the formation of prostaglandins and other essential body regulators. Although linolenic acid is a polyunsaturated fatty acid, and is essential to the human diet, it is less desirable because it oxidizes readily, giving off-flavors and reduced shelf life. Lower levels of saturates are nutritionally desirable, but margarines made from oils having a high proportion of fatty acids with the same 18-carbon chain length, have a tendency to form crystals on storage. Blending with other vegetable oils can prevent this problem, but there is considerable interest in increasing the amount of the saturated fatty acids, palmitic and palmitoleic acids, in the profile of canola to alleviate the crystallization problem (Downey and Rakow 1987).

The meal which comprises approximately 60% of the original seed weight, contains 36 to 44% crude protein, and can provide a good balance of essential amino acids when used as an animal feed supplement. However, a high proportion of glucosinolates present in the meal of all *Brassica* species, reduces its feedability. Glucosinolates are sulphur containing oximine thioesters predominantly composed of glucose. The amino acid derived R group can be aliphatic, cyclic, or heterocyclic (Uppström, 1995). Of the more than 100 glucosinolates discovered within the plant kingdom, 6 occur in a significant amount in the seed of *B. napus* and *B. rapa.* (Bell, 1995).

Glucosinolates are readily hydrolized after the oil extraction process by myrosinase, an enzyme present within separate compartments of the seed. The biproducts of hydrolized glucosinolates reduce the palatability of meal and in non-ruminant animals, have been found to produce goiterogenic effects (Downey & Röbbelen, 1989). By selecting for lower levels of glucosinolates, breeders have improved the marketability of the meal. Further reductions in the glucosinolate content of meal should encourage producers to increase the proportion of canola meal in swine and poultry diets without adverse effects (Bell, 1995).

Hulls represent 30% of the total oil-free meal weight, are high in fibre and low in protein. The fibre is primarily composed of cellulose, pentosans and lignin, and therefore can not be digested by non-ruminant animals. The presence of hulls depresses the level of available energy and protein as well as amino acids and minerals when the meal is fed to non-ruminant animals. Yellow seeded cultivars of

B. rapa tend to have thinner hulls and therefore lower fibre content compared to brown and black seeded cultivars of *B. napus* and *B. rapa* (Stringam et al., 1974). Since the hulls contain up to 10% of the total protein and 25% of the gross energy contained in the meal, many breeding programs are attempting to develop pure yellow seeded *B. napus* canola cultivars (Bell, 1995). Rashid et al., (1994) was able to produce stable yellow seeded *B. napus* through interspecific crosses with *B. juncea* L. and *B. carinata* L.

Downey (1983) noted that increased seed size also reduced the hull percentage of the seed. He reported that *B. campestris* (*B. rapa*) Indian sarson types (yellow seeded) that are exceptionally larger seeded than *B. napus* cultivars, have higher oil and protein with less fibre than *B. napus* cultivars. The larger seeded *B. rapa* varieties were also found to contain more oil and protein than yellow or brown seeded *B. rapa* cultivars with small seed size. Selection for seed colour as well as seed size may improve the oil and protein content of new varieties beyond what is currently obtainable.

1.3. Agronomics:

The two species of canola produced commercially for oil and meal, have unique agronomic traits that enable them to be grown in different areas. Table 1.1. illustrates several of the differing agronomic characters of Argentine canola (*B.*

napus) cultivars and Polish canola (*B. rapa*) cultivars.

Table 1.1. Agronomic characteristics of Polish and Argentine canola.

Characteristic	Argentine	Polish
Seed Yield	Provincial average of 25 bu/acre but under optimal growing conditions 40 to 50 bu/acre are possible	Under good conditions, 15-20% less than Argentine, under frost or drought about equal to Argentine
Days to Maturity	Provincial average 105 days	10 days to 3 weeks earlier
Height	75 to 175 cm	50 to 125 cm
Frost Damage	Slightly more susceptible to late spring frost than wheat, susceptible to early fall frost	More resistant to late spring frost and usually mature before fall frost
Drought Tolerance	May suffer loss in yield and quality from late summer drought	Often matures early enough to escape late summer drought
Shattering	Shatters readily when ripe	More resistant to shattering, may be straight combined
Flowering	May flower during high temperature periods in July with reduced seed set	Usually finishes flowering before high temperatures occur

(Modified from Thomas, 1984).

Several fungal pathogens affect the quality and yield of canola across Canada. Blackleg and white rust are two fungal diseases, which can be found infecting canola annually. *Leptosphaeria maculans* (Desm.) Ces & de Not. (asexual state *Phoma lingam*) is a fungus that attacks the leaves, stems, and siliques of

many species within the Brassicaceae. This pathogen produces stem cankers, which is a common symptom of blackleg. The disease can be found on spring rapeseed in Australia, winter rapeseed in Europe, and in wide-spread areas across western Canada. Stem cankers result from the systemic growth of *L. maculans* (Hammond et al., 1985). The lesions begin forming at the base of the plant prior to flowering, and completely girdle the stem by the time silique filling occurs. Because it is cut off from it's root system, the plant ripens prematurely, producing shrivelled seeds. Siliques on infected plants tend to shatter more easily, resulting in further yield losses. Partial control of this disease is achieved by growing canola once in a three-year rotation. Due to the short-lived nature of the organism, proper rotation can greatly reduce the amount of inoculum on crop residue, in the soil (Martens et al., 1988).

The most effective and economical method of controlling this pathogen is through breeding for genetic resistance (Bansal et al., 1994). There remains controversy regarding the genetics of blackleg resistance. Delwiche (1980) identified two dominant linked genes (*Lm* 1 and *Lm* 2) governing cotyledon resistance to blackleg in two French winter *B. napus* breeding lines, while Sawatsky (1989) identified a single recessive gene governing cotyledon resistance in two summer oilseed rape breeding lines. Sawatsky (1989) also identified adult-plant resistance to a Manitoba isolate of *L. maculans* in two spring rapeseed lines (R83-14 and R83-17), which was governed by two dominant, complementary genes. These findings indicated that adult and seedling resistance in *B. napus* may be

independent. However, Stringam et al. (1992) found that blackleg resistance, transferred from the Austrailian *B. napus* cultivar Maluka to a canola quality *B. napus* breeding line, is controlled by either a major gene or tightly linked polygenes that are inherited as a unit. Bansal et al. (1994) went on to demonstrate that the genetic resistance to *L. maculans* transferred from Maluka was present at both the cotyledon and adult plant stages. The blackleg resistant canola cultivar Quantum, developed by the University of Alberta canola breeding program, was obtained from the crosses with the Australian cultivar Maluka (Stringam et al., 1995).

Blackleg resistance, observed in *Brassica* species with the **B** genome (*B. juncea* and *B. nigra* L.), appears to be different from that seen in many of the resistant *B. napus* rapeseed cultivars, as it is absolute and stable at all stages of growth (Sacristan and Gerdemann, 1986). Roy (1978, 1984) was able to transfer a high level of resistance from *B. juncea* to *B. napus* through an interspecific cross. Although many, but not all of Roy's interspecific lines were found by Rimmer and van den Berg (1992) to be addition lines, it was still evident that disease resistance could be transferred between species. Based on the fact that adult-stage resistance was readily transferred while complete resistance was only recovered in a few lines, Roy (1978) suggested that resistance is governed by two loci, with adult-stage resistance residing on the **A** genome while seedling resistance may be on the **B** genome. Roy believed that the presence of seedling resistance on the **B** genome explains the difficulty in obtaining complete resistance through interspecific crosses as *B. napus* does not have a **B** genome complement and therefore

seedling resistance would be expected to be transferred through a non-homologous recombination event only (Roy 1978).

Keri et al. (1997) attempted to determine the genetics controlling blackleg resistance in *B. juncea*. In the cross they made, 2 gene pairs interacting in a dominant/recessive manner, determined both seedling and stem resistance to blackleg in *B. juncea*. They hypothesized that for *B. juncea*-type resistance to be maintained in the progeny of interspecific crosses with the susceptible members of the Brassicaceae, both genes may have to be transferred because the dominant gene has major effects on the expression of the resistant phenotype, and the recessive gene has modifying effects on the expression of the dominant gene. Another mechanism proposed by Keri et al. (1997) was that transfer of one of the two genes might result in expression of resistance in the recipient species as either a dominant or a recessive character.

B. napus varieties with strong resistance to the blackleg pathogen have been developed, however no strong resistance has been found in canola or rapeseed cultivars of *B. rapa*. Rouxel et al. (1991) has identified wild *B. rapa* accessions from Sicily that demonstrate a blackleg resistance reaction similar to that seen in *B. juncea* and *B. nigra*. Crouch et al. (1994) was able to resynthesize *B. napus* by combining one of the wild *B. rapa* lines with *B. oleracea* L. The synthetic *B. napus* type demonstrated a high degree of blackleg resistance indicating that this wild relative may be a useful source of resistance genes (Crouch et al., 1994).

There is evidence which suggests *L. maculans* posses a high degree of

variability in its capacity to cause severe disease on a range of *B. napus* types (McGee and Petrie, 1978). Based on cotyledon tests with *B. napus* cultivars Westar, Glacier, and Quinta, Mengistu et al. (1991), was able to classify isolates of *L. maculans* into 4 pathogenicity groups (PG 1 to 4). Based on their identification of the first avirulence gene of *L. maculans*, as well as the findings that virulence and resistance appear to be controlled by one or a few genes, Ausan-Melayah et al. (1995) suggested that this plant-pathogen system follows a gene-for-gene model.

Another fungal pathogen that can be found affecting canola is *Albugo candida* (Pers. ex Hook.) Kuntze, the causal agent for the white rust and staghead diseases. There are at least 10 pathotypes of *A. candida*, two of which affect *Brassica* species of economic importance. Race 2, identified by Pound and Williams (1963), can be found on *B. juncea*, while race 7, identified by Petrie (1988), affects *B. rapa.* Although no pathotypes are known whose primary host is *B. napus*, some genotypes of this species, primarily from China, are susceptible to race 7 (Fan et al., 1983).

Seed or soil borne oospores of *A. candida* germinate under moist conditions and infect young seedlings. Raised white pustules form on the underside of cotyledons and leaves, and from these pustules, zoosporangia produce a secondary inoculum capable of infecting leaves higher up on the plant as well as the leaves of other plants. When infection occurs on the flowering meristem, distorted stems and floral parts called stagheads are produced. These stems which are infected by the pathogen, produce little or no seed (Rimmer & Buchwaldt, 1995).

On average, 6 to 10% of flowering stems are infected under normal field conditions, while under more favourable conditions, the number of infected stems can become significant (Martens et al., 1988). Although yield loss due to infection by *A. candida* usually tends to be minimal, the presence of conspicuous white pustules and malformed flowering structures cause concern for canola producers (Bansal VK, personal communication). To be registered as a canola variety, the Western Canadian Canola/ Rapeseed Recommendation Committee (WCC/RRC) has set the standard that, cultivars of *B. napus* can not show greater than 10% infection with white rust, while varieties of *B. rapa* must score better than the *B. rapa* check variety (Tobin).

Studies regarding the genetics governing the host-parasite interaction involving white rust have focused on the level of specificity among races (Saharan and Verma, 1992). Resistance to race 2, governed by single dominant genes, has been chracterized in *B. nigra, B. campestris, B. carinata, and B. juncea* (Delwichie and Williams, 1981, 1974; Ebrahimi et al., 1976; Thukral and Singh, 1986). Fan et al. (1983) identified 3 genes in the *B. napus* cultivar Regent that governed resistance to white rust. They also found that a dominant gene at any one of these loci is sufficient enough to give a resistant phenotype. Canadian cultivars of *B. napus* are mostly resistant to indigenous populations of the pathogen while only a few *B. rapa* cultivars like Tobin show some resistance to the pathogen. Downey and Rimmer (1993) indicated that resistance to *A. candida* in Tobin is under the control of a single dominant gene.

Developing *B. rapa* cultivars with complete resistance to white rust is difficult due to the self-incompatibility system present in the species. Breeding *B. rapa* as an open-pollinating species reduces inbreeding depression, but results in segregation of resistance genes within the plant population selected as a cultivar. Ten to twenty five percent of the plants within the best resistant cultivars can be expected to show disease symptoms (Rimmer & Buchwaldt, 1995). Fan et al. (1983) and Liu et al. (1996) believe that resistance to white rust in *B. napus* has not broken down because of the number of genes involved. It appears that the fungus has not yet been able to evolve sufficiently to overcome the multiallelic resistance in *B. napus.*

1.4. Genomic Relationships Among the Brassicaceae:

Analysis of meiotic configurations and differential chromatin condensation can be utilized to aid in classification of species. Chromosome preparations, particularly at the diakinesis stage of meiosis are most effective as chromosomes are condensed enough to be observed under the microscope and pairs have not yet separated (Cheng et al., 1994).

Analysis of species with small chromosomes, like the Brassicaceae, requires special techniques for fixing and staining chromosomes, which tend to be lengthy and labour intensive. Chromosomes, within the pollen mother cells of *Brassica* species, have distinct heterochromatic and euchromatic regions, creating a situation

where adequate staining of euchromatin, without loss of detail by overstaining is difficult (Stringam, 1970). Stringam (1970) discovered that Newcomer's solution, supplemented with 3% (w/v) basic ferric acetate as a mordant in the fixative, produced improved staining with acetocarmine. Historically, the textiles industry utilized basic ferric acetate as a mordant to fix natural dyes to fabric (Casey and Doyel, 1967). With the production of synthetic dyes, there has been a movement away from using ferric acetate, and therefore commercially produced, pure sources of the chemical are difficult to obtain. Lack of a reliable source, providing acceptable staining, resulted in the need to find other sources of mordants for the present study.

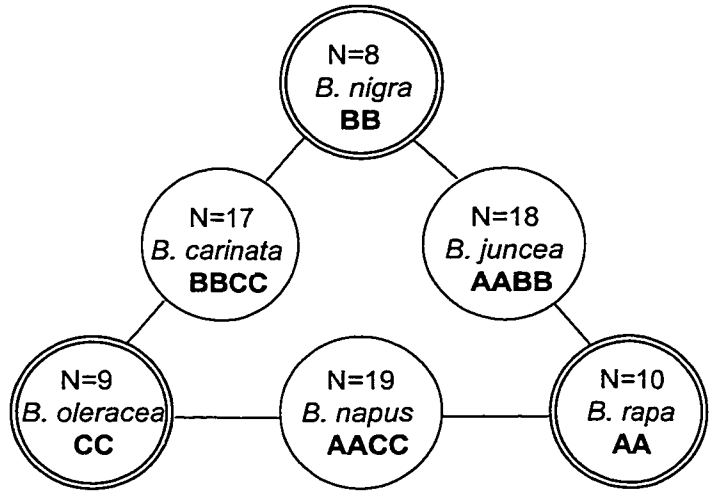
Extensive cytological analysis has been conducted on members of the Brassicaceae. During the 1920's and early 1930's, the chromosome numbers of the *Brassica* species were determined (reviewed by U, 1935). While studying meiotic chromosome pairing in the hybrids obtained through interspecific crosses between the *Brassica* species, Morinaga (1929a,b,c & 1934) hypothesized that the three tetraploid species may have evolved from natural interspecific hybrids amongst the three existing diploid species. Morinaga (1929a,b,c & 1934) assigned the **A** genome to *B. rapa*, the **B** genome to *B. nigra*, and the **C** genome to *B. oleracea*, and therefore the genomes of the tetraploids, based on the amphidiploid theory became **AB** of *B. juncea*, **BC** of *B. carinata*, and **AC** for *B. napus*.

Diploids: *B. nigra* 2n=16, *B. oleracea* 2n=18, *B. rapa* 2n=20

Tetraploids: *B. carinata* 2n=34, *B. juncea* 2n=36, *B. napus* 2n= 38

U (1935) confirmed the genomic relationship by artificially resynthesizing the amphidiploid species from the diploid species, and by observing chromosome pairing in the interspecific hybrids he produced. U (1935) illustrated the inter-relatedness of the *Brassica* species in what is now referred to as U's triangle (Figure 1.1.).

Figure 1.1. U's Triangle. Diagrammatic representation of the genomic relationships among the species of *Brassica*, indicating the gametic chromosome number (N) and genome designations assigned by Morinaga (1929a,b,c and 1934) (after U, 1935).



Studies by Röbbelen (1960) of chromosome structure in the diploid species revealed that there are only six distinctly different chromosomes. This indicates that the three elemental species may actually be segmental alloploids that evolved from a common progenitor species with a basic chromosome number of 6 (from Downey

and Röbbelen, 1989). Lagercrantz and Lydiate (1996) have recently challenged this theory. Using molecular markers, Lagercrantz and Lydiate (1996) discovered that the diploid genome of *B. nigra* consists of 3 complete, but rearranged copies of a fundamental ancestral genome. This suggests that this species descended from a hexaploid ancestor rather than through the addition/duplication of individual intact chromosomes from an n=6 ancestor. Considering the fact that the diploid *Brassica* species contain similar amounts of nuclear DNA (Arumuganathan and Earle, 1991), with the observed conservation of genome content across the three genomes, lead Lagercrantz and Lydiate (1996) to conclude that all the *Brassica* genomes may have inherited a complete but rearranged copy of a duplicated ancestral genome. There is evidence of chromosome fission and fusion occurring during the divergence of the 3 genomes, which may have been responsible for changes in chromosome number, rather than the duplication and/ or elimination of whole chromosomes proposed by Röbbelen (1960).

Although U (1935) initially reported that chromosome pairing in *B. napus* (**AACC**) by *B. rapa* (**AA**) interspecific hybrids was confined to the **A** genome (autosyndetic pairing), reports of pairing between chromosomes from the different *Brassica* genomes (allosyndetic pairing) have since been reported in many interspecific crosses. Busso et al. (1987) showed that although pairing occurs less frequently with the **B** genome, both autosyndetic and allosyndetic pairing occurs between the **A**, **B**, and **C** genomes. Several authors including Prakash and Chopra (1993), Song et al. (1993), McGrath and Quiros (1990), Tai and Ikonen (1988),

Busso et al. (1987), Prakash and Hinata (1980), U (1935), and Catchside (1934), have reported quadrivalent chromosome associations in interspecific *Brassica* progeny. These authors concluded that multivalent associations such as quadrivalents are the result of non-homologous recombination and intragenomic pairing. The genomes of the naturally occurring amphidiploid *Brassica* species are suspected to have undergone enough genetic differentiation to lose their ability to pair allosyndetically (Prakash and Hinata, 1980), or a genetic system which suppresses non-homologous (allosyndetic pairing) may be present (Prakash, 1974), resulting in fewer observed multivalent associations.

Because of the inter-relatedness of these species, and their subsequent ability to form interspecific hybrids, several researchers have reported success in transferring traits between *Brassica* species. Scarth et al. (1992) reported on the development of an *Albugo candida* race 7 resistant *B. rapa* cultivar, which was developed through interspecific crosses between *B. rapa* and *B. napus*. Roy (1984) transferred blackleg resistance from *B. juncea* to *B. napus* through interspecific crosses. Clubroot resistance was transferred to *B. napus* from *B. rapa* by Growers (1982), Johnston (1974), and Lammerink (1970). Low glucosinolate *B. juncea* has been created by interspecific crosses between *B. rapa* and *B. juncea* (Love et al. 1990). Rashid et al. (1994) was able to produce stable yellow seeded *B. napus* through interspecific crosses with *B. juncea*, *B. carinata*, and *B. napus*.

1.5. Doubled Haploid Breeding Method:

Microspore culture is known to be an effective means of hastening the in-breeding process in a number of crops. By culturing immature pollen grains to produce haploid plants, and then later doubling their chromosome number by treating with colchicine, 5 to 6 generations of self-pollinating normally required to reach homozygosity can be eliminated. This procedure can reduce the time required to produce a new cultivar by 3 to 4 years (Stringam, 1992).

Haploid-derived populations are ideal for quantitative genetic and linkage studies (Tanhuanpää et al., 1994). Since a doubled haploid (DH) population contains no heterozygous individuals, genetic ratios are much simpler than in conventional genetic populations, and differences between classes are more distinct (Choo et al., 1985).

Because of the success in the U of A breeding program of using doubled haploidy in breeding conventional *B. napus* and in interspecific crosses between *B. juncea* and *B. napus*, this technique was used to study the progeny of interspecific crosses between *B. napus* and *B. rapa*. Doubled haploidy has the potential to eliminate fertility problems caused by unpaired chromosomes, which can arise in these interspecific plants (Thiagarajah et al., 1994; Stringam et al., 1995).

The outcrossing nature and relative poor performance of *B. rapa* when compared to *B. napus*, coupled with the fact that most of the centres developing canola tend to be in areas predominated by *B. napus*, has contributed to a lag in the

breeding of *B. rapa* compared to *B. napus*. By using the Doubled Haploid breeding technique in an interspecific breeding program centred around crosses between self-compatible *B. rapa* and *B. napus*, it may be possible to produce a *B. rapa* germplasm base containing lines that are self-compatible, and display some of the better traits from *B. napus*. This approach has the potential of altering the breeding behaviour of *B. rapa* from self-incompatible to self-compatible (Stringam GR, personal communication) and could result in the development of high yielding, disease resistant cultivars with the potential for surpassing current yield plateau's in this species.

1.6. Genetic Characterization Using Random Amplified Polymorphic DNA Markers (RAPD's):

DNA markers have become useful tools for genetic mapping of traits, gene cloning, and marker assisted selection in many species. Some of the first molecular markers were phenotypic markers which relied on variations in expressed proteins, termed isozymes (Stuber, 1992). Discovery of techniques capable of exposing genetic differences between individuals has lead research towards molecular markers in screening for traits of interest. Molecular markers are more reliable as they are phenotypically neutral and are not affected by environmental interactions (Lander and Botstein, 1989). Molecular markers are not usually the gene of interest, but are closely linked with the trait (Weeden, 1993).

Restriction fragment length polymorphism (RFLP) are one type of molecular markers which detects differences due to nucleotide substitution, insertion, deletion, or rearrangement of genomic DNA sequences (Chang and Meyerowitz, 1991). RFLP's behave in a codominant manner, which allows for determination of genotype in a plant derived from any two parents (Lander and Botstein, 1989). RFLP's are being used to generate genetic maps in many *Brassica* species (Landry et al., 1991; Lydiate et al., 1993; Quiros et al., 1987; Song et al., 1991).

Molecular markers have provided a new technique for the study of genome evolution. Homoeologous loci and collinear segments of DNA can be identified by comparing genetic linkage maps, produced for two species using a common set of markers (Lagercrantz and Lydiate, 1996). Applying RFLP technology, Lagercrantz and Lydiate (1996) have been able to shed new light on the origins of the *Brassica* genomes.

A lack of saturated genetic maps for the Brassicaceae, results in difficulty in applying traditional mapping techniques. Williams et al. (1990) and Welsh and McClelland (1990) simultaneously discovered that arbitrary nucleotide sequences could be used as primers in PCR reactions to reveal polymorphisms, which were useful as genetic markers. These types of markers have been named Random Amplified Polymorphic DNA (RAPD's).

The Polymerase Chain Reaction (PCR) is an enzyme mediated DNA amplification technique. Genomic DNA, oligodeoxynucleotide primer, thermostable *Taq* Polymerase, deoxyribonucleoside triphosphates (dNTP's), buffer, and salts are

combined in reaction tubes and placed in a thermocycler. One PCR cycle involves the thermocycler changing the temperature from 95°C to denature the DNA, to 36°C to promote annealing of the primer to the DNA, to 72°C to optimise DNA amplification by the *Taq* polymerase.

The first cycle can produce several products of different lengths, depending on the number of primer binding sites within the genomic DNA. The first produces variable length, double stranded regions along the two original strands of genomic DNA. In the second cycle, the same primer sites on the genomic DNA give rise to another set of variable length products. These products continue to accumulate arithmetically with each cycle. When a second primer binding site exists in an inverted orientation on the opposite strand of the genomic DNA, within 2000 to 3000 bases downstream, the fragment of DNA between the two primer sites is amplified exponentially (Ausebel et al., 1996). Because the genomic DNA as well as each new fragment amplified from these regions have primer binding sites, the number of templates available for amplifying new strands increases exponentially with each cycle and therefore these fragments accumulate more quickly.

When the PCR products are separated by gel electrophoresis, DNA bands can be visualised by ethidium bromide fluorescence (Sambrook et al., 1989). Scoring presence versus absence of bands visualised in the gel identifies a polymorphism. Polymorphisms are the result of single base pair changes, deletions of primer sites, insertions that increase the separation of primer sites over the 3000 base pair limit, and insertions or deletions that result in changes in the size of the

fragment length (Yu et al., 1993).

By using arbitrary nucleotide sequences as primers, this technique eliminates the need for target DNA sequence information which is required to build primers for other PCR based systems, and provides an alternative to the labour intensive and lengthy use of southern blotting to detect RFLP's. The markers revealed by these primers behave as dominant traits as a single fragment is either amplified or not at one locus and segregate in Mendelian fashion (Williams et al., 1990). This can be a disadvantage to the technique as different alleles at the same locus are rarely amplified (Landry, 1993).

Quiros et al. (1991) found that 3 sets of decamer oligonucleotide primers disclosed 65 genome-specific markers for the diploid species *B. rapa*, *B. oleracea*, and *B. nigra* as outlined by U (1935). These markers provided Quiros et al. (1991) with the opportunity to map the **B** genome and to determine the feasibility of introgressing the **B** genome into the **A** and **C** genomes of *B. napus* through interspecific aneuploidy.

1.7. Objective:

This project was developed to evaluate the potential for introgressing traits of agronomic importance, between *B. napus* and *B. rapa* during interspecific crosses. Microspore culture was utilized to accelerate the inbreeding process by rapidly "fixing" desirable genotypes, and eliminate fertility problems caused by

unpaired chromosomes, which are known to arise in interspecific plants (Thiagarajah et al., 1994; Stringam et al., 1995). Interspecific doubled haploid lines were evaluated for their field agronomics, oil and protein quality, disease resistance, and chromosome number. Genome-specific RAPD markers were utilized to demonstrate whether an exchange of genetic material occurred during the introgression process.

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Chapter 2

Chromosome Number of Doubled Haploid Lines Derived from Interspecific Crosses between *Brassica napus* and *Brassica rapa*.

2.1. Introduction:

Prior to 1996, *Brassica rapa* L. (Polish Canola) varieties dominated in Alberta because of the short growing season in a major proportion of the canola producing areas (Thomas P, personal communication). Agronomic advances in *B. rapa* have been hindered by the difficulties of breeding a self-incompatible species, while in *B. napus* L., yield performance and disease resistance have been improved dramatically over the past ten years. The potential yield benefit of *B. napus* has attracted more Alberta farmers to growing cultivars of this species, although there is a risk that the crop may be damaged by early fall frost because of its tendency to be late maturing. Alberta farmers and the canola industry could greatly benefit from new cultivars of *B. rapa* containing a number of the superior agronomic traits found in *B. napus*, or *B. napus* cultivars with early maturity. As part of a larger study to determine the potential for transferring traits of agronomic importance between *B. napus* and *B. rapa*, the chromosome number of doubled haploid lines produced from an interspecific modified backcross (Figure 2.1.) was determined.

The genomic relationship among the *Brassica* species was determined in the 1930's. Through cytological studies, Morinaga (1929a,b,c & 1934)

hypothesized that the tetraploid *Brassica* species evolved from natural interspecific hybrids amongst the three existing diploid species, i.e. *B. napus* evolved from a natural hybrid between *B. oleracea* and *B. rapa*. U (1935) later confirmed the genomic relationship by artificially resynthesizing the amphidiploid species from the diploid species, and by observing chromosome pairing in the interspecific hybrids he produced through crossing these species. U (1935) illustrated the inter-relatedness of the *Brassica* species in what is now referred to as U's triangle (Figure 1.1.). Because *B. napus* and *B. rapa* share a common **A** genome which consists of 10 chromosomes, interspecific crosses can be used to transfer genetic material between the two species.

U (1935) reported that interspecific crosses could be made between *B. napus* and *B. rapa* using either species as the female parent, although seed germination in the hybrid was better when *B. napus* was used as the female parent. Similar crosses made in the years to follow revealed that the success of interspecific crosses between *B. napus* and *B. rapa*, may be influenced by the varieties used as parents. Becker (1950), Hoffmann and Peters (1958), McNaughton (1963), and Röbbelen (1965), found that crosses between *B. campestris* (*B. rapa*) and *B. napus* were unsuccessful when *B. campestris* (*B. rapa*) was used as the female parent although the reciprocal was fertile. However, Calder (1937), Palmer (1962), and Lammerink (1970) successfully crossed these two species using either species as the female parent (from Quazi, 1988).

Crosses between *B. rapa* and *B. napus* were made extensively in the 1930's, to transfer early maturity to *B. napus* varieties (U, 1935). Growers (1982), Johnston (1974), and Lammerink (1970) were able to transfer clubroot-resistance from *B. campestris* (*B. rapa*) (turnip) to oilseed *B. napus*, through 3 different types of interspecific crosses. The first low erucic acid and low glucosinolate *B. rapa* cultivars were developed from interspecific crosses made between *B. rapa, B. napus,* and *B. juncea* (Downey et al., 1975). Low glucosinolate *B. juncea* was also developed through interspecific crosses between *B. rapa* and *B. juncea* (Love et al., 1990). Roy (1984) reported the transfer of blackleg resistance from *B. juncea* to *B. napus*. Although many, but not all of the *B. napus* cultivars were found to be addition lines and subsequently lost their resistance in later generations (Rimmer and van den Berg, 1992), there is obvious potential for transferring traits of agronomic importance between species through interspecific crosses.

The transfer and subsequent loss of blackleg resistance in the *B. napus* X *B. juncea* crosses made by Roy (1984) underscores the importance of performing cytological analysis on the progeny of interspecific crosses. Determining chromosome behavior within the progeny and backcross progeny may indicate the stability of traits that appear to have been transferred.

Special techniques for fixing and staining chromosomes in species with small chromosomes, like the Brassicaceae, are essential. Although root tip and other somatic chromosomal preparations are available, to gain insight into

chromosome pairing and behavior during meiosis, meiotic preparations such as anther squashes are required.

Chromosomes, within the pollen mother cells of *Brassica* species, have distinct heterochromatic and euchromatic regions, creating a situation where adequate staining of euchromatin, without loss of detail by overstaining is difficult (Stringam, 1970). Stringam (1970) discovered that Newcomer's solution, supplemented with 3% (w/v) basic ferric acetate as a mordant in the fixative (see materials and methods) resulted in improved staining with acetocarmine. Historically, the textiles industry utilized basic ferric acetate as a mordant to fix natural dyes to fabric (Casey and Doyel, 1967). With the production of synthetic dyes, there has been a movement away from using ferric acetate, and therefore commercially produced, pure sources of the chemical are difficult to obtain. Lack of a reliable source, which provided acceptable staining resulted in the need to find other sources for the present study.

Recent studies have shown that the use of doubled haploidy in interspecific crosses between *B. juncea* and *B. napus* is effective in overcoming the fertility problems associated with chromosomal and genic imbalances (Thiagarajah et al., 1994; Stringam et al., 1995). Accordingly, we have applied the doubled haploid method after the initial cross (*B. napus* X *B. rapa*), as well as after the second cross (Interspecific DH_1 X *B. rapa*) to alleviate some of the fertility problems in this modified backcross scheme. Essential improvements in the mordanting process in addition to the cytological analysis of the interspecific DH progeny are reported.

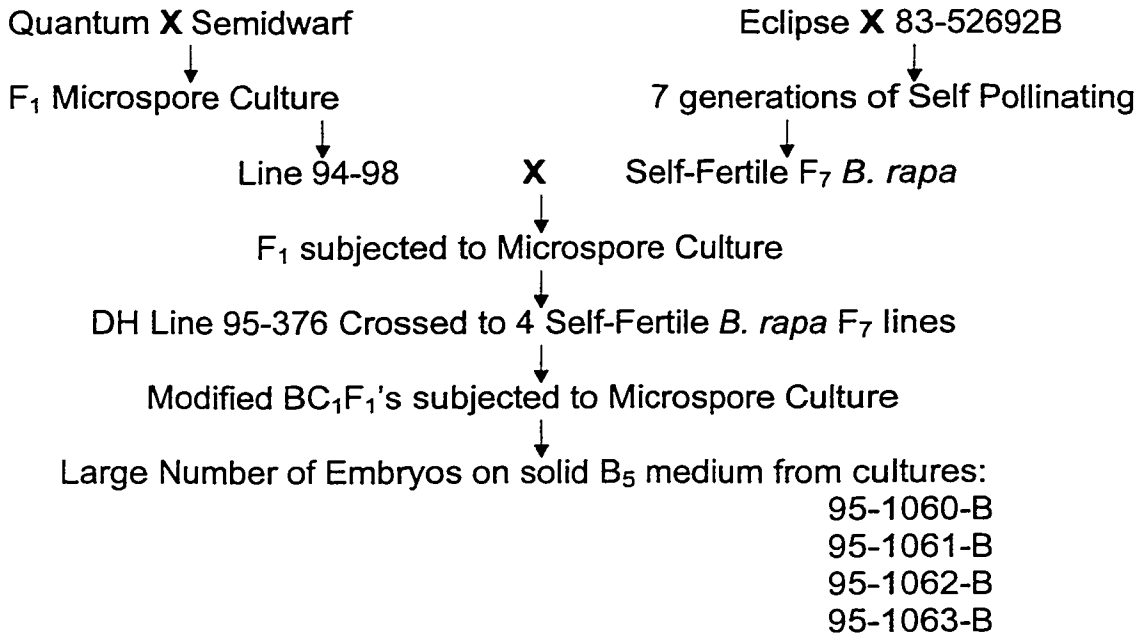
2.2. Materials and Methods:

2.2.1. Crossing Scheme:

Initial background germplasm development for this project was completed under Dr. Stringam's canola program at the University of Alberta (Figure 2.1.). Buds from four F_1 generation plants of a cross between *B. napus cv* Quantum, and a semidwarf *B. napus* line with early maturity, were bulked and subjected to microspore culture, and the doubled haploid line 94-98 was isolated. Line 94-98 was 2 to 3 days earlier maturing than Quantum, had blackleg and white rust (race 2 and 7) disease resistance, and was highly embryogenic, but had lower oil content and was lower in yield than Quantum. Line 94-98 was crossed to a self-fertile F_7 *B. rapa* line, derived from an interspecific cross between the cultivar Eclipse and U of A *B. napus* breeding line number 83-52692B. Line number 83-52692B was a Dwarf F_6 line, derived from a cross between *B. napus* cultivar Tower and a colchicine induced dwarf *B. napus* line (Figure 2.1.), produced by Brian Fowler (Crop Development Centre, University of Saskatchewan). Numerous doubled haploids ranging in fertility from completely fertile to partially fertile were obtained from microspore culture of the F_1. Line 95-376, selected from these, had partially yellow seed, upright silique angles, and was fertile. Line 95-376 was crossed to 4 sister lines of the self-fertile F_7 *B. rapa* line used in the original cross, in a modified backcross scheme. The *B. rapa* lines used in the crosses, had acceptable oil and protein levels, but also had some residual female

sterility. Buds collected from four plants within each backcross were bulked, and microspore culture was performed. Responsibility of the project was assumed when the microspores from the 4 backcross cultures (Figure 2.1.), were beginning to germinate into plantlets, on solid B_5 medium, and carried them through the rooting and doubling stages.

Figure 2.1. Interspecific crossing scheme.



2.2.2. Microspore Culture:

The F_1 and backcross F_1 donor plants produced through interspecific crosses were grown in soil free medium (Stringam, 1971), in a greenhouse at 20/ 18°C day/night temperature, under natural light supplemented with high intensity

sodium vapor lights (HID Sylvania, Canada). A minimum light intensity of 450 µmol $m^{-2}s^{-1}$ was maintained, for a 16 hour photoperiod. Plants were fertilized with 20-20-20 fertilizer solution bi-weekly. Prior to bolting, donor plants were moved to a Conviron growth cabinet (model number E15). The plants were grown at 10°C day / 8°C night temperature, under very high output fluorescent and 40 watt incandescent lights which supplied a minimum of 425 µmol $m^{-2}s^{-1}$ of light for a 16 hour photoperiod. Microspore culture was performed according to Coventry et al. (1988), modified for *B. rapa* by Ferrie and Keller (1995) with the following exception. Embryos which developed into plantlets, with true leaves, were transferred to Majenta boxes containing solid Murashige and Skoog medium (2 % sucrose, 1 % agar), prior to being transferred to soil, to allow the plantlets to become more hardy.

2.2.3. Plant Material Screened for chromosome number, and Growth Conditions:

Fifty microspore-derived modified backcross F_1 individuals (Doubled Haploid lines), obtained from the above crossing scheme, and the parents used to generate these DH lines were grown in an Enconaire Environmental Chamber (model GRB-168). The temperature regime was 17°C day to 15°C night, and the plants were provided with 350 µmol $m^{-2}s^{-1}$ of light from very high output fluorescent and 40 watt incandescent bulbs, over a 16 hour photoperiod. Four

plants of each DH line were grown in sterile soil free medium (Stringam, 1971), and fertilized with 20-20-20 fertilizer bi-weekly.

2.2.4. Terminal Bud Selection and Preservation:

Terminal buds were harvested at approximately 11:00 am, prior to elongation of the main stem, when a broad range of individual flower bud sizes were still present within the bud cluster. The plants ranged in age from 28 to 63 days old. Time of bud harvest is crucial as it greatly influences the ease at which the proper meiotic stage can be identified. Harvested buds were fixed in Newcomer's solution (Newcomer, 1953) to which powdered basic ferric acetate was added, for 24 hours at room temperature (Stringam, 1970). Fixed buds were then stored in the fixative at 4°C until analyzed.

2.2.5. Preparation of Fixing Solution:

One commercial source of basic ferric acetate, obtained would not disolve in the fixative and therefore an attempt was made at preparing it fresh. Basic ferric acetate was prepared according to Casey and Doyle (1967), by heating an Iron II acetate solution and exposing the precipitate to air. Twelve grams of iron powder (reduced by hydrogen; BDH Prod 28602 4P) was combined with 400 ml of glacial acetic acid in a 1000 ml beaker. A magnetic stir bar was added, and the beaker was covered with Parafilm to minimize evaporation. The reactants

were mixed using a Fisher Model 310T Thermix Stirring hot plate, until a color change from milky white, through chocolate brown, to black was observed (approximately 24 to 48 hours). The unreacted iron was removed with the magnetic stir bar. The black solution of ferrous acetate was warmed to 58°C using a hot plate, and air was gently bubbled through it while mixing continued. Because glacial acetic acid posses a moderate fire hazard when exposed to heat or flame, has a flash point of 39°C, and produces explosive fumes when heated beyond 59°C, all reactions were carried out in the fume hood with close monitoring of temperature. As the volume of acid decreased, a reddish brown precipitate of basic ferric acetate began to form. Heating was continued with aeration until a thick slurry of ferric acetate was produced. The slurry was stored uncovered until the remaining acetic acid evaporated. Basic ferric acetate is light sensitive and therefore was stored in the dark. Approximately 35 grams of reddish brown scale, which had a slight acetic acid smell, was recovered and crushed into fine powder.

The final product was sent for chemical analysis to determine if ferric acetate had been produced. Infrared spectrum and combustion analyses were performed on the ferric acetate by Jim Hoyle (Analytical Service Laboratory, Dept. Chemistry, U of A). The Alberta Research Council analyzed the sample using Scanning Electron Microscopy and X-ray diffraction.

The basic ferric acetate was added to Newcomer's fluid in a ratio of 3% w/v as a mordant for improved staining in plants with small chromosomes (Stringam, 1970). Newcomer's solution consists of 2-propanol, propionic acid,

petroleum ether, acetone, and 1,4-dioxane in a ratio of 6:3:1:1:1 (Newcomer, 1953). The Newcomer's solution and ferric acetate were stirred together for 24 hours. The dark purple to amber colored solution obtained was decanted to remove any basic ferric acetate that did not dissolve. The mordant was stored in a brown bottle at 4°C until needed.

2.2.6. Staging, Anther Squash and Photography:

Buds were removed from the fixative, placed in a disposable plastic petri dish, and kept moist with 70% ethanol. A single anther was dissected from the oldest bud, placed onto a clean glass slide, in a drop of 1% acetocarmine, and the anther was quickly macerated using an iron needle. The slide was observed under 200X magnification to identify the meiotic stage of the pollen mother cells. By starting from the largest and working inward to the smallest bud, the proper meiotic stage for observing meiotic configurations was identified. Slides containing cells at diakinesis to metaphase I were covered by a cover slip after the tapetal debris was removed. The underside of the slide was heated over a steam jet. While still hot, the slide was inverted, placed on a piece of tissue paper, and pressed to flatten the cells. The slide was temporarily sealed with clear nail polish, and the pollen mother cells observed under oil emersion (600 and 1000X magnification). A minimum of 6 pollen mother cells at diakinesis to early metaphase I, were observed to determine the chromosome number of each DH line. Pollen mother cells at later meiotic stages were checked for the

presence of aberrant chromosome behavior. Photographs were taken under oil emersion, using an Olympus microscope (Model BH-2) equipped with a 35mm camera (Olympus C-35AD) and an Olympus Exposure control unit.

2.2.7. Pollen Fertility:

Pollen fertility was determined by differential staining, using acetocarmine as described by Lesley and Lesley (1939). Four flowers were harvested from the dominant raceme of the plants grown for cytological characterization. Pollen grains from dehiscing anthers were brushed onto a clean microscope slide and a drop of 1 % acetocarmine and a coverslip added. Under low magnification, the number of large, stained pollen grains and small, unstained pollen cells were recorded in 4 different microscope fields. The percentage viable pollen was calculated by dividing the number of fully developed, stained pollen grains by the total number of pollen grains counted.

2.3. Results:

Reciprocal crosses did not produce equal results in this study. Only crosses where the *B. napus* DH line 94-98, and interspecific DH line 95-376 were used as the female parent, resulted in seed production.

For the current study, the only obtainable source of basic ferric acetate did not produce the results desired, and therefore an attempt was made at preparing

it fresh. Preliminary chemical analysis revealed that the synthesized basic ferric acetate was not pure. SEM indicated that ferric and ferrous iron were present in the sample. The IR analysis and X-ray diffraction of the prepared ferric acetate were inconclusive because known profiles for this chemical could not be found in the chemical libraries.

Chromosome counts, pollen viability, and observations made were for each line (Table 2.1.). Pollen mother cells were observed at various stages, however the chromosome counts were made at diakinesis to early metaphase I.

2.3.1. Characterization of the *Brassica napus* and *Brassica rapa* Parents:

Pollen mother cells sampled from the five *B. rapa* parents (93-1005-3, 95-439-2, 95-440-2, 95-442-3) that were used in the crosses, all contained 10 bivalents at diakinesis (Figure 2.2.A), while those sampled from the *B. napus* parent (94-98) contained 19 bivalents (Figure 2.2.B). Chromosome stickiness was observed in the PMC's of the *B. rapa* parents. Average pollen viability was above 90% in all parents except *B. rapa* line 95-442-3 which had a pollen viability of 81% (Table 2.1.).

2.3.2. Characterization of the Doubled Haploid Lines:

The doubled haploid line 95-376 (Figure 2.2.C), obtained from the interspecific cross (*B. napus* X *B. rapa*), as well as the 50 DH lines obtained after

backcrossing, all contained 38 chromosomes. Within these lines, an array of 15 to 19 bivalents as well as univalents, quadrivalents and hexavalents were observed at diakinesis (Table 2.1.). A suitable stage for observing and counting chromosomes could not be identified for 6 of the DH lines.

A loose pairing of single homologous chromosomes was observed at varying degrees, in 11 of the 50 DH lines. All 38 chromosomes were present at diakinesis, however PMC's with 18 bivalents and 2 univalents, as well as PMC's with 17 bivalents, 1 univalent and 1 trivalent were also observed. Within these lines, the frequency of loose homologous pairing ranged from approximately 7 to 44%. The observation of 1 to 2 univalents located off the metaphase plate (Figure 2.3.A), and laggards at anaphase confirmed the univalents observed at diakinesis.

A high degree of chromosome stickiness made chromosome counts difficult in 3 of the 50 DH lines. One to two quadrivalents (rings of 4 chromosomes) were present in 37 of the DH lines (Figure 2.3.B), a hexavalent (ring of 6) was observed in 3 DH lines (Figure 2.4.C), and a chain of 6 was observed in 4 DH lines. Quadrivalents were also observed at early diakinesis (Figure 2.4.A). The cross shape indicates that chiasma formed between the 4 chromomsomes. Chiasma were apparent between the chromosomes of the rings as they began to terminalize later in diakinesis (Figure 2.4.B). Ring formations often carried through to metaphase. Non-disjunction, and chromosome bridge / fragment associations were observed at anaphase I, as well as telophase I and II (Figure 2.5.A,B,C). Although the frequency of ring

formation was high, the frequency of non-disjunction and improper disjunction was low. However, anaphase was not observed for every line and therefore these results are inconclusive.

Twenty-two DH lines had an average pollen viability greater than or equal to 90%, 16 had a viability greater than or equal to 80%, 5 had a viability greater than or equal to 70%, and 1 had a viability of 64%. When DH lines had higher frequencies of aberrant chromosome behavior, the pollen viability tended to be lower (Table 2.1.).

Table 2.1. Chromosome number, pollen viability, and observations in the parents, DH1, and modified backcross DH lines.

Line number	Parents	# PMC's Observed	Number bivalents	Pollen Viability (% ± Sterr)	Observations
93-1005-	F7 *B. rapa*	35	10	91 ± 1.6	stickiness
94-98	*B. napus*	10	19	96 ± 0.3	appeared normal
95-376	DH1	17	15,17,19	75 ± 3.7	1 to 2 Quadrivalents, non-disjunction observed
95-439-2	F7 *B. rapa*	25	10	91 ± 7.4	high amount of stickiness
95-440-2	F7 *B. rapa*	11	10	98 ± 0.3	stickiness
95-442-2	F7 *B. rapa*	9	10	97 ± 0.8	stickiness
95-442-3	F7 *B. rapa*	13	10	81 ± 7.0	stickiness
	DH Progeny				
96-1057-	95-1060-B	10	15,17,19	80 ± 2.2	1 to 2 Quad., high amount of stickiness, no laggards or non-disjunction
96-797-2	95-1060-B	7	19	98 ± 1.6	chains and stickiness
96-2015-	95-1060-B	0			
96-1062-	95-1061-B	8	17,18,19	84 ± 2.9	1 Quad.,3 PMC's showed a loose pair, laggards
96-1066-	95-1061-B	8	19	92 ± 3.0	stickiness
96-1069-	95-1061-B	12	17,18,19	92 ± 1.3	1 Quad, 1 PMC showed a loose pair
96-1179-	95-1061-B	13	15,17,19	97 ± 1.0	1 to 2 Quad., low frequency of non-disjunction at anaphase
96-1180-	95-1061-B	7	19	94 ± 1.1	high amount of stickiness
96-1183-	95-1061-B	11	19	92 ± 0.7	appears normal
96-1188-	95-1061-B	18	15,16,17,18,19	71 ± 5.1	1 to 2 Quad., 5 PMC's showed a loose pair, non-disjunction, chain of 6
96-1195-	95-1061-B	12	16,17,18,19	74 ± 5.5	1 Quad., 1 ring of 6, 2 PMC's showed a loose pair
96-1196-	95-1061-B	16	18,19	83 ± 1.3	stickiness, 7 PMC's showed a loose pair, anaphase appeared normal
96-1687-	95-1061-B	5	17,19	91 ± 3.4	1 Quad. low freq.,stickiness made counts difficult
96-1691-	95-1061-B	15	15,17,19	96 ± 1.1	1 to 2 Quad., non-disjunction noted at low frequency
96-1693-	95-1061-B	9	17,19	93 ± 1.1	1 Quad., stickiness made counts difficult
96-1696-	95-1061-B	14	17,18,19	91 ± 0.8	1 Quad. low freq., 2 PMC's showed a loose pair, no non-disjuction
96-1703-	95-1061-B	17	16,17,19	90 ± 4.0	1 Quad., non-disjunction noted at low frequency, chain of 6
96-1704-	95-1061-B	21	17,18,19	85 ± 3.4	stickiness, 1 Quad. at low freq., laggards and non-disjunction noted
96-1707-	95-1061-B	11	17,18,19	90 ± 2.7	stickiness ,1 Quad. low freq., 2 PMC's showed a loose pair, bridging
96-1711-	95-1061-B	0			

Table 2.1. continued

Line number	DH Progeny	# PMC's Observed	Number bivalents	Pollen Viability (% ± stderr)	Observations
96-1712-	95-1061-B	13	17,18,19	93 ± 0.8	1 Quad., 1 PMC showed laggards, and non-disjunction
96-1713-	95-1061-B	14	17,18,19	95 ± 0.8	1 Quad., 3 PMC's showed laggards, and non-disjunction
96-1718-	95-1061-B	12	15,17,18,19	85 ± 2.2	1 to 2 Quad., 3 PMC's showed laggards, bridging
96-1720-	95-1061-B	18	16,19	74 ± 3.7	stickiness, no anaphase observed, chain of 6
96-1727-	95-1061-B	13	17,19	83 ± 1.6	1 Quad.
96-1732-	95-1061-B	10	17,19	93 ± 0.9	1 Quad. low freq.
96-1734-	95-1061-B	11	17,19	84 ± 2.0	1 Quad. low freq.
96-1735-	95-1061-B	8	17,19	85 ± 1.8	1 Quad.
96-1743-	95-1061-B	8	17,19	96 ± 1.0	1 Quad.
96-1746-	95-1061-B	11	15,17,19	78 ± 1.5	1 to 2 Quad.
96-1747-	95-1061-B	12	17,19	93 ± 1.8	1 Quad., low freq of non-disjunction
96-1753-	95-1061-B	0			
96-1755-	95-1061-B	14	17,18,19	90 ± 1.6	1 Quad., 1 PMC showed a loose pair, 3 showed non-disjunction
96-1756-	95-1061-B	5	17,19	94 ± 1.1	1 Quad.
96-1758-	95-1061-B	0			
96-1759-	95-1061-B	29	15,17,18,19	85 ± 4.5	1 to 2 Quad., 2 PMC's showed a loose pair, 5 showed non-disjunction
96-1764-	95-1061-B	9	16,17,18,19	85 ± 0.6	1 Quad., ring of 6, 1 PMC showed a loose pair
96-1766-	95-1061-B	23	17,18,19	91 ± 0.5	1 Quad, 4 PMC's showed a loose pair, 5 PMC's showed non-disjunction
96-1767-	95-1061-B	10	19	86 ± 1.2	stickiness, bridging
96-1768-	95-1061-B	16	17,19	80 ± 2.2	1 Quad. low freq.
96-1773-	95-1061-B	13	17,19	98 ± 0.5	1 Quad. low freq.
96-1775-	95-1061-B	5	17,19	64 ± 5.5	1 Quad., a lot of stickiness and chaining made counts difficult
96-2016-	95-1061-B	16	15,17,19	83 ± 2.2	1 to 2 Quad., a lot of stickiness
96-2018-	95-1061-B	18	17,19	93 ± 0.5	1 Quad. at low freq.
96-2022-	95-1061-B	9	17,19	81 ± 4.4	1 Quad.
96-2025-	95-1061-B	24	15,16,17,18,19	70 ± 6.3	1 to 2 Quad., laggards in 4 PMC's, chain of 6
96-2027-	95-1061-B	27	16,17,18,19	82 ± 3.3	1 Quad., 5 PMC's showed laggards, ring of 6
96-1776-	95-1062-B	13	17,18,19	81 ± 3.8	1 Quad. low freq., 2 PMC's at diak & 5 at meta showed a loose pair
96-815	95-1062-B	0			
96-1698-	95-1063-B	0			

Figure 2.2. Diakinesis stage observed in *B. rapa*, *B. napus*, and interspecific DH_1 parents. **A** Diakinesis stage of *B. rapa* parent 93-1005-3 showing 10 bivalents, **B** *B. napus* parent 94-98 showing 19 bivalents (note the two pairs associated, indicated by arrows), **C** interspecific doubled haploid (DH) line 95-376 showing 15 bivalents and 2 quadrivalents (two quadrivalents indicated by arrows).

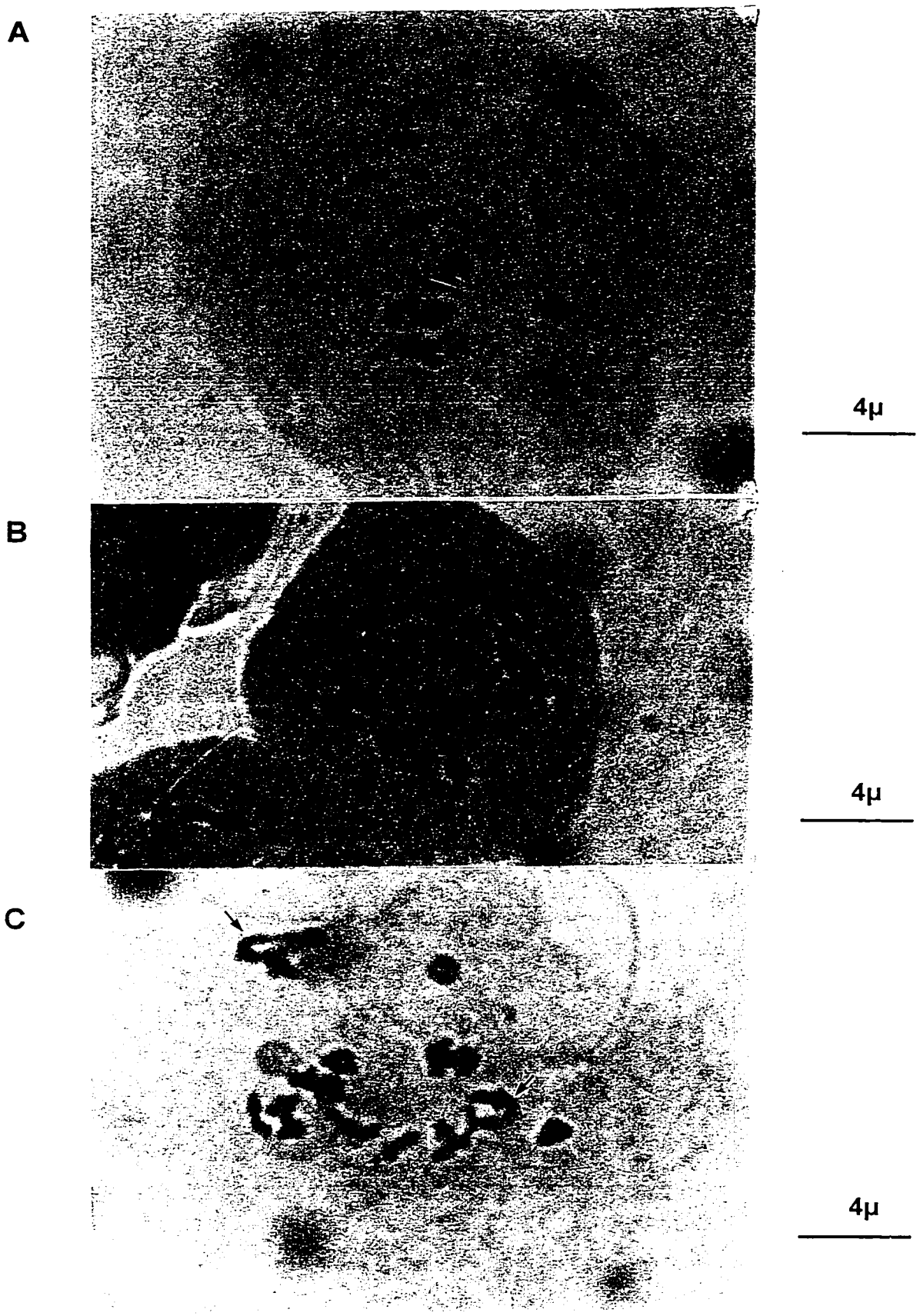
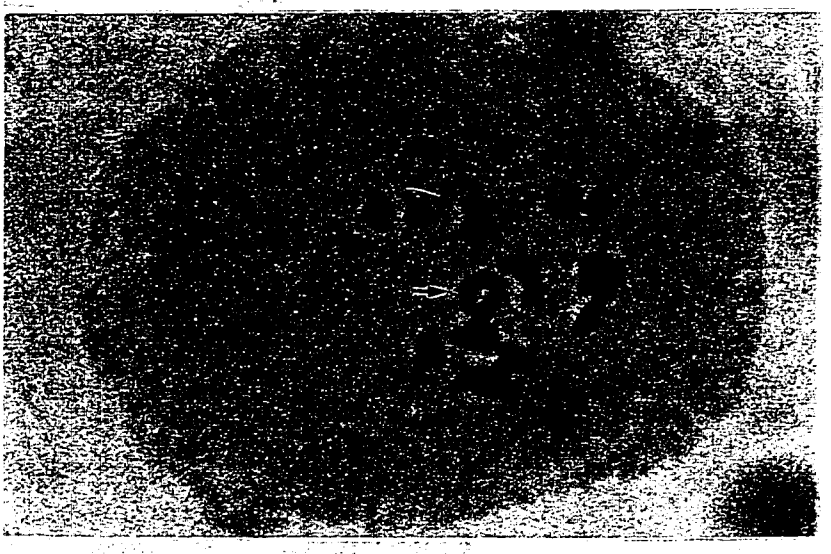

A
4μ
B
4μ
C
4μ

Figure 2.3. Univalents, quadrivalents, and hexavalents observed in the interspecific DH lines. **A** Two univalents off the metaphase plate in interspecific DH line 96-1776-2, **B** Quadrivalent observed in interspecific DH line 96-1711-3, **C** Hexavalent observed in interspecific DH line 96-1764-2.

A

4μ

B



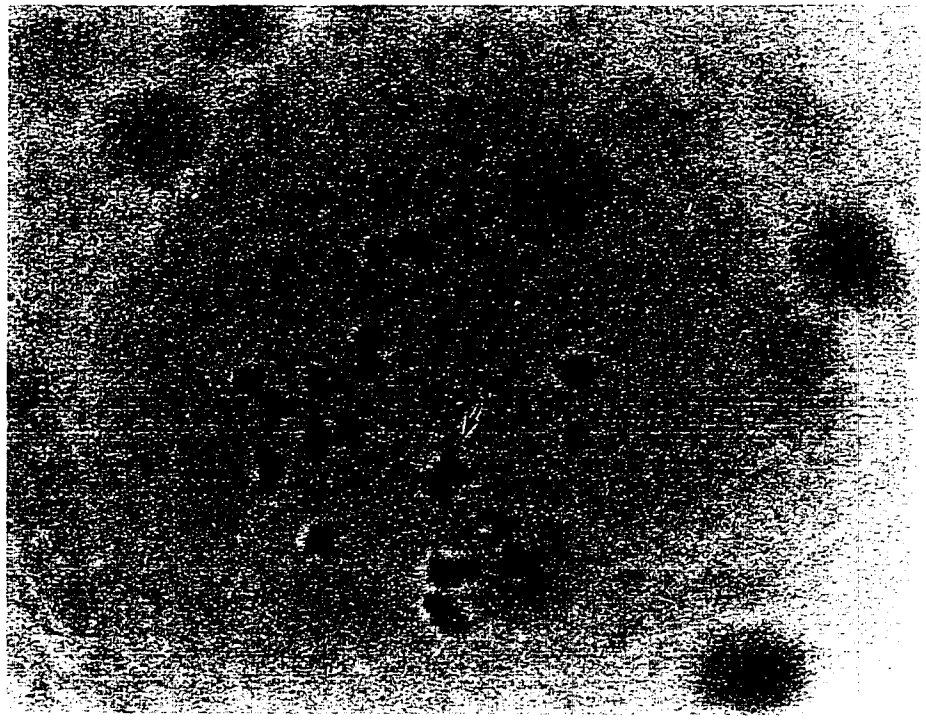
4μ

C

4μ

Figure 2.4. Quadrivalents at early and late diakinesis in the interspecific DH lines. **A** Quadrivalent (indicated by the arrow) in interspecific DH line 96-1704-3 early in diakinesis showing chiasmata, **B** Quadrivalent (indicated by the arrow) in interspecific DH line 96-2022-4 later in diakinesis showing chiasmata terminalizing.

A



B

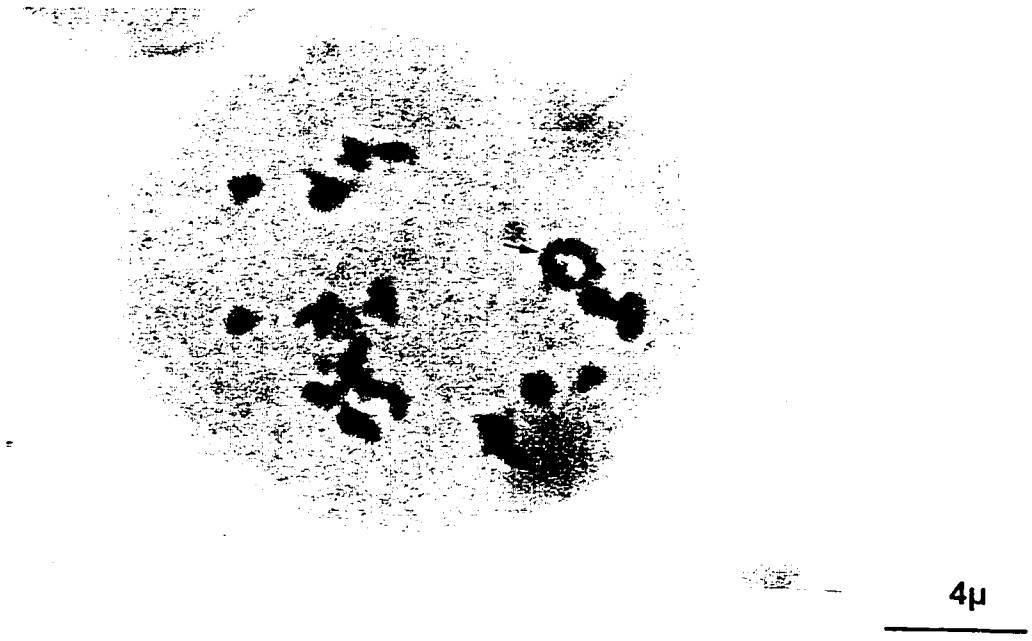
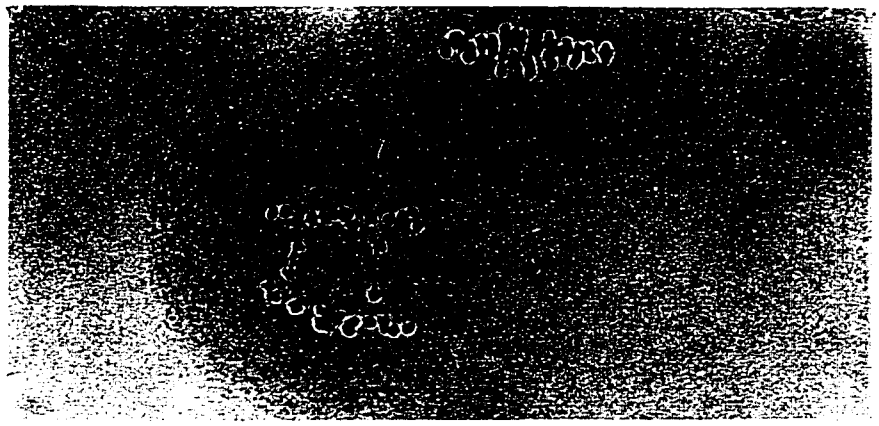


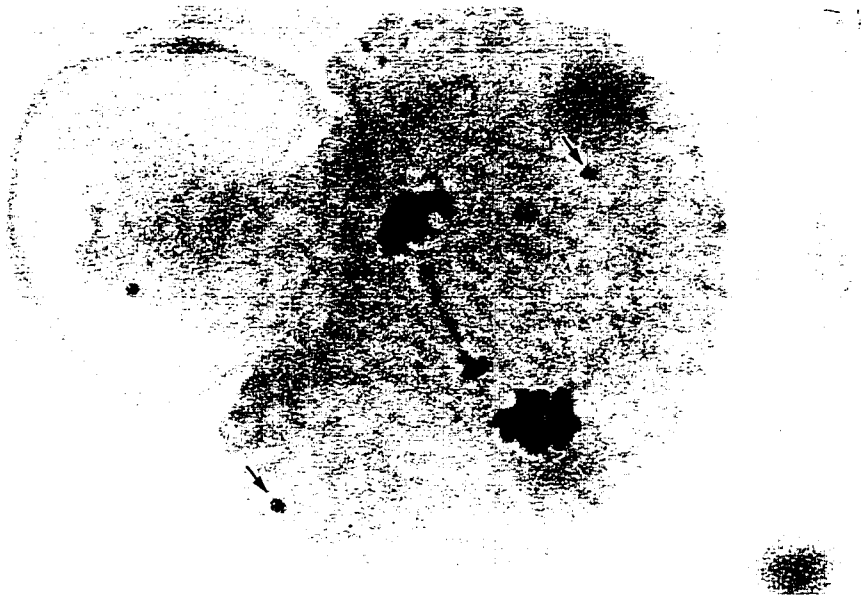
Figure 2.5. Non-disjunction and bridge / fragment associations observed in the interspecific DH lines. **A** Non-disjunction and bridges during anaphase in interspecific DH line 96-1691-3, **B** Bridge / fragment associations observed in interspecific DH line 96-1766-1 at telophase I (arrows indicated fragments), **C** Bridging observed in telophase II in interspecific DH line 96-1718-3.

A



4μ

B



4μ

C

4μ

2.4. Discussion and Conclusions:

Chemical analysis revealed that the basic ferric acetate prepared for the present study was not pure. SEM revealed both ferric and ferrous iron in the sample, which suggested that ferrous acetate and ferric acetate, two intermediates in the production of basic ferric acetate, were present in the final product. The presence of intermediate products suggested that the reaction was not driven to completion. Although chemical analysis did not provide a more conclusive answer to what the chemical is, the superior chromosome staining observed when it was used as the mordant, demonstrated the usefulness of the chemical in the form it was made.

The inability to obtain seed from *B. rapa* X *B. napus* crosses when *B. rapa* was used as the female parent during this study supports the theory of Quazi (1988), that there may be a genotypic or cultivar specific influence on seed set in interspecific crosses between *Brassica* species. Meng and Lu (1993) reported variation in the success of pollinating 3 genotypes of *B. napus* with 2 genotypes of *B. juncea*. They found that maternal genotypes differed from each other in their reproductive behavior after pollination with *B. juncea.* Most notably, endosperm failed to form properly, or the hybrid embryo degenerated at an early stage. Since successful fertilization, zygote and embryo formation, and endosperm development, each could be controlled by many independent genes, a failure to set seed could occur for different reasons depending on the genetics of different plants (Meng and Lu, 1993). A genotypic influence on seed set in the

Brassica species would explain the lack of seed recovered during the present study when *B. rapa* was used as the female parent.

Application of embryo rescue techniques in future *B. rapa* X *B. napus* crosses, when *B. rapa* is used as the female parent, could improve the chances of obtaining plants from seeds in which the endosperm does not develop properly. The survival of interspecific hybrids between *B. alboglabra* Bailey and *B. campestris* (*B. rapa*). (Chen et al., 1988), and between *B. napus* and *B. oleracea* (Quazi, 1988) was greatly enhanced by rescuing embryos from seeds in which endosperm did not form.

The mechanism by which the interspecific doubled haploid lines produced for the present study maintained a *B. napus* chromosome number despite having two cross with *B. rapa* is unknown. Little is known of the response of interspecific *Brassica* species to the doubled haploid breeding technique and therefore a maternal influence on embryogenesis may exist.

Song et al. (1993) reported that cytoplasmic genomes are suspected to influence changes in nuclear genomes, especially when foreign cytoplasm and nuclei are brought together in the case of the formation of amphidiploid *Brassica* species. Cytoplasmic genetic male sterility in the *Brassica* species is one well-documented example of the interaction between the nuclear genome of the paternal parent, and the cytoplasm of the female parent (Buzza, 1995). In an earlier study using RFLP's, Song et al. (1988) found that when the maternal diploid species of *Brassica* amphidiploids had highly differentiated cytoplasms, the nuclear genome originating from the paternal diploid species was altered

considerably more than the nuclear genome of the maternal parent. This led them to believe that stabilization of newly synthesized amphidiploids may occur through modifications to the paternal nuclear genome and/or the maternal cytoplasmic genome.

Genome stabilization suggested by Song et al. (1993) may occur because pollen grains, or pre-pollen cells in the case of the doubled haploid system, with less than a complete haploid set of chromosomes, are aborted. If this theory is true, all the haploid embryos obtained through culturing the anthers of the interspecific F_1 would be expected to have 19 chromosomes. McGrath and Quiros (1990) observed that both male and female fertility improved each time the interspecific F_1 from a cross between their synthetic *B. napus* (Hakuran) and *B. campestris* (*B. rapa*), was backcrossed to *B. campestris* (*B. rapa*). They hypothesized that fertility increased with backcrossing because chromosome number was decreased, or the chromosome abnormalities seen in the synthetic *B. napus,* which were causing detrimental effects, were removed. Aborted pollen grains, which are produced in high quantities by interspecific F_1's are often the result of unpaired chromosomes leading to unbalanced reduction division during meiosis (Iwanami et al., 1988). Because many of the aborted pollen grains are presumed to have resulted from improper division during meiosis, it can be postulated that most of the viable pollen produced by the F_1 have a full chromosome complement and therefore stabilization of the genome is achieved through self-pollination.

A maternal effect could also be influencing the development of the haploid embryo during microspore culture. Once pollen cells are isolated from the anthers, a heat shock is used to switch normal pollen development to embryogenesis. A number of rapid cell divisions result in a globular embryo that begins to differentiate. Development continues through the heart-shaped and torpedo-shaped embryo stages, until the cotyledonary stage is reached. A cold treatment or high dose of abscisic acid is often used to enhance plant regeneration from the embryos (Coventry et al., 1988). These critical steps in microsporogenesis may be influenced by environmental factors, but the genes passed to the pre-pollen cells from their mother plant ultimately govern development. The interaction between the cytoplasm and nuclear genome during microspore culture is not fully understood. It appears that having less than a complete haploid set of *B. napus* genetic material, in a *B. napus* cytoplasm may result in incomplete embryo differentiation and development, prevent regeneration of plants from the cotyledonary stage, or both. The number of developing plant embryos isolated from the anthers of the interspecific plants, and regeneration of whole plants from the embryos that did develop, was low (data not presented). Although the low embryogenic response seen in this study is not conclusive evidence for the theory that an interaction between the maternal and paternal genomes occurs during microspore culture, it would be expected.

An objective of the present study was not to prove that there might be a maternal influence on microspore culture. The findings that the DH lines, obtained from culturing the anthers of *B. napus* X *B. rapa* interspecific plants, all

contain a genetic complement similar to the maternal parent indicates that there are interactions occurring during embryogenesis that are not yet fully understood. To determine if this influence is specific to the genotypes of the plants used in the crosses, the test could be repeated using the DH progeny derived from crosses between a number of different *B. napus* and *B. rapa* cultivars. Further insight into the existence of a maternal influence on microspore culture would be obtained by culturing the anthers of F_1's obtained when *B. rapa* is the female parent, and by observing the number of chromosomes in the DH progeny. Since seed did not set when *B. rapa* was used as the female parent, embryo rescue could be used to improve the survival of the hybrid plants.

The diploid *Brassica* species are secondarily balanced polyploids, which evolved from a common progenitor believed to have had 6 chromosomes (Röbbelen, 1965). Because there is considerable duplication within and between the **A**, **B**, and **C** genomes of the *Brassica* species (Lagercrantz and Lydiate, 1996), there is an affinity for non-homologous pairing inherent in these species. Catchside (1934) suggested that 10 bivalents and 9 univalents reported by Morinaga (1929a) is the least amount of pairing possible between *B. napus* and *B. rapa* if the 10 **A** genome chromosomes common to both species were pairing. Catchside (1934) reported a range in bivalent formation from 7 to 12 in addition to 0 to 3 trivalents and the rare presence of 1 quadrivalent during meiosis in the F_1. This indicates that pairing occurs between homologous **A** genome chromosomes, between non-homologous **C** chromosomes (observed as any number of bivalents greater than 10), and between the **A** and **C** genome

chromosomes through the formation of trivalents. Based on the presence of 1 to 2 quadrivalents in many of the DH lines produced for the present study, it appears that non-homologous recombination did occur in the F_1 and modified backcross F_1. Quadrivalent chromosome associations have been reported in the Brassicaceae by several authors including Prakash and Chopra (1993), Song et al. (1993), McGrath and Quiros (1990), Tai and Ikonen (1988), Busso et al. (1987), Prakash and Hinata (1980), U (1935), and Catchside (1934). These authors concluded that multivalent associations such as quadrivalents are the result of non-homologous recombination and intragenomic pairing. Application of the doubled haploid technique is expected to stabilize the recombined chromosomes by making them homozygous through the process of doubling. Therefore, multivalent associations would be expected in the interspecific F_1, but not the DH lines unless non-homologous or homeologous associations had occurred after the doubling process, resulting in chiasma formation and chromatid exchange. After doubling, every chromosome has a homologue with which it can pair. Since homologous chromosomes are completely identical, it would be expected that preferential pairing would occur between homologues. Within the *B. napus* X *B. rapa* interspecific DH lines produced for the present study, there appears to be a tendency for increased non-homologous pairing as evident by 1 to 2 quadrivalents observed in many of the DH lines as well as a low frequency of hexavalents. Prakash (1974) suggested that there might be a system that evolved in the natural amphidiploid *B. juncea,* which suppresses non-homologous pairing. In wheat, a dominant gene termed *Ph* is responsible

for suppression of non-homologous pairing (Riley and Chapman, 1958; Sears, 1976). Prakash and Hinata (1980) indicated that the gemones of the naturally occurring alloploids may have undergone significant genetic differentiation during their evolution. The fact that in the haploids of synthetically produced amphidiploid *Brassica* species, there is a higher bivalent frequency than in natural ones, suggests that differentiation occurred within the natural amhidiploid *Brassica* species as they evolved. Suppression of a genetic system which prevents homoeologous pairing, or loss of differentiation between the **A** and **C** genomes in the interspecific progeny may be responsible for the increase in non-homologous pairing and therefore increase in quadrivalent associations in the DH lines.

Two univalents were observed at a low frequency in both *B. napus* and the *B. napus* by *B. rapa* interspecific F_1 studied by Catchside (1934). The author reasoned that smaller chromosomes with subterminal centromeres may fail to form chiasmata, or chiasmata may fail to hold the homologous chromosomes together until anaphase. A pair of chromosomes lacking a chiasmata would separate early in meiosis and therefore appear as univalents in metaphase. Catchside (1934) concluded that univalents may not necessarily lead to deficient gametes as there is a 50% chance that they may line up at metaphase and pass to opposite polls. Lagging of univalents during anaphase I and II does occur, and results in the chromosomes being stranded between the dividing cells.

Chromosome bridges were observed in 3 of the DH lines. Bridge / fragment production is known to result from inversions (Burnham, 1962). Olson

and Hagberg (1955) observed bridges and fragments during anaphase I and II in *B. napus*. They attributed these associations to structural differences such as inversions between paired chromosomes. They reported that the consequence of bridges was a breakdown in normal meiosis, which often lead to pollen sterility.

Inversions, which may have resulted during the interspecific crosses and / or microspore culture process, are not expected to appear in the DH progeny. An inversion occurs when a chromosome breaks in two places and the ends of the central fragment reunite with the opposite break point from which it arose (Burnham, 1962). When a normal and inverted chromosome in an inversion heterozygote pair, an inversion loop is formed. Depending on the type of inversion, and if crossing over occurs within that loop, governs the types of bridge / fragment products that result. Inversions may be expected in the interspecific F_1 plants (heterozygous), but not the DH progeny as microspore culture renders every chromosome homozygous. An inversion carried in a DH line would not be expected to appear as a loop or bridge / fragment association because both homologues are the same. The fact that there are bridge / fragment associations in the DH lines therefore suggests that inversions have occurred after the microspore culture event.

DH lines that were found to have bridges also had lower pollen viability (Table 2.1.), which may have been partially due to inversions. Inversions can cause pollen and egg abortion due to the production of gametes with chromosome duplications and deficiencies as a result of crossing over (Fehr,

1991). Because anaphase I and II were not found for every line, the frequency and phenotypic effects of inversions in this population were not fully characterized. Franzke and Ross (1952, 1957) found that colchicine could produce mutations and other unexpected breeding behaviors in the diploid progeny of *Sorghum*. Because colchicine was used as the chromosome-doubling agent in this study, inversions may have been chemically induced. An inversion, caused by colchicine before the doubling would still be expected to be homozygous after doubling and should not be detected as bridges and fragments. Therefore the mutation would have had to occur after the doubling event.

It can be assumed that the doubled haploid lines in this study, produced by culturing both the interspecific F_1 from a cross between *B. napus* and *B. rapa*, and the modified backcross F_1, all carry 38 chromosomes. These chromosomes formed 15 to 19 bivalents, as well as 1 to 2 quadrivalents, 2 univalents, and occasionally a hexavalent or chain of 6 chromosomes. The mechanism by which the DH lines maintained a *B. napus* chromosomes number is unknown. One theory is that there may be a maternal influence on pollen formation or microspore embryogenesis in the progeny of this interspecific *B. napus* X *B. rapa* cross. Preliminary cytological analysis suggests that recombination may still be taking place in the DH lines produced for this study because of multivalent formation and other chromosomal aberrations. The presence of multivalent associations in many of the DH lines indicates that homeologous pairing and potential recombination is occurring in these lines. Whether bridges were the

result of inversions and how they were caused is unknown although non-homologous pairing cannot be ruled out. One theory as to the persistance of univalents in some of the DH lines proposed by Catchside (1934) is that they are the result of early separation of small chromosomes prior to anaphase. Multivalents, bridging, and univalents appear to have influenced fertility levels in some of the DH lines despite having no obvious effect on other phenotypic traits. The persistence of these aberrant chromosome configurations and their effects on the phenotypic stability of the lines needs to be explored if these lines are to be utilized as cultivars, or in future breeding efforts.

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Chapter 3

Greenhouse Screening for Resistance to *Leptosphaeria maculans* and *Albugo candida* in Doubled Haploid lines Derived from Interspecific Crosses between *Brassica napus* and *Brassica rapa*.

3.1. Introduction:

Fungal diseases, such as the stem canker or blackleg caused by the virulent form of *Leptosphaeria maculans* (Desm.) Ces. & de Not. (asexual state *Phoma lingam*), and white rust / staghead caused by *Albugo candida* (Pers. ex Hook.) Kuntze can be found infecting fields of canola in Canada each year.

Since the discovery of the virulent strain of *L. maculans* in central Saskatchewan in 1975, blackleg has spread to most of the canola growing areas of Saskatchewan, as well as into Manitoba and Alberta (Thomas, 1984). The potential impact *L. maculans* can have on canola production was seen in 1989 in Saskatchewan when a survey detected blackleg in 92% of the fields surveyed, at an average infection level of 56%. Reports of 100% infection and yield losses estimated at greater than 50% were also recorded (Jesperson, 1989; 1990). Since then, growers have been encouraged to switch to Argentine canola varieties (*Brassica napus*) with some level of resistance, discontinue growing Westar and Alto which are highly susceptible, and to closely follow the recommended 4-year rotation (Harrison and Kharbanda, 1996). Certain areas

such as central Saskatchewan, and the Peace River Region of Alberta continue to be significantly effected by *L. maculans* (Petrie, 1995; Evans et al., 1996).

Leptosphaeria maculans attacks the leaves, stems, and siliques of many species within the Brassicaceae. Stem cankers result from the systemic growth of *L. maculans* (Hammond et al., 1985). The most destructive effect of the pathogen is observed when developing stem cankers completely girdle the stem. Because it is deprived of it's root system, the plant ripens prematurely, is often subject to lodging, and produces shriveled seeds. Siliques of infected plants tend to shatter more readily, resulting in further losses (Martens et al., 1988).

The most effective and economical method of controlling this pathogen is through breeding for genetic resistance (Bansal et al., 1994). Stringam et al. (1995) reported the successful transfer of high degrees resistance to *L. maculans,* from the blackleg resistant Australian rapeseed cultivar Maluka into an agronomically superior canola cultivar. Stringam et al. (1992) found that the blackleg resistance transferred from Maluka is controlled by either a major gene or tightly linked polygenes that transmit from generation to generation in a block. Bansal et al. (1994) demonstrated that the genetic resistance to *L. maculans*, transferred from Maluka, could be detected at the cotyledon stage, as well as the adult plant stage. They credited the rapid development of blackleg resistant, *B. napus* canola to the utilization of the doubled haploid breeding technique to increase the speed at which homozygosity is reached, and application of an efficient and reliable greenhouse screening technique for identification of resistance to *L. maculans.*

White rust, and more importantly staghead, resulting from infection by *Albugo candida,* has been observed on many *Brassica* species, primarily *B. rapa* cultivars of rapeseed and canola, in Western Canada. When the fungus attacks the plant, blister like pustules are produced on the undersides of the leaves. The pustules contain zoosporangia, which release zoospores that infect other leaves and adjacent plants. Infection of individual flowers, or the entire raceme results in hypertrophy of the tissue, which produces the disease symptom, referred to as the staghead (Martens et al., 1988).

Concern that white rust may pose a threat to the canola industry has lead the Western Canadian Canola / Rapeseed Recommendation Committee (WCC/RRC) to set a white rust resistance standard which must be met by potential new cultivars. *B. napus* cultivars, which typically show strong resistance to the pathogen, must not show more than 10% infection by *A. candida*. *B. rapa* cultivars must perform better than the white rust check (Tobin). Registration of partially resistant cultivars such as Tobin, reduced the losses to white rust (Rimmer and Buchwadlt, 1995). Although yield losses are not high in Canada, the presence of conspicuous white pustules and stagheads in a field can cause serious concern to farmers (Bansal VK, personal communication). The emergence of a new pathotype of race 7 that is virulent on Tobin has raised new concern as it may be responsible for heavy local infections by *A. candida* in Alberta (Conn and Tewari, 1991). Resistance and selection for the new pathotype has also been identified within Tobin, but Rimmer and Buchwaldt (1995) do not believe that this resistance can be sustained. Most European and

Canadian cultivars of *B. napus* are resistant to all known races of *A. candida* (Fan et al., 1983), while some Chinese cultivars, believed to have originated from *B. napus* X *B. rapa* interspecific crosses, are susceptible to race 7 (Downey and Rakow, 1987).

Downey and Rimmer (1993) indicated that resistance to *A. candida* race 7 in Tobin is under the control of a single dominant gene. Fan et al. (1983) reported that *A. candida* race 7 resistance in *B. napus* cultivar Regent, is controlled by at least 3 genes with resistance being dominant. They also found that any one of the dominant alleles at one of the loci would produce a resistant response. Fan et al. (1983) and Liu et al. (1996) concluded that resistance to white rust in *B. napus* has not broken down because of the number of genes involved. It appears that the fungus has not yet been able to evolve enough to overcome the multiallelic resistance in *B. napus*. Transfer of *B. napus*, multiallelic type *A. candida* resistance into *B. rapa* could eliminate producer concerns about this pathogen.

Although Argentine canola cultivars are more resistant to white rust, and are partially resistant to blackleg, Polish canola cultivars dominated in Alberta prior to 1996 (Thomas P, personal communication), because of the short growing season in a major portion of the canola producing areas. Agronomic deficiencies in *B. rapa* such as little to no observed resistance to *L. maculans*, loss of Tobin's resistance to *A. candida* race 7, and the lower yield potential of *B. rapa* canola varieties compared to *B. napus*, have attracted more Alberta farmers to growing *B. napus.* Because there is a risk that *B. napus,* grown in the short season areas

may be damaged by early fall frost, Alberta farmers and the canola industry as a whole could benefit from new cultivars with a combination of *B. rapa's* early maturity and *B. napus's* disease resistance and yield.

Interspecific crosses have been used in the past to transfer disease resistance between *Brassica* species. Reward summer turnip rape (*B. rapa*) was developed through an interspecific cross between *B. rapa* cultivar Polar and *B. napus* cultivar Bronowski (Scarth et al., 1992). Reward has a high level of *A. candida* race 7a resistance. Roy (1984) reported on the interspecific transfer of *B. juncea*-type high blackleg resistance to *B. napus*. Although Rimmer and van den Berg (1992) found many of Roy's lines to be addition lines, which lost their resistance in subsequent generations, the potential for transferring disease resistance between species was demonstrated.

As a component of a larger study which focused on transferring superior agronomic traits between *B. napus* and *B. rapa*, the potential for transferring backleg and white rust resistance to *B. rapa* was examined using greenhouse screening techniques. Doubled haploid progeny derived from an interspecific modified backcross (see Figure 2.1.), were screened for resistance to *Leptosphaeria maculans* and *Albugo candida*, and the results are reported in this chapter.

3.2. Materials and Methods:

3.2.1. Plant Material Screened and Growth Conditions:

Disease resistance screening was conducted in an Enconaire Environmental Chamber (model GRB-168), using the cotyledon assays described by Bansal et al. (1994) for blackleg resistance, and Bansal et al. (1998) for white rust resistance. Forty DH lines derived from the interspecific crossing scheme described in Chapter 2, the seven parents used to generate the DH lines, the *B. rapa* cultivar Eclipse, and *B. napus* cultivar Quantum, were screened for resistance to the pathogens, *Leptosphaeria maculans* (Desm.) ces. & de Not. (conidial form *Phoma lingam*) and *Albugo candida* (Pers. ex Hook.) Kuntze. Growth cabinet temperatures were maintained at 18°C day/ 15°C night, and the plants were provided with 350 µmol $m^{-2}s^{-1}$ of light from very high output fluorescent and 40 watt incandescent bulbs, over a 16 hour photoperiod.

3.2.2. Blackleg Inoculation Procedure and Symptom Scoring:

A pycnidiospore suspension consisting of five single spored *L. maculans* isolates was prepared and used to inoculate seven day old plants according to Bansal et al. (1994).

The host pathogen reaction was visibly scored on two reps of 8 plants using the 0 to 4 susceptibility scale outlined by Bansal et al. (1994) (Table 3.1.), after 10 days of incubation in the growth cabinet.

Table 3.1. Disease rating values and the visible symptoms of blackleg associated with each class of infection.

Class	Symptom
0	No visible expression of the disease
1	Necrotrophic hypersensitive response around the wound
2	Gray – green tissue collapse with distinct margin
3	Gray – green tissue collapse with diffused margin
4	Most of the tissue collapsed with pycnidia formation

The disease severity index was calculated for each line in both reps, as follows.

$$\text{Disease severity index (DSI)} = \frac{\text{SUM (No. of plants in category X category value)}}{\text{total No. of plants}}$$

Statistical analysis was performed using the univariate and mixed procedures of SAS version 6.12 (1996) statistical software.

3.2.3. White Rust Inoculation Procedure and Symptom Scoring:

White rust inoculations were carried out according to Bansal et al. (1998) with the following modification. *Albugo candida* race 7 was maintained and increased on the cotyledons of *B. rapa* cultivar Torch.

The total number of plants with pustules on their cotyledons and/ or leaves was recorded. A line was scored resistant only if all nine of the inoculated plants in the pot were free of the pathogen after the 11 day incubation period.

3.3. Results:

Thirty-five of the 40 backcross F_1 derived Doubled Haploid lines, were obtained when the interspecific DH_1 (95-376), between *B. napus* and *B. rapa* was crossed again to a *B. rapa* line 95-440-2 (microspore culture 95-1061-B). Three additional DH lines were obtained when *B. rapa* line 95-439-2 was used in the modified backcross (culture 95-1060-B), and 2 DH lines were obtained when *B. rapa* line 95-442-2 was used in the modified backcross (culture 95-1062-B). Although no fertile DH lines were obtained when *B. rapa* line 95-442-3 was used in the modified backcross (culture 95-1063-B), this parent was also screened for disease resistance.

3.3.1. Blackleg Resistance Screening Results:

The *B. napus* parent 94-98 had the best disease resistance rating based on the 0 to 4 scale (score of 0.7) (Table 3.2.), while the *B. rapa* parent 93-1005-3 had the worst rating (3.5). The interspecific DH line 95-376 (DSI score of 1.7), used in the second cross (modified backcross) with the *B. rapa* line 95-440-2 (DSI score of 2.8), produced 35 DH progeny that had disease severity scores ranging from 0.8 to 2.8, intermediate between *B. napus* line 94-98 and *B. rapa* line 95-440-2. The DSI scores for the other five DH lines, 3 *B. rapa* parents used to produce them, Quantum, Eclipse, and the blackleg check cultivars (Quantum and Profit) were also determined (Table 3.2.)

The cumulative frequency distribution plot for the DSI scores for the DH lines from the 95-1061-B microspore culture (Figure 3.1.) demonstrates the continuous nature of disease resistance in this population. Statistical analysis of blackleg resistance, based on the calculated Disease Severity Index, in the DH lines derived from this backcross, is not significantly different from a normal distribution, with a mean of 2.0. Analysis of variance reveals that there is a significant line effect ($Pr>|Z|=0.0002$). The estimate of variance due to the line effect, with a 95% confidence interval was 0.33 ± 0.18.

B. rapa parents 95-439-2 and 95-442-3 unexpectedly had DSI scores of 1.7 and 1.5 respectively (Table 3.2.). Examining the lineage of these parents (see Chapter 2) reveals that they are self-fertile F_7 *B. rapa* lines, derived from an interspecific cross between the *B. rapa* cultivar Eclipse and U of A *B. napus*

breeding line number 83-52692B. Line 83-52692B was a Dwarf F_6 line, derived from a cross between *B. napus* cultivar Tower and a colchicine induced dwarf *B. napus* line, produced by Brian Fowler (Crop Development Center, University of Saskatchewan). Tower is known to express some resistance to blackleg (Stringam GR, personal communication), and therefore this resistance may have been transferred to the *B. rapa* parents.

3.3.2. White Rust Resistance Screening Results:

Albugo candida race 7 did not infect or sporulate on the *B. napus* parent 94-98, the interspecific DH line 95-376, or any of the interspecific modified backcross DH lines inoculated. All of the *B. rapa* lines (93-1005-3, 95-439-2, 95-440-2, 95-442-2, and 95-442-3) showed heavy infection by the pathogen as evident by numerous white rust pustules on the cotyledons and first true leaves. The number of white rust infected plants out of the total number of plants inoculated, and the white rust score for the 7 parents, 40 DH lines, Quantum, Eclipse and white rust test checks (Commercial Brown and Torch) were recorded (Table 3.2.).

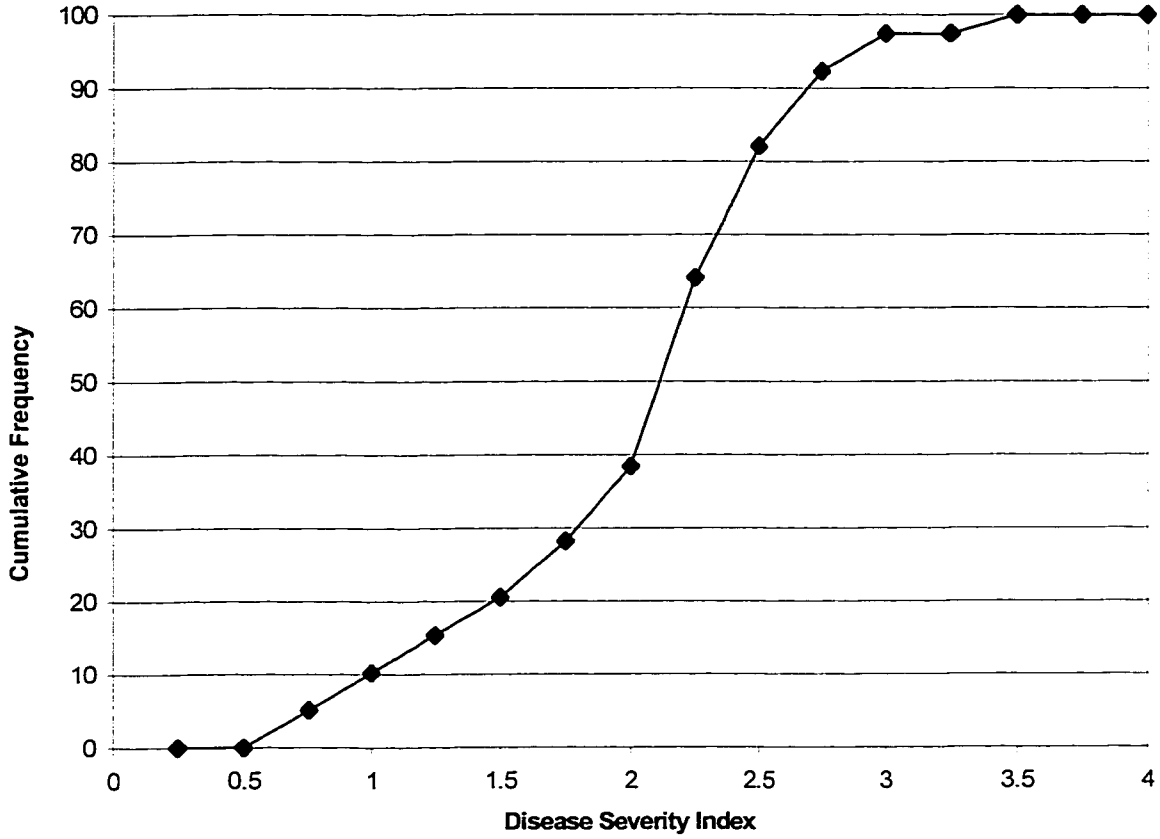
Table 3.2. Mean DSI and Standard error for blackleg scores, white rust infection and white rust resistance ratings recorded in the parents, DH_1, and modified backcross DH lines.

Line	Parent/Culture	Blackleg Mean DSI	SE	# infected/ # inoculated	White Rust Rating
93-1005-3	F7 *B. rapa*	3.5	0.1	9-9	S
94-98	*B. napus*	0.7	0.2	0-9	R
95-376	DH1	1.7	0.1	0-9	R
95-440-2	F7 *B. rapa*	2.8	0.3	9-9	S
96-1066-2	95-1061-B	2.2	0.2	0-9	R
96-1069-1	95-1061-B	2.8	0.1	0-9	R
96-1179-1	95-1061-B	2.1	0.1	0-9	R
96-1180-1	95-1061-B	2.6	0.1	0-9	R
96-1183-1	95-1061-B	2.1	0.2	0-9	R
96-1195-1	95-1061-B	1.9	0.1	0-9	R
96-1196-3	95-1061-B	1.1	0.1	0-9	R
96-1687-3	95-1061-B	2.3	0.3	0-9	R
96-1691-3	95-1061-B	2.0	0.3	0-9	R
96-1693-3	95-1061-B	2.0	0.4	0-9	R
96-1696-1	95-1061-B	0.9	0.4	0-9	R
96-1703-2	95-1061-B	2.0	0.5	0-9	R
96-1704-3	95-1061-B	1.3	0.1	0-9	R
96-1707-3	95-1061-B	2.7	0.1	0-9	R
96-1711-3	95-1061-B	2.1	0.1	0-9	R
96-1712-2	95-1061-B	2.0	0.4	0-9	R
96-1713-3	95-1061-B	2.4	0.4	0-9	R
96-1718-3	95-1061-B	1.7	0.2	0-8	R
96-1732-2	95-1061-B	2.2	0.2	0-9	R
96-1734-2	95-1061-B	2.6	0.1	0-9	R
96-1735-3	95-1061-B	2.3	0.3	0-9	R
96-1743-2	95-1061-B	2.4	0.2	0-9	R
96-1746-2	95-1061-B	1.4	0.5	0-9	R
96-1747-2	95-1061-B	0.8	0.0	0-9	R
96-1753-3	95-1061-B	2.4	0.4	0-9	R
96-1755-2	95-1061-B	2.3	0.0	0-9	R
96-1756-3	95-1061-B	2.6	0.0	0-9	R
96-1759-2	95-1061-B	2.5	0.2	0-8	R
96-1768-3	95-1061-B	1.2	0.4	0-9	R
96-1773-1	95-1061-B	1.7	0.2	0-9	R
96-2016-3	95-1061-B	2.1	0.3	0-9	R
96-2018-1	95-1061-B	1.8	0.2	0-9	R
96-2022-4	95-1061-B	0.9	0.4	0-9	R
96-2025-1	95-1061-B	2.1	0.3	0-9	R
96-2027-1	95-1061-B	2.2	0.1	0-9	R

Table 3.2.continued.

Line	Parent/Culture	Blackleg Mean DSI	SE	# infected/ # inoculated	White Rust Rating
95-439-2	F7 *B. rapa*	1.9	0.1	9-9	S
95-442-2	F7 *B. rapa*	3.2	0.1	9-9	S
95-442-3	F7 *B. rapa*	1.3	0.3	8-9	S
96-1057-1	95-1060-B	3.1	0.1	0-9	R
96-797-2	95-1060-B	2.3	0.6	0-9	R
96-2015-4	95-1060-B	2.1	0.5	0-9	R
96-1776-2	95-1062-B	1.6	0.2	0-9	R
96-815	95-1062-B	2.5	0.1	0-9	R
Quantum	Quantum	0.9	0.1	0-9	R
Eclipse	Eclipse	2.6	0.1	9-9	S
Quantum	Blackleg	1.0	0	NT	NT
Profit	Checks	3.6	0.1	NT	NT
Commercial Brown	White rust	NT	NT	0-45	R
Torch	Checks	NT	NT	88-90	S
NT=Not tested					

Figure 3.1. Cumulative Frequency distribution of Mean Disease Severity Index in the DH Progeny derived from the 95-1061-B Microspore Culture.



3.4. Discussion and Conclusions:

There is considerable speculation concerning the number of genes involved in blackleg resistance, and on which genome the genes are located. The source of genes believed to be conferring resistance in the present study were transferred from the Australian rapeseed variety Maluka (*B. napus*) to

advanced breeding lines from the University of Alberta canola breeding program (Stringam et al., 1995). The resistance observed in the *B. napus* canola lines such as Quantum, derived from Maluka could be detected at both the cotyledon and adult plant stages (Bansal et al., 1994). However this *B. napus* resistance is not absolute as seen in *B. juncea* type resistance, which suggests the *B. napus* resistance from Australia is unique (Stringam et al., 1995). Segregation analysis of crosses between Maluka and U of A breeding lines, by Stringam et al. (1992), and molecular mapping performed by Mayerhofer et al. (1997) indicate that the resistance genes transferred from Maluka segregate in a 1:1 ratio, and map to a single major locus.

Roy (1978) suggested that adult blackleg resistance was on the **A** genome in *B. juncea* (**AABB**) parent BJ168, as it was readily transferable to *B. napus* through interspecific crosses, whereas seedling resistance may reside on the **B** genome. However, in some of his crosses between *B. juncea* and *B. napus*, complete resistance was transferred to *B. napus*, possibly through non-homologous recombination between the **B** genome and either the **A** or **C** genomes of *B. napus*. Cytological analysis (outlined in Chapter 2) revealed that the interspecific DH lines screened for disease resistance during the present study all contained 38 chromosomes, which suggests that a *B. napus* chromosome complement has been maintained. Since a single dominant gene is suspected to be governing blackleg resistance in the *B. napus* parent used for these crosses, it was assumed that two distinct classes of DH lines (resistant and susceptible) derived from the *B. napus* X *B. rapa* interspecific cross would be

observed. U (1935) proved that 10 of the chromosome pairs in *B. napus* came from *B. rapa* and the other 9 pairs from *B. oleracea* during a natural hybridization event. Within the interspecific F_1 plants produced for this study by crossing *B. napus* with *B. rapa*, the 10 **A** genome chromosomes common to both species are assumed to pair homologously, potentially recombine through normal crossing over events, and pass independently to the progeny. If the blackleg resistance gene is on one of the **A** genome chromosomes from *B. napus*, it was expected that progeny from the interspecific crosses would either have the gene and be resistant, or lack the gene and be susceptible to blackleg. The 9 **C** genome chromosomes passed from *B. napus*, do not have homologues in *B. rapa* with which to pair, therefore it was expected that the genetic make up of the **C** genome would remain relatively unaltered in the interspecific progeny. If blackleg resistance genes resided on the **C** genome, it was expected that all the interspecific DH progeny would maintain a high level of resistance. The ranged of DSI scores from resistant to susceptible in the backcross DH lines suggests that neither of these theories are adequate to explain the results. The continuous nature of blackleg resistance in this population makes it difficult to determine the number of genes involved in resistance and to which genome they are associated. Recombination within the blackleg resistance gene complex may have contributed to variation in the level of resistance observed. Cytological analysis revealed considerable non-homologous pairing and recombination within the interspecific DH lines. The continuous range of DSI scores in this population may therefore be the result of recombination between the *B. napus*

and *B. rapa* **A** genome chromosome conferring resistance, different chromosomes within the **A** genome, as well as between the **A** and **C** genomes.

The DSI scores of *B. rapa* parents 95-439-2 and 95-442-3 suggest that there may be some resistance present in the *B. rapa* parents that likely came from the *B. napus* cultivar Tower. Preliminary field evaluation of this *B. rapa* type blackleg resistance suggests that it is only effective at the seedling stage. Further characterization of this resistance is required. Additive effects of resistance genes from the *B. rapa* parents in the interspecific progeny may also have contributed to the range of DSI scores observed.

The amount of variability seen in the disease severity scores suggests there is considerable genetic variability with respect to blackleg resistance in this cross that could be exploited. Because blackleg resistance is normally distributed in the DH population derived from the interspecific crosses, it can be concluded that approximately 13.5% of lines should have a disease score between 1 and 2 standard deviations from the mean. This corresponds to mean DSI scores of 0.9 and 1.4. Since Quantum scores 1.0 on average, it is suggested that blackleg resistance similar to Quantum, was maintained in a certain proportion of the DH population. Since we were unable to recover *B. rapa* types from the interspecific crosses, this indicates the potential for maintaining blackleg resistance while incorporating *B. rapa* traits such as early maturity, into *B. napus*.

It appears that resistance to *Albugo candida* race 7 has been maintained in the interspecific DH lines. The recombination seen in the cytological study

(Chapter 2) that appears to have also influence the inheritance of blackleg resistance, may also have influenced the number of white rust genes maintained in the interspecific progeny. Whether this resistance is governed by the three genes reported by Fan et al. (1983) to be conferring *B. napus* type *A. candida* race 7 resistance in Regent was not determined in the present study. As indicated previously, cytological analysis indicated that all the DH progeny contain 38 chromosomes during meiosis as seen in *B. napus*. Cytological evidence suggested that a full **C** genome complement was maintained in the DH progeny. Although the mechanism that maintained a *B. napus* chromosome number in the DH progeny is unclear, it appears that the 9 **C** genome chromosomes and a mixture of the 10 **A** genome chromosomes from *B. napus* and *B. rapa* have been introgressed to the interspecific progeny. The fact that all the DH progeny were resistant to white rust, and all contained a full complement of **C** genome chromosomes may be evidence that at least one of the resistance alleles is on the **C** genome.

Chinese accessions of *B. napus* reported to be susceptible to *A. candida* race 7 (Fan et al., 1983), were derived from interspecific *B. napus* X *B. rapa* crosses (Downey and Rakow, 1987). This raises some concern that white rust resistance transferred through this cross could breakdown over time in these lines if they do not contain multiallelic resistance. Evaluation of these lines under field conditions, where the disease was prevalent revealed that the lines maintained their resistance. Long term evaluation of the strength of this resistance is required.

Although the original objective of transferring *B. napus* (Quantum) disease resistance to *B. rapa* was not achieved, a potential for maintaining disease resistance in *B. napus* DH progeny derived through interspecific crosses was determined.

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Chapter 4

Agronomic Performance and Quality of Doubled Haploid Lines Derived from Interspecific Crosses between *Brassica napus* and *Brassica rapa*.

4.1. Introduction:

Brassica rapa (formerly *B. campestris*) and *B. napus* are two species of *Brassica* that have been bred for improved oil and meal. Reducing the levels of erucic acid in the oil to less than 2% and glucosinolates to less than 30 µmoles per gram air-dried oil free meal, makes the oil fit for human consumption and allows the meal to be utilized as a high protein feed supplement for animals. Species of *B. napus* and *B. rapa* that meet these quality requirements are given the designation of canola quality (Thomas, 1984). The low levels of saturated fatty acids, high levels of mono-unsaturated fatty acids, and moderate levels of polyunsaturated fatty acids of canola oil, coupled with the high proportion of linoleic and alpha-linoleic fatty acids makes canola oil a desirable edible oil (Uppström, 1995).

The fatty acid profiles of *B. napus* and *B. rapa* are slightly different. *B. napus* canola varieties tend to have approximately 5% more oleic fatty acid (18:1) in their profiles but have a higher total saturate content than *B. rapa* varieties, which tend to have a higher proportion of linolenic fatty acid (18:3) and approximately 2% less total saturates (Downey and Rimmer, 1993).

An increased level of oleic acid improves the cooking quality of the oil (Dhillon et al., 1993), while linoleic acid (18:2) or vitamin F is an important building block for the formation of prostaglandins and other essential body regulators. Although linolenic acid is a polyunsaturated fatty acid, and is essential to the human diet (cannot be synthesized in the human body), it is less desirable because it oxidizes readily, giving off-flavors and reduced shelf life. Lower levels of saturates are nutritionally desirable, but margarines made from oils having a high proportion of fatty acids with the same 18-carbon chain length, have a tendency to form crystals on storage. Blending with other vegetable oils can prevent this problem however, there is considerable interest in increasing the amount of the saturated fatty acids, palmitic and palmitoleic acids in the profile of canola that may alleviate the crystallization problem (Downey and Rakow, 1987).

The meal remaining after the oil is extracted from canola seed makes up approximately 60% of the original seed weight, contains 36 to 44% crude protein, and can provide a good balance of essential amino acids when used as an animal feed supplement. Reducing the levels of glucosinolates in the meal has improved its palatability and reduced the potential for producing goiterogenic effects in non-ruminant animals (Downey and Röbbelen, 1989). Current breeding strategies are focused on reducing the levels of glucosinolates even further to allow a higher proportion of canola meal to be added to swine and poultry diets without adverse effects (Bell, 1995).

Hull represents 30% of the total oil-free meal weight, is high in fibre and low in protein. The fibre is primarily composed of cellulose, pentosans and lignin,

therefore, can not be digested by non-ruminant animals. The presence of hulls depresses the level of available energy and protein as well as amino acids and minerals when the meal is fed to non-ruminant animals. Yellow seeded cultivars of *B. rapa* tend to have thinner hulls and therefore have lower fibre content compared to brown and black seeded cultivars of *B. napus* and *B. rapa* (Stringam et al., 1974). Since the hulls contain up to 10% of the total protein and 25% of the gross energy contained in the meal, many breeding programs are attempting to develop pure yellow seeded *B. napus* canola cultivars (Bell, 1995).

Downey (1983) noted that increased seed size also reduced the hull percentage of the seed. He reported that *B. campestris* (*B. rapa*) Indian sarson types (yellow seeded) that are exceptionally larger seeded than *B. napus* cultivars, have higher oil and protein with less fibre than *B. napus* cultivars. The larger seeded *B. rapa* varieties were also found to contain more oil and protein than yellow or brown seeded *B. rapa* (*B. campestris*) cultivars with small seed size. Selection for seed colour as well as seed size may improve the oil and protein content of new varieties beyond what is currently obtainable.

B. napus and *B. rapa* are very different in terms of their agronomics in addition to their quality (Table 1.1.) The differences in agronomic importance make the two species suitable for production in different regions. Thomas (1984) indicates that the short frost-free period in Western Canada is one of the major factors affecting canola production. By selecting varieties that mature within the average frost-free period of their area, producers can reduce the risk of crop loss due to early fall frost. In Western Canada, the average number of frost-free days

in a growing season is less than 100 (Downey and Rakow, 1987). In Alberta prior to 1996, much of the land available for canola production is in the short season zone therefore *B. rapa* (Polish canola) varieties which mature earlier than *B. napus* tended to dominate the seeded area (Thomas P, personal communication). The potential yield benefit of *B. napus* has attracted more Alberta farmers to grow cultivars of this species although there is a risk that the crop may be damaged by early fall frost (Stringam GR, personal communication).

Since the release of the first low erucic acid, low glucosinolate *B. napus* variety "Tower" by the University of Manitoba in 1974, the focus of many breeding programs has shifted to other traits of agronomic importance. The University of Alberta Canola breeding program is interested in improving traits such as time to flowering, yield, oil and protein content of the seed, disease resistance, shattering resistance, silique angle, and lodging resistance. One approach to improving the performance of these two species is to introgress the superior traits of *B. napus* into *B. rapa* and *visa versa* (Stringam GR, personal communication). Since many of the traits of agronomic interest are controlled by one or a few genes, and are often influenced significantly by the environment, it is necessary to evaluate all traits under field conditions.

Interspecific crosses have been used previously to transfer traits of agronomic interest between *Brassica* species. Scarth et al. (1992) reported on the development of an *Albugo candida* race 7 resistant *B. rapa* cultivar, developed through interspecific crosses between *B. rapa* and *B. napus*. Roy (1984) transferred blackleg resistance from *B. juncea* to *B. napus* through

interspecific crosses. Clubroot resistance was transferred to *B. napus* from *B. rapa* by Growers (1982), Johnston (1974), and Lammerink (1970). Low glucosinolate *B. juncea* was produced through interspecific crosses between *B. rapa* and *B. juncea* (Love et al., 1990). Rashid et al. (1994) was able to produce stable yellow seeded *B. napus* through interspecific crosses between *B. napus*, *B. juncea* and *B. carinata*.

Cytological studies conducted in the 1930's by Morinaga (1929a,b,c & 1934) and U (1935) showed the inter-relatedness of the *Brassica* species (reviewed by Prakash and Hinata, 1980). Because the species within U's triangle share a common ancestry, interspecific crosses can be used to transfer genetic material between the species.

The objective of the current study was to determine whether traits of agronomic importance could be transferred between species, utilizing an interspecific modified backcross scheme (see Figure 2.1.). The doubled haploid technique was utilized at the F_1 and BC_1F_1 stages to reduce the time required to reach homozygosity, and to alleviate some of the fertility problems associated with unpaired chromosomes known to arise in interspecific crosses (Thiagarajah et al., 1994; Stringam et al., 1995).

4.2. Materials and Methods:

4.2.1. Field Design:

Field evaluation of plant material was conducted at the Edmonton Research Station in 1997. The dominant soil type of the area was black Chernozemic. An interspecific DH_1 derived from a cross between *B. napus* and *B. rapa* was again crossed to four *B. rapa* types in a modified backcross scheme (see Chapter 2). The *B. rapa* parents were all self-fertile F_7 *B. rapa* lines, derived from an interspecific cross between the cultivar Eclipse and U of A *B. napus* breeding line number 83-52692B. Line 83-52692B was a Dwarf F_6 line, derived from a cross between *B. napus* cultivar Tower and a colchicine induced dwarf *B. napus* line, produced by Brian Fowler (Crop Development Center, University of Saskatchewan). Forty-three interspecific individuals (Doubled Haploid lines), the parents used to generate these DH lines, and two commercial cultivars (Quantum and Eclipse) were grown in two separate tests. One test was set up as a 4 replicate Randomized Complete Block design with 17 of the DH lines, the 7 parent lines (*B. rapa* parents 93-1005-3, 95-439-2, 95-440-2, 95-442-2, 95-442-3; *B. napus* 94-98; interspecific DH_1 95-376), and the 2 commercial checks. The second test, set up as an Augmented design featured 12 DH lines, the 7 parents, and 2 commercial checks all seeded in all 4 replicates, 6 DH lines in two replicates, and 8 DH lines in one replicate. The Augmented design included lines from which there was insufficient seed available to plant a 4-replicate test. The

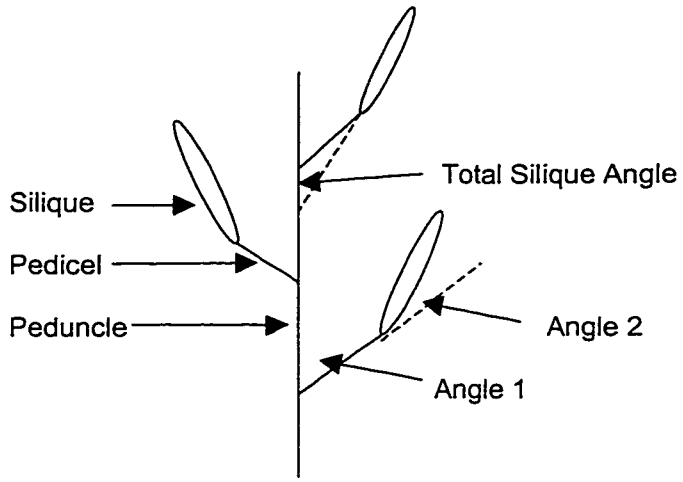
two-row plots were one meter long, with 22.8 cm (nine-inch) row spacing and 1 meter alleys between replicates. Seeds were sown one row at a time with a manual push hoe seeder on May 9th 1997. The tests were sown with an 8 row border of a 50:50 mixture of *B. napus B. rapa* seed, to eliminate edge effects. Weeds were controlled by manual rouging.

4.2.2. Phenotypic Characterization:

General plant morphology was recorded using the descriptors outlined by the IBPGR (1990). Leaf color, leaf shape, leaf division (margin), and leaf hairiness were recorded prior to bolting. Flowering data were recorded when the first flower opened in each plot. During full flowering, the degree of leaf clasping, bud position, and flower type were recorded. The plants in one replicate of both tests were self-pollinated using Delnets, to multiply self-pollinated seed. The length of the main raceme, total plant height, and the number of siliques on the main raceme were recorded on 3 plants in each plot, after flowering was complete. The two angles governing the total angle between the silique and the main raceme were measured on the middle third of the main raceme. Four measurements on three plants were made in each plot, using a protractor. The first measurement was the angle between the pedicel and the peduncle, while the second angle was the number of degrees the silique was deflected up from parallel with the pedicel (Figure 4.1.). The siliques measured for silique angle were harvested for determination of carpel length, beak length, and seeds per

silique. The number of seeds per silique was determined by placing the unopened siliques over a bright light. At maturity, plants were harvested by hand. Three individual plants and a bulk sample were harvested where there was greater than 5 plants in a plot. When there was 1 to 5 single plants in a plot, the seed from each was harvested separately. Seed color was recorded, and 1000-kernel weight determined on seed dried to 5% moisture. Three samples of 100 seeds were weighed for each plot to determine 1000-seed weight. For each plot, the 3 weights were averaged and used to calculate 1000-seed weights. To estimate fertility and determine seed spacing within the silique, the carpel length was divided by the number of seeds per silique and reported as distance between seeds (DBS).

Figure 4.1. Main raceme of a canola plant depicting the two angles governing total silique angle. Angle 1 is the angle between the pedicel and peduncle (main stem), while angle 2 is the number of degrees the silique is deflected up from being parallel with the pedicel. These angles were used to extrapolate the angle between the silique and peduncle (Total Silique Angle).



4.2.3. Determination of Oil Content:

Oil content was determined on a whole seed basis by nuclear magnetic resonance (NMR) analysis, using an Oxford 4000 NMR Analyzer (Oxford Analytical Instruments Ltd). One bulk sample of 1.2 g was analyzed per replicate for each line grown during the 1997 field season. The measured oil content was multiplied by 0.95 to correct for the 5 % moisture present in the air dried seed.

4.2.4. Determination of Protein Content:

Protein content was determined on a whole seed basis using a Leco FP-2000 Analyzer fitted with a nitrogen probe. One bulk sample of 0.5 g was analyzed per replicate for each line grown during the 1997 field season. The protein value measured was corrected for the 5 % moisture present in the seed by dividing by 0.95. The dry protein value was then converted to an oil free value by dividing by 1 minus the percent oil (expressed as a decimal).

4.2.5. Analysis of Fatty Acid Profiles:

Three single plants from each line per replicate were analyzed for the proportion of each fatty acid. Fatty acid content was determined by the ISO 5508 (1990) procedure for animal and vegetable oils, using gas chromatography as. A Gas Liquid Chromatograph (HP model 5890 series 1) equipped with an autosampler and an integrator was used to analyze the fatty acid profiles. Fatty acids were expressed as a percentage of the total fatty acid profile.

4.2.6. Determination of Glucosinolate Content in the Meal:

Analysis of glucosinolate content was performed on the defatted meal obtained after oil was extracted for fatty acid analysis, according to the trimethysilyl (TMS) derivatization of desulphated glucosinolate procedure (Raney

and McGregor, 1990). Analysis was performed using a Gas Liquid Chromatograph (HP model 5890 series 1) equipped with an autosampler and an integrator. Glucosinolates were reported as µmol per gram of oil extracted meal and glucosinolates expressed on a µmole per gram of air dried oil free meal basis.

2.4.7. Statistical Analysis:

Least-squares means (ls means) were calculated using SAS version 6.12 (1996), as missing data, and the augmented field design lead to unbalanced data sets. Mean, standard deviation, and Wilk's statistic, for each of the quantitative traits within the 95-1061-B population were generated by the univariate procedure of SAS.

Chi-square analysis was performed to test whether the observed segregation ratios of 5 morphological characteristics in the 95-1061-B population fit the expected frequencies of homozygous recessive alleles in a DH population, proposed by Pauls (1996).

The glm procedure of SAS (version 6.12 1996) was utilized to test for correlation between morphological characters which demonstrated similar segregation ratios in the 95-1061-B population.

4.3. Results:

Seed from three of the lines in the second test did not germinate and therefore only 40 out of 43 lines could be evaluated. Thirty-five of the forty modified backcross DH lines came from the 95-1061-B microspore culture. Analysis of variance could not be performed because all of the data sets violated the assumptions of normally distributed residuals and/or homogeneity of variance. Log, inverse, squared, square root, exponent, and arcsin transformations did alleviate the heterogeneity of variation and normality violations.

4.3.1. Leaf and Flower Morphology:

Descriptions of leaf and flower morphology and seed color were made for the parents and lines within the 95-1061-B population (Table 4.1.), as well as for the lines from the other 3 cultures, their *B. rapa* parents, Quantum, and Eclipse (Appendix 1). The appearance of the backcross DH lines resembled the *B. napus* parent with some *B. rapa* influence on leaf shape, margin serration, upper leaf clasping, flower size, and the position of the bud cluster in relation to newly opened flowers. The *B. napus* parent (94-98), DH_1 line (95-376), and all of the backcross DH progeny had waxy gray-green leaves while the *B. rapa* parents were glossy green.

The leaf shape of the *B. napus* parent and DH_1 lines (95-376) was classified as elliptic since leaves were broad in the middle and tapered towards the apex and the petiole. The leaves of the *B. rapa* parents were classified as ovate as they were broad at the petiole end, and tapered to a point at the apex. The leaves of Quantum differed from both these shapes, more closely fitting the description of orbicular since they were almost completely round. Within the backcross DH progeny, 4 DH lines had ovate leaves while 31 had elliptic shaped leaves (Table 4.1.). The ratio of *B. napus* (elliptic) leaf shape to *B. rapa* (ovate) leaf shape was 7.8:1. Based on the model for the frequency of a homozygous recessive genotype in a doubled haploid population ($1/2^n$ where n=#genes) described by Pauls (1996), it appears that leaf shape in this population is governed by 3 genes with *B. rapa* (ovate) leaf shape being recessive. Chi-square analysis revealed P values greater than 0.05 for 2, 3, and 4 gene inheritance ratios (Table 4.2.).

The *B. napus* parent and interspecific DH_1 parent (95-376) had a dentate leaf margin while leaf margins of the *B. rapa* parents tended to be doubly dentate. Twelve DH lines had *B. napus* (dentate) leaf margins and 20 lines had doubly dentate leaf margins similar to the *B. rapa* parent (Table 4.1.). Three of the DH progeny had leaf margins that were doubly dentate to the extent of being slightly toothed. Because *B. rapa* can have either doubly dentate or toothed leaf margins, these classes were combined for chi-square analysis.

The upper leaves of the *B. rapa* parent 95-440-2 clasped the stem approximately 75% of its circumference. The upper leaves of the *B. napus*

parent and DH_1 line (95-376) clasped the stem 50% of its circumference. Within the backcross DH lines, 12 lines had *B. rapa* (75%) leaf clasp and 23 lines had *B. napus* (50%) leaf clasp (Table 4.1.). The segregation ratios of 1.9:1 for leaf margin type (*B. rapa*:*B. napus*), and upper leaf clasp (*B. napus*:*B. rapa*) suggests that these traits are governed by 1 to 2 genes each, with *B. napus* margin type and *B. rapa* leaf clasp being recessive. Chi-square analysis (Table 4.2.) revealed P values greater than 0.05 for segregation ratios expected if 1 or 2 genes govern these traits.

The *B. rapa* parents were the only lines with leaf pubescence. The hairs tended to be on the upper leaf surface, near the apex.

The cluster of unopened flower buds in the *B. rapa* parents did not protrude above the newly opened flower buds as is the case with *B. napus*. The unopened bud cluster of the *B. napus* parent and DH_1 line (95-376) protruded above the newly opened flower buds. Three of the thirty-five backcross DH lines had bud clusters that remained below the last opened flowers, while 32 had a bud cluster that protruded above the newly opened flower buds (Table 4.1.).

The flower petals of the *B. rapa* parents were small, thin and a deeper yellow color than the petals of the *B. napus* and DH_1 line which were large, broad and pale yellow in color. Thirty-two of the backcross DH lines had flower color and petal size similar to the *B. napus* parent, while 3 had flower color and size similar to the *B. rapa* parent (Table 4.2.). One out of the thirty-five lines had both a *B. rapa* flower type (color and size) and bud position. The segregation ratios of 10.7:1 for flower bud cluster position, and flower type suggest that these traits

are governed by at least 3 genes, with *B. rapa* bud cluster position and flower type being recessive. Chi-square analysis revealed significant P values for segregation ratios expected if 3, 4, or 5 genes govern each of these traits.

Similar segregation ratios for pairs of traits were observed in the population. Leaf margin type and upper leaf clasp both had a segregation ratio of 1.9:1, while flower type and bud position both had a segregation ratio of 10.7:1. Statistical analysis revealed a significant correlation (Pr>P of 0.002) of flower type and bud position, with an R^2 value of 0.28, and an insignificant correlation (Pr>P of 0.4876) of leaf margin type and upper leaf clasp with an R^2 value of 0.02.

4.3.2. Quality and Quantity of Oil and Protein, Agronomic Traits, and Yield Components:

Least-squares means (ls means) for the dominant fatty acids, expressed as a percent of the total fatty acid profile were determined for the parents and lines from the 95-1061-B population (Table 4.3.). Ls means for the dominant glucosinolates were calculated and expressed on a µmole per gram of air dried, oil free meal basis (Table 4.4.). Ls means for agronomic traits such as days to flower, silique angle, length of the main stem, and height were calculated (Table 4.5.). Ls means for yield components such as carpel length, beak length, number of siliques on the main stem, seeds per silique, 1000 seed weight, distance between seeds (DBS) were determined (Table 4.6.). Seed color as well

as Ls means for percent oil content (dry basis), % protein content (dry oil free meal basis), and were also determined (Table 4.7.). The population mean, standard deviation and Wilk's statistic for normality were also determined for each of the quantitative traits (Tables 4.3. to 4.7.).

Ls means for the fatty acid profiles, glucosinolates, days to flower, silique angle, length of the main stem, height, yield components, oil and protein content, as well as visual description of seed color for the 5 lines which were derived from the other populations, as well as for Quantum and Eclipse were determined (Appendices 2 to 6).

The distribution of alkenyl, indoyl, and total glucosinolate content within the parents and interspecific DH lines from the 95-1061-B population was plotted (Figure 4.2.).

Table 4.1. Leaf and flower morphology within the parents and DH lines from the 95-1061-B population based on IBPGR descriptors.

		Leaf Characteristics					Flower Characteristics	
Line	Parent	Color	Shape	Margin	Hairiness	Clasp	Bud position	Flower Type
93-1005-3	*B. rapa*	Glossy	Ovate	Doubly dentate	Apex	Full	Below	*B. rapa*
94-98	*B. napus*	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
95-376	DH1	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
95-440-2	*B. rapa*	Glossy	Ovate	Doubly dentate	Apex	75%	Below	*B. rapa*
DH lines								
96-1066-2	95-1061-B	Waxy	Ovate	Doubly dentate	None	75%	Above	*B. napus*
96-1069-1	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1179-1	95-1061-B	Waxy	Elliptic	Toothed	None	50%	Above	*B. rapa*
96-1180-1	95-1061-B	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
96-1183-1	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1195-1	95-1061-B	Waxy	Elliptic	Doubly dentate	None	75%	Above	*B. napus*
96-1196-3	95-1061-B	Waxy	Elliptic	Doubly dentate	None	75%	Above	*B. napus*
96-1687-3	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1691-3	95-1061-B	Waxy	Elliptic	Toothed	None	50%	Above	*B. napus*
96-1693-3	95-1061-B	Waxy	Ovate	Doubly dentate	None	50%	Above	*B. napus*
96-1696-1	95-1061-B	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
96-1703-2	95-1061-B	Waxy	Elliptic	Doubly dentate	None	75%	Above	*B. napus*
96-1704-3	95-1061-B	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
96-1707-3	95-1061-B	Waxy	Elliptic	Dentate	None	75%	Above	*B. napus*
96-1711-3	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Below	*B. rapa*
96-1712-2	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1713-3	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1718-3	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1732-2	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1734-2	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1735-3	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1743-2	95-1061-B	Waxy	Elliptic	Toothed	None	50%	Above	*B. napus*
96-1746-2	95-1061-B	Waxy	Elliptic	Dentate	None	75%	Above	*B. napus*
96-1747-2	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Above	*B. napus*
96-1753-3	95-1061-B	Waxy	Elliptic	Doubly dentate	None	75%	Above	*B. napus*
96-1755-2	95-1061-B	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
96-1756-3	95-1061-B	Waxy	Elliptic	Dentate	None	75%	Above	*B. napus*
96-1759-2	95-1061-B	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
96-1768-3	95-1061-B	Waxy	Ovate	Dentate	None	75%	Below	*B. napus*
96-1773-1	95-1061-B	Waxy	Elliptic	Doubly dentate	None	50%	Below	*B. napus*
96-2016-3	95-1061-B	Waxy	Elliptic	Dentate	None	50%	Above	*B. rapa*
96-2018-1	95-1061-B	Waxy	Ovate	Doubly dentate	None	75%	Above	*B. napus*
96-2022-4	95-1061-B	Waxy	Elliptic	Dentate	None	50%	Above	*B. napus*
96-2025-1	95-1061-B	Waxy	Elliptic	Dentate	None	75%	Above	*B. napus*
96-2027-1	95-1061-B	Waxy	Elliptic	Doubly dentate	None	75%	Above	*B. napus*

Table 4.2. Chi-square tests for five morphological characters within the 95-1061-B interspecific DH population.

# genes predicted	Predicted ratio	Phenotype	Leaf Shape			Leaf Margin			Leaf Clasp		
			Observed frequency	X^2	P	Observed frequency	X^2	P	Observed frequency	X^2	P
1	1:1	*B. rapa*	4	20.829	<0.01	23	3.457	0.20-0.05	12	3.457	0.20-0.05
		B. napus	31			12			23		
2	3:1	*B. rapa*	4	3.438	0.20-0.05	23	1.610	0.30-0.20	12	1.610	0.30-0.20
		B. napus	31			12			23		
3	7:1	*B. rapa*	4	0.037	0.95-0.80	23	15.188	<0.01	12	15.188	<0.01
		B. napus	31			12			23		
4	15:1	*B. rapa*	4	1.602	0.30-0.20						
		B. napus	31								
5	31:1	*B. rapa*	4	7.971	<0.01						
		B. napus	31								
6	63:1	*B. rapa*									
		B. napus									

Table 4.2. Continued

# genes predicted	Predicted ratio	Phenotype	Bud Position			Flower Type		
			Observed frequency	X^2	P	Observed frequency	X^2	P
1	1:1	*B. rapa*						
		B. napus						
2	3:1	*B. rapa*	3	5.038	<0.01	3	5.038	<0.01
		B. napus	32			32		
3	7:1	*B. rapa*	3	0.494	0.50-0.30	3	0.494	0.50-0.30
		B. napus	32			32		
4	15:1	*B. rapa*	3	0.322	0.70-0.50	3	0.322	0.70-0.50
		B. napus	32			32		
5	31:1	*B. rapa*	3	3.429	0.20-0.05	3	3.429	0.20-0.05
		B. napus	32			32		
6	63:1	*B. rapa*	3	11.179	<0.01	3	11.179	<0.01
		B. napus	32			32		

Table 4.3. Ls means for the dominant fatty acids (% Total Profile) in the parents and DH lines from the 95-1061-B population, population mean, standard deviation (stdev.), and Wilk's Statistic (Pr<W).

Line	Parent	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Erucic	Saturates
93-1005-3	*B. rapa*	3.40	1.57	58.57	21.69	12.24	0.03	5.89
94-98	*B. napus*	4.07	1.70	56.77	18.67	15.74	0.04	6.96
95-376	DH1	4.48	1.67	52.64	21.79	16.42	0.05	7.36
95-440-2	*B. rapa*	3.51	1.51	54.97	24.53	12.81	0.04	5.97
DH lines								
96-1066-2	95-1061-B	4.56	1.55	53.16	20.50	17.23	0.05	7.24
96-1069-1	95-1061-B	4.99	1.45	50.95	22.67	16.93	0.07	7.60
96-1179-1	95-1061-B	4.04	1.51	54.43	21.32	15.81	0.04	6.64
96-1180-1	95-1061-B	3.98	1.70	59.23	17.63	14.67	0.02	6.81
96-1183-1	95-1061-B	4.49	1.42	50.95	22.82	17.47	0.04	7.02
96-1195-1	95-1061-B	5.45	2.04	46.84	26.87	15.31	0.02	9.00
96-1196-3	95-1061-B	4.56	1.78	47.24	25.40	17.74	0.02	7.72
96-1687-3	95-1061-B	4.54	1.71	54.04	22.65	14.16	0.03	7.48
96-1691-3	95-1061-B	4.44	1.70	50.66	21.62	17.87	0.44	7.34
96-1693-3	95-1061-B	4.60	1.50	56.23	19.43	15.57	0.03	7.12
96-1696-1	95-1061-B	4.35	1.79	58.38	16.75	15.64	0.06	7.35
96-1703-2	95-1061-B	4.31	1.84	56.84	19.13	15.03	0.04	7.38
96-1704-3	95-1061-B	4.25	1.58	54.97	20.25	16.01	0.06	6.96
96-1707-3	95-1061-B	4.37	1.38	48.54	23.97	18.45	0.06	6.92
96-1711-3	95-1061-B	4.40	1.70	55.06	21.51	14.68	0.04	7.21
96-1712-2	95-1061-B	4.14	1.68	54.94	20.23	16.08	0.02	7.00
96-1713-3	95-1061-B	4.23	1.71	58.09	18.24	14.81	0.03	7.08
96-1718-3	95-1061-B	4.99	1.56	48.56	25.06	16.78	0.04	7.77
96-1732-2	95-1061-B	4.09	1.83	55.09	21.26	14.87	0.02	7.13
96-1734-2	95-1061-B	4.72	1.69	47.37	24.02	18.14	0.63	7.69
96-1735-3	95-1061-B	4.42	1.49	56.44	19.97	14.87	0.02	7.01
96-1743-2	95-1061-B	4.69	1.75	51.44	22.30	16.75	0.03	7.70
96-1746-2	95-1061-B	4.55	1.61	53.26	22.65	14.73	0.08	7.42
96-1747-2	95-1061-B	5.19	1.32	47.34	22.62	20.40	0.06	7.57
96-1753-3	95-1061-B	4.48	1.74	56.73	18.80	15.31	0.06	7.25
96-1755-2	95-1061-B	4.28	1.89	55.87	20.10	14.86	0.05	7.41
96-1756-3	95-1061-B	4.55	1.54	51.68	22.18	16.92	0.04	7.24
96-1759-2	95-1061-B	4.38	1.69	52.66	20.84	17.23	0.05	7.31
96-1768-3	95-1061-B	4.42	1.79	54.59	20.95	14.98	0.04	7.51
96-1773-1	95-1061-B	4.37	1.58	53.76	21.77	15.59	0.05	7.12
96-2016-3	95-1061-B	4.70	1.99	53.71	21.41	15.11	0.04	7.83
96-2018-1	95-1061-B	4.50	1.72	54.93	19.02	16.90	0.04	7.33
96-2022-4	95-1061-B	4.53	1.70	50.88	24.14	15.54	0.04	7.50
96-2025-1	95-1061-B	5.37	1.58	45.81	25.97	18.04	0.04	8.26
96-2027-1	95-1061-B	4.68	1.80	49.95	23.97	16.46	0.05	7.72
Mean		4.46	1.66	53.17	21.66	16.00	0.07	7.30
Stdev		0.39	0.14	3.36	2.15	1.50	0.05	0.50
Pr<W		0.0500	0.7928	0.1433	0.9531	0.6110	0.0001	0.0119

Table 4.4. Ls means for glucosinolate content of air dired oil free meal (µmol/g) the parents and DH lines from the 95-1061-B population, population mean, standard deviation (stdev.), and Wilk's Statistic (Pr<W).

LINE	Parent	T. Alkenyl	T. Indoyl	T. Gluc.	Butanyl	OHIndoyl	OHButanyl
93-1005-3	*B. rapa*	6.1	9.7	15.8	2.4	9.4	2.6
94-98	*B. napus*	12.0	10.9	22.9	2.8	10.5	6.6
95-376	DH1	16.9	9.7	26.6	3.6	9.2	11.5
95-440-2	*B. rapa*	9.4	10.6	19.9	2.3	10.2	4.3
DH lines							
96-1066-2	95-1061-B	4.6	12.2	16.7	1.1	11.1	3.4
96-1069-1	95-1061-B	1.5	11.3	12.8	0.1	10.9	1.2
96-1179-1	95-1061-B	0.9	10.4	11.3	0.0	9.8	0.6
96-1180-1	95-1061-B	0.0	10.3	9.9	0.0	9.9	0.0
96-1183-1	95-1061-B	0.0	8.9	8.4	0.0	8.7	0.0
96-1195-1	95-1061-B	5.0	12.0	17.0	0.4	11.3	4.4
96-1196-3	95-1061-B	5.3	12.0	17.3	1.6	11.5	3.6
96-1687-3	95-1061-B	2.3	9.2	11.5	0.7	8.8	1.2
96-1691-3	95-1061-B	1.1	9.8	10.9	0.5	9.1	0.3
96-1693-3	95-1061-B	1.9	13.2	15.1	0.5	12.7	1.0
96-1696-1	95-1061-B	9.4	11.4	20.8	1.3	10.9	7.1
96-1703-2	95-1061-B	0.9	8.9	9.8	0.3	8.7	0.3
96-1704-3	95-1061-B	2.1	9.5	11.6	0.4	8.9	1.3
96-1707-3	95-1061-B	24.1	13.0	37.1	2.9	12.6	19.8
96-1711-3	95-1061-B	0.2	7.5	7.7	0.0	7.3	0.0
96-1712-2	95-1061-B	1.5	10.0	11.5	0.7	9.6	0.4
96-1713-3	95-1061-B	2.1	11.9	14.0	0.7	11.3	0.9
96-1718-3	95-1061-B	5.0	10.2	15.1	1.4	9.7	3.4
96-1732-2	95-1061-B	0.9	8.0	8.9	0.0	7.8	0.7
96-1734-2	95-1061-B	7.8	6.4	14.2	1.5	6.1	5.2
96-1735-3	95-1061-B	0.0	10.6	10.2	0.0	10.3	0.0
96-1743-2	95-1061-B	3.2	10.3	13.5	1.0	9.7	1.6
96-1746-2	95-1061-B	9.4	6.0	15.4	1.3	5.7	8.6
96-1747-2	95-1061-B	7.5	10.7	18.3	1.2	10.0	6.2
96-1753-3	95-1061-B	6.9	9.8	16.7	1.0	9.6	6.0
96-1755-2	95-1061-B	4.7	10.3	15.1	1.2	9.8	3.7
96-1756-3	95-1061-B	6.9	9.4	16.3	1.7	9.1	4.9
96-1759-2	95-1061-B	2.8	8.4	11.2	0.7	8.1	2.3
96-1768-3	95-1061-B	6.6	9.1	15.7	2.6	8.6	4.0
96-1773-1	95-1061-B	5.4	11.3	16.7	1.1	10.6	4.3
96-2016-3	95-1061-B	10.1	10.2	20.3	3.0	9.6	6.2
96-2018-1	95-1061-B	3.7	10.3	14.0	1.0	9.9	2.7
96-2022-4	95-1061-B	6.1	10.9	16.9	2.0	10.3	4.1
96-2025-1	95-1061-B	10.7	10.2	21.0	2.6	9.3	8.0
96-2027-1	95-1061-B	7.1	8.9	16.0	1.5	8.3	5.3
Mean		5.4	10.1	15.5	1.2	9.6	3.7
Stdev		4.0	0.9	4.6	0.7	0.8	3.4
Pr<W		0.0002	0.4734	0.0003	0.0438	0.4426	0.0001

Table 4.5. Ls means for agronomic traits in the parents and DH lines from the 95-1061-B population, population mean, standard deviation (Stdev), and Wilk's Statistic (Pr<W).

LINE	Parent	Days to Flower	Silique Angle (Degrees)	Angle 1 (Degrees)	Angle 2 (Degrees)	L.main (cm)	Height (cm)
93-1005-3	*B. rapa*	36.4	40	68	28	59.0	97.1
94-98	*B. napus*	46.5	50	57	7	40.4	66.8
95-376	DH1	39.6	51	62	11	42.8	75.9
95-440-2	*B. rapa*	38.9	36	67	30	51.2	75.1
DH lines							
96-1066-2	95-1061-B	44.5	56	64	8	60.6	100.4
96-1069-1	95-1061-B	50.9	50	60	10	55.4	87.6
96-1179-1	95-1061-B	42.4	71	78	7	42.9	82.7
96-1180-1	95-1061-B	47.4	58	73	14	55.7	91.9
96-1183-1	95-1061-B	44.1	60	66	6	61.1	98.0
96-1195-1	95-1061-B	39.5	44	64	20	41.9	70.6
96-1196-3	95-1061-B	44.7	.	.	.	.	.
96-1687-3	95-1061-B	36.4	45	58	13	48.7	81.1
96-1691-3	95-1061-B	48.4	51	62	12	52.6	80.0
96-1693-3	95-1061-B	36.9	47	58	12	51.7	86.9
96-1696-1	95-1061-B	45.1	55	65	10	48.9	80.1
96-1703-2	95-1061-B	43.1	49	57	7	57.1	95.8
96-1704-3	95-1061-B	46.9	53	60	7	39.2	63.6
96-1707-3	95-1061-B	50.9	45	60	15	.	.
96-1711-3	95-1061-B	38.6	58	66	8	45.0	70.9
96-1712-2	95-1061-B	40.6	52	62	10	57.3	94.2
96-1713-3	95-1061-B	40.6	55	68	14	58.4	95.6
96-1718-3	95-1061-B	43.1	55	66	12	39.2	59.5
96-1732-2	95-1061-B	37.9	48	60	12	42.9	77.3
96-1734-2	95-1061-B	48.1	52	67	15	71.5	97.8
96-1735-3	95-1061-B	38.4	56	65	9	56.1	90.6
96-1743-2	95-1061-B	43.6	68	72	4	48.3	83.3
96-1746-2	95-1061-B	42.9	43	59	16	31.4	69.6
96-1747-2	95-1061-B	45.9	57	67	10	69.1	101.1
96-1753-3	95-1061-B	42.6	51	60	9	47.9	81.8
96-1755-2	95-1061-B	40.4	54	70	16	55.9	91.3
96-1756-3	95-1061-B	41.3	56	61	6	57.6	85.6
96-1759-2	95-1061-B	46.1	50	67	18	38.5	79.1
96-1768-3	95-1061-B	42.3	58	67	9	41.6	69.1
96-1773-1	95-1061-B	38.4	59	68	9	47.4	85.4
96-2016-3	95-1061-B	40.9	55	70	14	45.4	72.1
96-2018-1	95-1061-B	51.1	48	60	13	63.6	92.1
96-2022-4	95-1061-B	39.4	59	72	13	36.6	63.1
96-2025-1	95-1061-B	40.5	.	.	.	.	.
96-2027-1	95-1061-B	43.9	52	64	11	40.5	71.3
Mean		42.8	53	65	12	50.1	82.3
Stdev		3.7	6	3	5	8.2	10.1
Pr<W		0.1784	0.6248	0.3730	0.0002	0.5913	0.2380

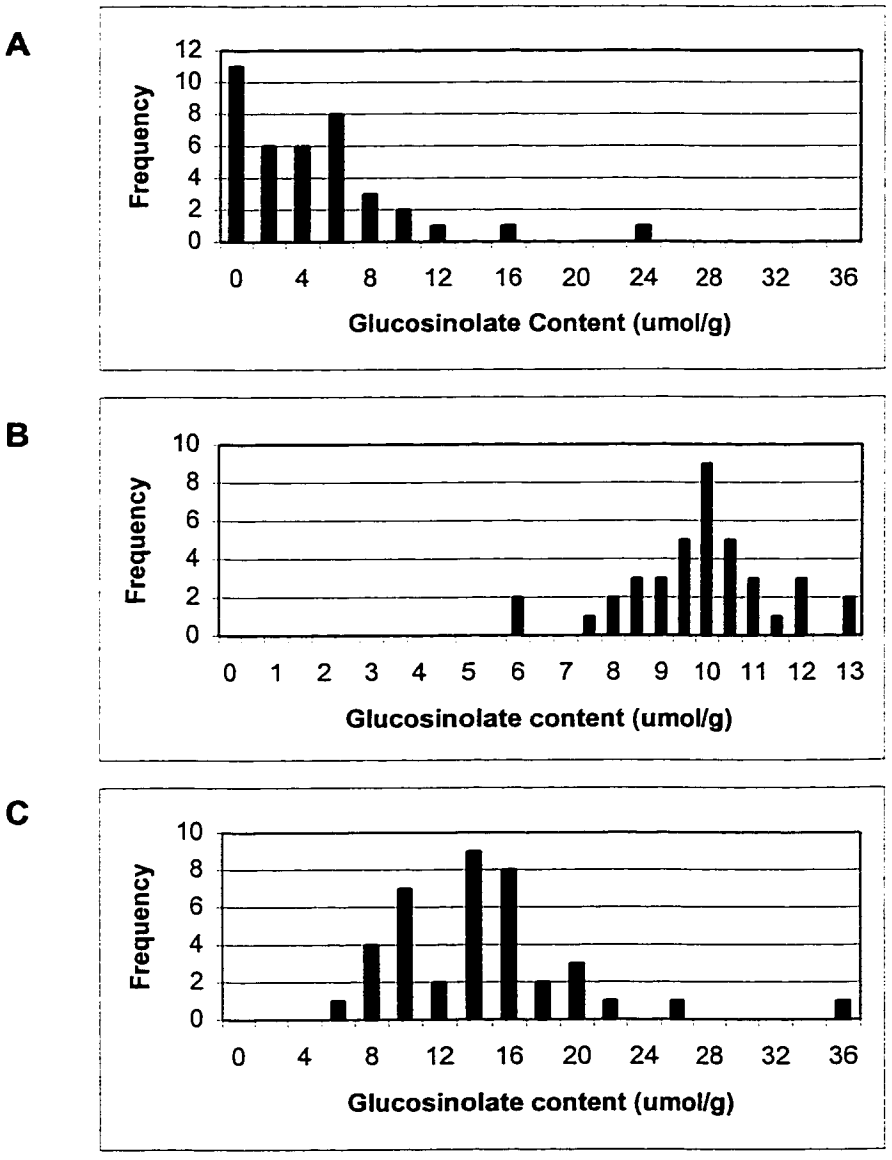
Table 4.6. Ls means for length of the carpel and beak, number of siliques on the main stem, seeds / silique, 1000 seed weight, and distance between seeds (DBS) in the parents and DH lines from the 95-1061-B population, population mean, standard deviation (Stdev), and Wilk's Statistic (Pr<W).

LINE	Parent	L.Carpel (mm)	L.Beak (mm)	Number Siliques	Seed/ Silique	Seed weight (g)	DBS (mm)
93-1005-3	*B. rapa*	42.6	16.6	52.8	19.5	3.31	2.3
94-98	*B. napus*	63.9	9.1	23.8	21.6	4.37	3.1
95-376	DH1	52.3	8.8	32.6	15.7	4.89	3.7
95-440-2	*B. rapa*	24.7	14.2	36.3	10.2	3.24	3.6
DH lines							
96-1066-2	95-1061-B	56.6	11.8	46.2	19.0	4.71	3.2
96-1069-1	95-1061-B	61.9	10.2	49.3	23.4	3.57	2.8
96-1179-1	95-1061-B	77.1	8.3	30.1	30.5	4.49	2.5
96-1180-1	95-1061-B	70.5	11.9	42.2	28.3	3.67	2.5
96-1183-1	95-1061-B	79.7	11.4	45.7	31.2	4.41	2.5
96-1195-1	95-1061-B	47.2	8.2	31.6	10.8	4.31	5.1
96-1196-3	95-1061-B	.	.	.	.	4.31	.
96-1687-3	95-1061-B	51.3	10.5	31.3	19.0	3.89	3.0
96-1691-3	95-1061-B	65.4	12.6	29.4	22.8	4.88	3.1
96-1693-3	95-1061-B	54.9	13.0	39.0	17.6	4.45	3.7
96-1696-1	95-1061-B	63.3	11.3	34.5	22.2	4.25	3.0
96-1703-2	95-1061-B	60.0	10.8	43.0	25.7	5.02	2.3
96-1704-3	95-1061-B	62.4	11.6	27.8	20.7	4.08	3.3
96-1707-3	95-1061-B	41.1	7.7	.	9.6	4.70	5.2
96-1711-3	95-1061-B	51.1	12.0	30.6	17.1	4.82	3.3
96-1712-2	95-1061-B	71.6	11.5	46.5	25.3	4.60	2.9
96-1713-3	95-1061-B	75.8	11.2	42.6	29.0	4.33	2.6
96-1718-3	95-1061-B	49.2	7.6	28.5	12.5	4.96	5.9
96-1732-2	95-1061-B	48.8	10.9	28.2	15.8	5.13	3.9
96-1734-2	95-1061-B	54.1	10.7	42.1	15.7	4.68	4.0
96-1735-3	95-1061-B	60.1	12.4	38.0	19.5	4.17	3.3
96-1743-2	95-1061-B	80.5	10.4	33.1	27.5	4.77	2.9
96-1746-2	95-1061-B	36.9	7.2	25.3	3.2	4.17	8.9
96-1747-2	95-1061-B	61.0	11.5	51.4	19.3	4.77	3.3
96-1753-3	95-1061-B	54.6	11.8	34.0	18.7	4.31	3.2
96-1755-2	95-1061-B	56.2	8.0	37.5	23.3	4.64	2.6
96-1756-3	95-1061-B	48.2	10.8	42.1	15.5	4.90	3.4
96-1759-2	95-1061-B	53.5	9.9	31.0	16.4	4.93	3.7
96-1768-3	95-1061-B	45.4	8.0	31.5	9.4	4.64	6.3
96-1773-1	95-1061-B	59.1	7.8	33.2	21.2	5.04	3.0
96-2016-3	95-1061-B	38.3	5.4	33.8	8.2	4.73	5.5
96-2018-1	95-1061-B	55.4	12.8	45.5	17.6	4.73	3.3
96-2022-4	95-1061-B	48.8	5.6	27.6	12.5	4.54	4.4
96-2025-1	95-1061-B	.	.	.	.	5.10	.
96-2027-1	95-1061-B	42.7	7.3	28.5	10.9	4.65	4.4
Mean		55.8	10.3	36.3	18.5	4.49	3.7
Stdev		11.9	2.3	7.1	5.8	0.44	0.8
Pr<W		0.8178	0.3953	0.0607	0.8357	0.0033	0.001

Table 4.7. Ls means for percent oil (dry basis) percent protein (dry oil free meal), and seed color description in the parents and DH lines from the 95-1061-B population, population mean, standard deviation (Stdev), and Wilk's Statistic (Pr<W).

LINE	Parent	% Oil	% Protein	Seed color
93-1005-3	*B. rapa*	42.9	46.0	yellow
94-98	*B. napus*	35.8	51.1	black
95-376	DH1	32.9	50.1	brown
95-440-2	*B. rapa*	40.5	47.9	yellow
DH lines				
96-1066-2	95-1061-B	37.8	49.4	black
96-1069-1	95-1061-B	35.6	45.2	black
96-1179-1	95-1061-B	37.6	48.7	black
96-1180-1	95-1061-B	37.5	47.0	black
96-1183-1	95-1061-B	34.2	44.9	black
96-1195-1	95-1061-B	26.3	45.1	brown
96-1196-3	95-1061-B	30.0	48.5	brown
96-1687-3	95-1061-B	35.5	48.1	black
96-1691-3	95-1061-B	35.6	46.7	black
96-1693-3	95-1061-B	36.2	48.2	black
96-1696-1	95-1061-B	36.8	50.0	black
96-1703-2	95-1061-B	36.9	47.5	black
96-1704-3	95-1061-B	35.3	50.0	black
96-1707-3	95-1061-B	32.1	46.9	black
96-1711-3	95-1061-B	36.3	50.7	black
96-1712-2	95-1061-B	36.6	49.1	black
96-1713-3	95-1061-B	39.2	48.4	black
96-1718-3	95-1061-B	31.5	50.9	brown
96-1732-2	95-1061-B	36.8	49.1	black
96-1734-2	95-1061-B	31.4	45.4	black
96-1735-3	95-1061-B	37.8	49.5	black
96-1743-2	95-1061-B	34.2	46.3	black
96-1746-2	95-1061-B	30.3	50.2	brown
96-1747-2	95-1061-B	32.3	48.8	brown
96-1753-3	95-1061-B	38.3	47.8	black
96-1755-2	95-1061-B	36.1	49.2	black
96-1756-3	95-1061-B	35.5	49.8	black
96-1759-2	95-1061-B	34.0	50.4	black
96-1768-3	95-1061-B	32.7	49.4	brown
96-1773-1	95-1061-B	35.2	48.9	black
96-2016-3	95-1061-B	29.9	48.6	brown
96-2018-1	95-1061-B	37.9	49.2	black
96-2022-4	95-1061-B	32.7	51.0	brown
96-2025-1	95-1061-B	30.1	48.6	brown
96-2027-1	95-1061-B	30.5	49.1	brown
Mean		34.8	48.5	
Stdev		3.0	1.6	
Pr<W		0.7559	0.0379	

Figure 4.2. Distribution of alkenyl **A**, indoyl **B**, and total glucosinolate **C** contents, within the parents and interspecific DH lines from the 95-1061-B population.



4.4. Discussion and Conclusions:

Due to poor seed germination, uneven stands were prevalent in most of the plots. All data should be considered preliminary as it is based on one field season. Transforming the data could not reduce heterogeneity of variance, observed in all the data collected. Heterogeneity of variance coupled with the fact that this is only one field season of data prevented the use of statistical backing for significant difference between lines. However, inferences into the potential for transferring traits of agronomic importance between these species can be made.

Thirty-five of the forty backcross DH lines came from the 95-1061-B microspore culture. Cytological analysis in Chapter 2 revealed that the interspecific DH_1 line 95-376, and all backcross DH lines contained a *B. napus* chromosome number. These lines were analyzed to determine the potential for introgression of morphological, agronomic and quality traits of interest in interspecific crosses.

The appearance of the backcross DH lines resembled the *B. napus* parent with some *B. rapa* influence (Table 4.1.). Leaf waxiness and absence of hairs, which tend to be associated with *B. napus*, were present in all backcross DH progeny. There appears to be a dominant *B. napus* influence on these traits in this interspecific cross. Leaf shape, upper leaf clasp, the position of the cluster of buds with respect to the last opened flowers, and the flower type were strongly influenced by the *B. napus* parent, while leaf margin type tended to resemble the

B. rapa parent. The segregation ratio of *B. napus* leaf shape was 7.8:1 in the interspecific DH population. Based on the model for the frequency of homozygous recessive genotype in a doubled haploid population described by Pauls (1996), it appears that leaf shape in this population is governed by 3 genes with the *B. rapa* shape, recessive. Chi-square analysis revealed significant P values for 2, 3, and 4 gene inheritance ratios, which suggests that leaf shape could be governed by 2 to 4 genes. The small population size may have contribute to variation in the analysis.

The segregation ratios for leaf margin type and and upper leaf clasp of 1.9:1, suggest that these traits are controlled by 1, possibly 2 genes with *B. napus* leaf margin and *B. rapa* upper leaf clasp as recessive traits. Chi-square analysis revealed significant P values for 1 and 2 gene inheritance ratio, which supports the theory that inheritance of these traits is governed by 1 or 2 genes.

The ratios of *B. napus* bud position and flower type were 10.7:1 each. These ratios suggest that inheritance of bud position and flower types may be governed by at least 3 genes and possibly 4 genes with *B. rapa* bud position and flower types recessive. Chi-square analysis suggested that 3, 4, or 5 genes may be involved in the inheritance of each trait. The small population size made it difficult to determine the exact number of genes involved.

Although leaf margin type and upper leaf clasp demonstrated similar segregation ratios in the interspecific population, statistical tests of association revealed no significant correlation between these traits. A statistically significant correlation between bud cluster position and flower type was observed. Looking

at the square root of the R^2 value, this correlation could account for 53% of the variation in this trait. This suggests that these traits have a slight tendency to be inherited together in this population.

Oleic (C18:1), Linoleic (C18:2), and Linolenic (C18:3) fatty acids made up the largest proportion of the fatty acid profiles. Typical ratios of oleic, linoleic, and linolenic fatty acids would be those seen in the Quantum and Eclipse checks. Most *B. napus* fatty acid profiles consisted of 60%, 20%, 11% oleic, linoleic, and linolenic acid while most *B. rapa* profiles consisted of 55%, 25%, 13% oleic, linoleic, and linolenic fatty acids (Downey, 1983). The fatty acid profiles of the backcross DH lines did not fit either a typical *B. napus* or *B. rapa* type profile. Most noticeable was the high proportion of linolenic acid in the profiles (Table 4.3.) and no line had less than 14% linolenic acid however, this may be a reflection of climatic conditions of the growing season. The proportion of linoleic acid varied from approximately 17% to 25% in the DH population, while the proportion of oleic acid varied from approximately 46 to 58%. The means and standard deviations in Table 4.3. for the proportion of oleic, linoleic, and linolenic acid in the 95-1061-B population appears similar to a typical *B. rapa* fatty acid profile. The Wilk's statistic (Pr<W), also in Table 4.3., indicates that these three fatty acids in this backcross population are not significantly different from a normal distribution. The assumption can thus be made that 68% of this population falls within ± 1 standard deviation of the mean. This may suggest that the population as a whole has a fatty acid profile that resembles a profile expected for a *B. rapa* cultivar. A major breeding objective is to reduce the level

of linolenic acid, as it tends to oxidize more quickly in storage, leading to spoilage (Uppström, 1995). This cross does not appear to be an effective method for reaching that goal.

The different levels of total saturated fatty acids are characteristic of *B. napus* and *B. rapa*. *B. rapa* varieties tend to have less total saturates than *B. napus* varieties (Downey and Rimmer, 1993). Despite having two crosses with *B. rapa*, a high level of total saturates was maintained in all interspecific DH lines. The high mean for total saturates within the 95-1061-B population, and more importantly, the Wilk's probability indicates a deviation from normality (Table 4.3.). The two saturated fatty acids which make up the largest proportion of the total saturates are C16:0 (Palmitic), and C18:0 (Stearic). The proportion of stearic fatty acid in the DH population, ranges from 1.45 to 2%, with the parents falling approximately in the middle at 1.60 to 1.70%. The Wilk's statistic indicates no significant difference from a normal distribution. However, in the case of palmitic acid, it is apparent that nearly all of the DH lines had palmitic acid levels similar to and higher than *B. napus*, which skewed the distribution. The slightly lower Wilk's probability reflects the large number of lines with a palmitic acid content near the mean of 4.46%. The reason why a high level of saturates was maintained in this population is unclear.

A requirement of any potential canola cultivar is that it must be canola quality, i.e, it must have less than 2% erucic acid (C22:1) in its fatty acid profile. The fatty acid profiles recorded in Table 4.3. reveal that a very low proportion of erucic acid was maintained in the backcross DH population. The apparent

skewness observed in the Wilk's value (Table 4.3) can be explained by the fact that the lower limit of detection by the GC was being reached, and many of the values were near zero.

In addition to having less than 2% erucic acid, a potential cultivar must also have less than 30 µmoles of glucosinolate per gram of air-dried oil free meal. Total glucosinolate content for typical canola *B. napus* is on average 17 µmoles/gram while that for *B. rapa* is slightly higher at 21 µmoles/gram (Uppström, 1995). The mean glucosinolate content of the 95-1061-B interspecific population was 15.5 µmoles/gram, lower than that expected for either species, and strikingly lower than the content observed in the *B. napus* parent 94-98 and interspecific DH_1 line 95-376 (Table 4.4.). The overall reduction in total glucosinolate content in the backcross population coupled with the large standard deviation, suggests that there is considerable genetic diversity that can be exploited within this cross in regards to glucosinolate content. Further examination of glucosinolate profiles of individual plants within the plots of 95-376 grown during the 1997 field season and from the 1996 field season, revealed that despite being a doubled haploid line, the interspecific parent (95-376) is segregating for glucosinolate content. Total glucosinolates ranged from 12.9 to 58.6 µmoles/gram with an average of 26.6 µmoles/gram. Cytological analysis presented in Chapter 2 revealed one to two quadrivalents at metaphase along with non-disjunction at anaphase. The segregation of glucosinolate content may be the result of non-homologous recombination, which has been shown to result in quadrivalent formation. Selecting an individual plant from the self-pollinated

seed of 95-376, may have lead to a lower mean glucosinolate content in the 95-1061-B modified backcross DH population.

Within the 95-1061-B population, mean total indoyl glucosinolate content followed a normal distribution (Figure 4.2. **B**), with a mean of 10.1 and standard deviation of 0.9, however the total alkenyl and subsequently total glucosinolate content did not follow a normal distribution. Two clusters of lines with total alkenyl glucosinolate contents between 0 and 1 µmoles/gram and between 6 and 7 µmoles/gram, resulted in a bimodal distribution (Figure 4.2. **A**). When the total alkenyl and total indoyl glucosinolates are summed, the distribution of total glucosinolates also becomes bimodal (Figure 4.2. **C**). The reason total alkenyl glucosinolate content was reduced to, or near zero through this cross is not apparent, but suggests that interspecific crosses may be effective in reducing glucosinolate levels in these two species. The extreme values recorded for the butanyl and OH-butanyl glucosinolates, two of the alkenyl glucosinolates, suggests that these interspecific crosses may have resulted in a recombination of genes controlling the synthesis of these glucosinolates.

B. napus X *B. rapa* interspecific crosses were utilized in the past by early Chinese breeders to improve the maturity of *B. napus* (U, 1935) therefore, it was of interest to determine if improvements on the number of days to flowering were made possible through this interspecific cross. Within the 95-1061-B backcross population, the mean number of days to flower followed a normal distribution, with a mean of 42.8 and standard deviation of 3.7 (Table 4.5). This suggests that the interspecific backcross population flowers somewhat intermediate between a

B. napus and *B. rapa* type, but tends towards the later side. The *B. rapa* backcross parent (95-440-2), appears to be later flowering than the original *B. rapa* parent which may account for the shift in the mean population days to flower towards the later side. Based on the number of lines that began to flower within 3.7 days of the mean (42.8), it appears that this interspecific cross did improve the number of days required to reach first flower over that of the *B. napus* parent.

Silique angle is suspected to affect the amount of seed lost due to shattering of the siliques at harvest, and the compact nature of swaths (Stringam GR, personal communication). *B. rapa* varieties have upright siliques (small total silique angle) therefore are less prone to shattering when straight combined. It is of importance to the U of A canola breeding program to determine if an upright silique angle can be transferred from *B. rapa* into *B. napus*. Silique angle followed a normal distribution in the 95-1061-B interspecific backcross population with a mean angle of 52.6 and standard deviation of 5.8. Considering the entire population, it appears that the interspecific DH progeny have inherited a more lax silique angle than that observed in either parent. Since the overall silique angle (angle between silique and main stem) is governed by two angles (Figure 4.1.), it is important to see how silique angle is achieved in the two species. Angle 1 is measured as the angle between the main stem (peduncle) and pedicel, while angle 2 is taken as the angle the silique is deflected up from parallel with the pedicel. In Table 4.5., the *B. napus* parent 94-98 has an average angle 1 of 57 degrees and angle 2 of 7 degrees while in the *B. rapa* parents, we see a slightly larger first angle and a very large second angle. Looking at the overall means for

the first and second angles within the 95-1061-B population, the mean for the first angle is intermediate between the two parents while the mean second angle is similar to that of the *B. napus* parent. By combining an intermediate first angle with a small second angle in many of the DH progeny, the overall silique angles in most of the lines tend to be larger than any of the parents. Individual lines with slightly smaller overall silique angles than the *B. napus* parent were identified.

To improve straw strength, the semi-dwarf *B. napus* parent 94-98, was selected for these crosses. The length of the main stem and overall plant height were measured to determine if the shorter stature could be maintained in the interspecific population. Based on the slightly larger population means for height and length of the main stem in Table 4.5., it appears that the DH lines within the 95-1061-B backcross population have maintained a tall nature. Based on the standard deviations for both these traits, it appears that there is a lot of genetic diversity for these traits that could be utilized. It is difficult to determine if the sparse plant number within plots affected the height and length of the main stem. Since no measure of tendency to lodge was taken, it may be of value to estimate lodging as well as height to determine the success of transferring stiff straw strength to *B. rapa*.

The inconsistent stands between plots coupled with the fact that this is only one site year of data, made assessment of yield impossible. To gauge the potential for combining yield component traits through interspecific crosses, measurements of carpel length, beak length, seeds per silique, and 1000 seed weight, were taken. Although beak length may not be directly related to yield, it

is a characteristic that can be used to distinguish between *B. napus* and *B. rapa* (Downey, 1983). To estimate fertility, the distance between seeds was calculated by dividing the carpel length by seeds per silique, with the assumption that when there are more aborted embryos, the average distance between seeds will increase. The population means in Table 4.6. for carpel length, suggest that a carpel length intermediate between the *B. rapa* parent 93-1005-3 and *B. napus* parent 94-98 was maintained in this backcross, while beak length similar to a *B. napus* type was maintained. Although the population mean for number of seeds per silique (Table 4.6.) was not as high as 93-1005-3 or 94-98, the standard deviation of 5.8 indicates that a significant proportion of the population had numbers of seeds per silique that compared to and even exceeded either parent. The Ls mean values for the individual lines, revealed that one line had an average seeds per silique number of 30.5. The mean number of siliques on the main stem in the 95-1061-B backcross progeny appears similar to the *B. rapa* parent 95-440-2 (Table 4.6.). Although there is no measured yield data, the potential for combining carpels that are larger and contain more seeds than a *B. rapa* type with a number of siliques on the main stem similar to *B. rapa*, may exist. Identifying early maturing individuals which also have these yield components needs to be explored further.

The skewed nature of the distance between seeds (DBS) parameter suggests that some infertility may be present in the 95-1061-B progeny. This infertility may be the result of some residual self-incompatibility transferred from

the *B. rapa* types, or of aberrant chromosome behavior observed in some of the DH lines in Chapter 2.

Another character that affects yield is the 1000-kernel weight. *B. napus* varieties tend to be large seeded with 1000-seed weights in the range of 3.5 to 4.5 grams/1000-seeds. The smaller seeded of *B. rapa* typically fall in the range of 2.0 to 3.0 grams/1000-seeds (Downey, 1983). Based on the values for 1000-seed weight in Table 4.6., it appears that the two *B. rapa* parents had slightly larger than average seeds while the *B. napus* parent had slightly smaller than average seeds. Within the 95-1061-B DH progeny, it appears that the population as a whole had a tendency towards larger seed. However, the thin stands may have contributed to the exceptionally large 1000-seed weights recorded. Re-evaluation of this trait over several seasons should indicate if the large seed size, reported during this field season was accurate. By examining yield and yield components within *B. napus*, Grosse et al. (1992) found that high yield was achieved through combinations of seeds per silique, number of siliques, and 1000-seed weight. Depending on the extent environment affected seed weight during this study, it appears that there is potential of combining superior yield components through these interspecific crosses.

Oil is the most valuable component of the seed, having 2 to 3 times the value of the meal (Downey and Rakow, 1987). Grami et al. (1977) found oil and protein content to be negatively correlated. Since sacrificing protein for oil content is not acceptable to the crushers, breeding has focused on maintaining an acceptable level of protein with increased oil content. One effective means to

achieve this goal is through breeding for yellow seed color. Stringam et al. (1974) found that yellow seeded varieties have a thinner seed coat, and therefore a larger proportion of the seed is made up of oil and protein rich embryo. A high level of protein was maintained in the 95-1061-B DH progeny as seen by the large number of progeny with protein levels similar to the population mean of 48.5% (Table 4.7.). However, the total oil content appears to be more variable and has a lower overall trend within the population. The oil content may be underestimated due to the high frequency of precocious germination within the DH lines. The prolonged maturation, due to increased branching may have resulted in seed beginning to germinate in the siliques prior to harvest. The data for oil content is inconclusive and requires further evaluation. Yellow seed color was not recovered in any of the backcross DH progeny. However, 10 out of 35 DH lines had brown seed coats as did the interspecific DH_1 parent 95-376. Whether brown seed color resulted in a lower fiber content was not determined. The absence of yellow seeded types despite backcrossing to a yellow seeded type may be a reflection that seed color genes were present in the **C** genome. Another possibility is that the population was too small to identify yellow seeded types.

It appears that with the exception of leaf hairiness, and leaf waxiness, many traits can be influenced through interspecific crosses. Further evaluation of yield potential and maturity of these lines will determine the overall potential for releasing new *B. napus* varieties which have a combination of *B. napus* and *B. rapa* characteristics.

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Chapter 5

Molecular Characterization of Doubled Haploid Lines Derived from Interspecific Crosses between *Brassica napus* and *Brassica rapa*, Using RAPD Markers.

5.1. Introduction:

The planting of early maturing *Brassica rapa* (Polish canola) varieties, has reduced the risk of late spring and early fall frosts, common in Alberta. However, the self-incompatibility of *B. rapa* has made it difficult to improve the agronomics of this species through conventional breeding. Because of the widening performance gap between *B. napus* and *B. rapa* cultivars, canola producers in the short season zones, are willing to risk the chance of frost in an attempt to obtain higher yields possible with disease resistant *B. napus* varieties such as Quantum. To provide new canola varieties which suit Alberta producers needs, we are attempting to combine the superior agronomic traits such as disease resistance and higher yield potential of *B. napus* with the early maturity and yellow seed color of *B. rapa* through interspecific crosses.

Interspecific crosses between these species are relatively simple to achieve, because *B. napus* and *B. rapa* share a common **A** genome. The original findings of researchers in the 1930's, revealed the inter-relatedness of the *Brassica* species, and were achieved through karyotype analysis and meiotic chromosome pairing affinities in haploids (Lagercrantz and Lydiate, 1996). U

(1935) confirmed the previous work of Takimine, Karpechenko, Nagai and Sasaoko, Olson, and Morinaga, regarding the genomic relationship within the *Brassica* species by artificially resynthesizing the amphidiploid species from the diploid species, and by observing chromosome pairing in the interspecific hybrids he produced (reviewed by Prakash and Hinata, 1980). U (1935) then illustrated the inter-relatedness of the *Brassica* species in what is now referred to as U's triangle.

Many plant species such as those in the Brassicaceae, have small chromosomes which do not stain well, making meiotic chromosome analysis difficult (Stringam, 1970). Advancements in molecular marker technology have provided a new means of studying genome evolution (Lagercrantz and Lydiate, 1996). Williams et al. (1990) and Welsh and McClelland (1990) simultaneously discovered that arbitrary nucleotide sequences could be used as primers in PCR reactions, to reveal polymorphisms which may be useful as genetic markers. This discovery provided a new tool for genetic mapping, gene cloning, and marker assisted selection in many species including the Family Brassicaceae where a lack of saturated genetic maps makes traditional mapping techniques difficult. These types of markers have been named Random Amplified Polymorphic DNA (RAPD's).

Using 3 sets of RAPD primers, Quiros et al. (1991) observed 65 genome-specific markers for the diploid species *B. rapa*, *B. oleracea*, and *B. nigra* as outlined by U (1935). These markers enabled Quiros et al., (1991) to map the **B** genome and to determine the feasibility of introgressing the **B** genome into the **A**

and **C** genomes of *B. napus* through interspecific aneuploidy. Thiagarajah et al., (1994) utilized several of these primers to demonstrate that intergenomic introgression of RAPD markers, and agronomic / quality traits occurs when interspecific crosses are made between *B. juncea* and *B. napus*.

In an attempt to determine the success of introgressing genetic material between *B. napus* and *B. rapa* in a doubled haploid system, we used RAPD primers found by Quiros et al., (1991) to reveal polymorphic differences in the *Brassica* species. One limitation of RAPD's is their dominant nature of inheritance. Because the interspecific progeny screened, were doubled haploids derived from crosses between parents that were doubled haploids or homozygous F_7 lines, the potential problems associated with the dominant nature of RAPD's could be avoided.

5.2. Materials and Methods:

5.2.1. Plant Material Screened and Growth Conditions:

Seed from thirty-eight doubled haploid (DH) lines were planted in six inch pots containing sterile soil-free medium (Stringam, 1971). The DH lines were derived from the 95-1061-B microspore culture (see Chapter 2 for crossing scheme), which was produced when the interspecific DH_1 line derived from a cross between *B. napus* and *B. rapa* was crossed with the *B. rapa* parent 95-440-2. The four parents used to produce this population were also grown. Four

plants from each line were raised in an Enconaire Environmental Chamber (model GRB-168) maintained at 18°C day/ 15°C night temperatures and provided the plants with 350 µmol $m^{-2}s^{-1}$ of light from very high output fluorescent and 40 watt incandescent bulbs, for a 16 hour photoperiod. Plants were fertilized with 20-20-20 fertilizer bi-weekly.

5.2.2. Tissue Harvest and DNA Extraction:

Leaf tissue was harvested and DNA extracted from 25 day old plants, according to Sommers (1997) with the following modifications. Tissue was wrapped in tin foil, flash frozen in liquid nitrogen and lyophilized. Dried tissue was stored at -17°C in a sealed container containing Dryrite, until DNA was extracted. Tissue was ground to a fine powder in liquid nitrogen then stored at -80°C in 50 ml tubes. A bulk tissue sample was created by combining equal amounts of tissue from four sister plants. DNA was extracted from 50 milligrams of lyophilized tissue. Purified DNA was dissolved in 1 ml of sterile distilled water and then quantified.

5.2.3. DNA Quantification, Amplification Conditions, and Electrophoretic Separation of PCR Products:

DNA quantification, amplification, and electrophoretic separation of PCR products were performed according to Sommers (1997) with the following

modifications. A bulk reaction mixture was prepared for each primer based on the number of genotypes to be screened. Each 25 µl reaction consisted of 2.5 mM Magnesium Chloride, 1X PCR Buffer, 0.2 mM dNTPs, 2U *Taq* DNA Polymerase (Gibco BRL), 10 ng DNA, and 0.2 µM Primer (final concentrations). Reactions were completed in 0.2 ml MicroAmp Reaction tubes (Perkin Elmer serial no. N801-0540). DNA was amplified using a Perkin Elmer GeneAmp 9600 PCR system. Nine primers from Operon Technologies were used to characterize the plant material (Table 5.1.).

Table 5.1. Operon Technologies primer numbers and sequences.

Primer number	Sequence
OPA 3	5' AGTCAGCCAC 3'
OPA 8	5' GTGACGTAGG 3'
OPA 9	5' GGGTAACGCC 3'
OPA 11	5' CAATCGCCGT 3'
OPA 12	5' TCGGCGATAG 3'
OPA 13	5' CAGCACCCAC 3'
OPA 16	5' AGCCAGCGAA 3'
OPA 17	5' GACCGCTTGT 3'
OPA 18	5' AGGTGACCGT 3'

Ethidium bromide was added to the gel at a concentration of 5 ng/ml prior to casting it into the gel tray. Products amplified using each primer separately, from the four parents and 38 DH lines were loaded into a 44 lane, 1.4% agarose gel. Seventy-five volts of current were passed through the gel for 3 to 3.5 hours to separate the DNA fragments. UV fluorescence (254nm) was used to visualize the DNA fragments and the results were recorded on type 55 Polaroid film as per Sambrook et al., (1989).

5.2.4. Statistical Analysis:

Chi-square analysis was performed to determine if the observed segregation of 6 markers in the interspecific population deviated from a 1:1 ratio, expected in a doubled haploid population.

The glm procedure of SAS statistical software version 6.12 (1996) was used to test for association between the observed polymorphic differences and the data collected for various traits during the field study outlined in Chapter 4.

5.3. Results:

The nine primers selected from Quiros et al. (1991) revealed 26 polymorphic differences. Consistent bands were scored by amplifying fragments with each primer over three runs. The sizes of polymorphic bands produced by each primer, the parent specificity of the polymorhic differences, and the segregation ratio's of the polymorphic differences in the modified backcross DH progeny were recorded (Table 5.2.). Nineteen separate polymorhic differences were identified by the presence of bands in *B. rapa* parent 93-1005-3, DH_1 parent 95-376, and *B. rapa* parent 95-440-2, which were absent in *B. napus* parent 94-98 (Table 5.2.). The 0.9 Kbp marker revealed by primer OPA 12, showed segregation in the modified backcross DH progeny despite being present in both of the parents used to generate the progeny. Two polymorphic differences appeared as bands present in the *B. napus* parent 94-98 and interspecific DH_1

parent 95-376. These markers were also observed to segregated in the modified backcross DH progeny. Two polymorphic differences appeared as a band present in *B. rapa* parent 95-440-2 that was absent in the three other parents. Both these differences segregated in the backcross DH progeny. The 3 remaining differences appeared as follows:

band present in *B. napus* (94-98) and backcross *B. rapa* (95-440-2)

band present in *B. rapa* (93-1005-3), *B. napus* (94-98), and DH_1 (95-376)

band present in *B. rapa* (93-1005-3) and backcross *B. rapa* (95-440-2).

All three of these banding patterns segregated in the backcross DH progeny. Primer OPA 18 revealed the most polymorphic differences between the parents, three of which also could be seen segregating in the 38 DH progeny (Figure 5.1.).

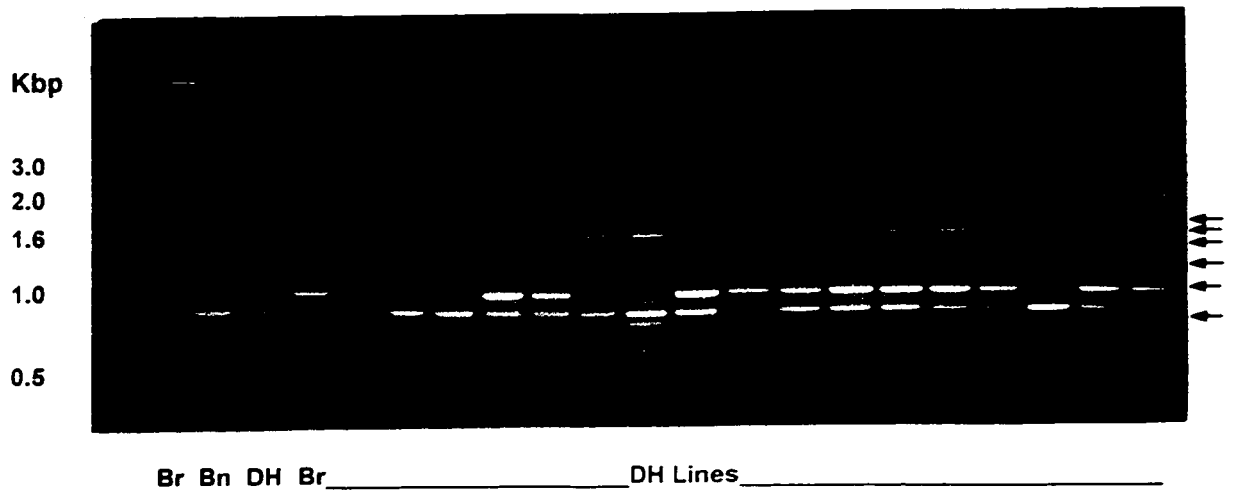
Chi-square analysis (Table 5.3.) revealed P values greater than 0.05 for a 1.0 Kbp marker revealed by primer OPA 12, and a 1.7 Kbp marker revealed by OPA18.

Correlation analysis revealed several significant associations (P values between 0.05 and 0.001) between the RAPD markers and the agronomic traits recorded in Chapter 4. The R^2 values for the significant correlations varied from 0.05 to 0.32 (Appendix 7).

Table 5.2. Sizes, parent specificity, and segregation of polymorphic differences revealed by nine RAPD primers.

Primer	Band Size (Kilo base pairs)	Parent Specificity	Segregation in BCDH (+/total)
OPA 3	1.4	*B. napus*, DH1	17/38
OPA 3	0.7	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 3	0.6	B.C. *B. rapa*	20/38
OPA 8	1.0	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 8	0.8	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 8	0.6	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 9	0.7	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 9	0.5	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 9	0.4	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 11	1.2	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 11	1.1	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 11	0.7	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 12	1.0	B.C. *B. rapa*	5/38
OPA 12	0.9	*B. rapa*, DH1, B.C. *B. rapa*	27/38
OPA 13	0.9	*B. rapa*, DH1, B.C. *B. rapa*	Present in all
OPA 13	0.8	*B. rapa*, DH1, B.C. *B. rapa*	present in all
OPA 16	1.4	*B. rapa*, DH1, B.C. *B. rapa*	present in all
OPA 16	1.0	*B. rapa*, DH1, B.C. *B. rapa*	present in all
OPA 17	2.1	*B. rapa*, DH1, B.C. *B. rapa*	present in all
OPA 17	0.6	*B. napus*, B.C. *B. rapa*	25/38
OPA 18	1.7	*B. rapa*, B.C. *B. rapa*	31/38
OPA 18	1.5	*B. rapa*, *B. napus*, DH1	28/38
OPA 18	1.4	*B. rapa*, DH1, B.C. *B. rapa*	present in all
OPA 18	1.1	*B. rapa*, DH1, B.C. *B. rapa*	present in all
OPA 18	0.9	*B. rapa*, DH1, B.C. *B. rapa*	present in all
OPA 18	0.7	*B. napus*, DH1	24/38

Figure 5.1. RAPD profiles of the four parents and thirty-eight modified backcross DH plants, using Primer OPA 18. Note that the gel has been split for ease of labeling. Three markers (arrows to the left of the gels at 1.4 Kb, 1.1 Kb, and 0.9 Kb), present in the *B. rapa* parent (93-1005-3), interspecific DH_1 parent (95-376), and backcross *B. rapa* parent (95-440-2) were absent in *B. napus* parent (94-98), but appeared in all 38 backcross progeny. A 0.7 Kb marker (indicated by arrow), present in the *B. napus parent* and interspecific DH_1 parent appeared in 24 of the 38 backcross DH progeny. A 1.5 Kb marker (indicated by arrow) present in the *B. rapa, B. napus*, and DH_1 parents, appeared in 28 of the 38 backcross DH progeny. A 1.7 Kb marker (indicated by arrow) present in the *B. rapa* and backcross *B. rapa* parents appeared in 31 of the 38 progeny.


Kbp
3.0
2.0
1.6
1.0
0.5
Br Bn DH Br
DH Lines

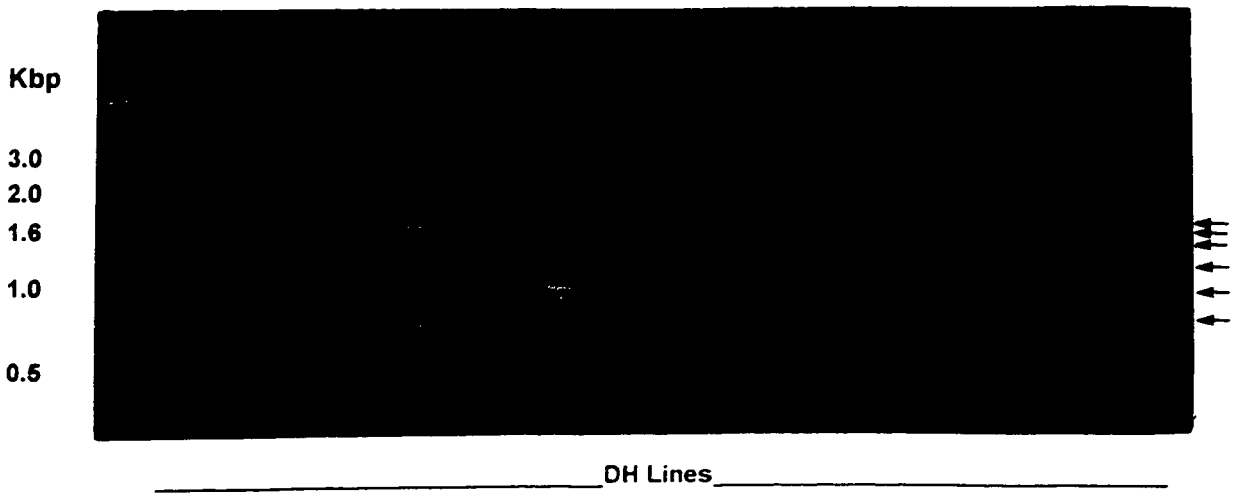

Kbp
3.0
2.0
1.6
1.0
0.5
DH Lines

Table 5.3. Chi-square analysis for 6 RAPD markers in the 95-1061-B population, to determine deviation from an expected 1:1 segregation ratio.

Marker Number	Phenotype	Observed Frequency	Expected Frequency	X^2	P
OPA3-1.4Kbp	+	17	19	0.421	0.70-0.50
	-	21	19		
OPA3-0.6Kbp	+	20	19	0.105	0.80-0.70
	-	18	19		
OPA12-1.0Kbp	+	5	19	11.158	<0.01
	-	23	19		
OPA17-0.6Kbp	+	25	19	3.789	0.20-0.05
	-	13	19		
OPA18-1.7Kbp	+	31	19	15.158	<0.01
	-	7	19		
OPA18-0.7Kbp	+	24	19	2.632	0.20-0.05
	-	14	19		

5.4. Discussion and Conclusions:

B. napus is an amphidiploid, which evolved from natural interspecific hybridization between *B. rapa* (**A** genome), and *B. oleracea* (**C** genome) (U, 1935). The set of 10 bivalents, common to *B. napus* (**AACC**) and *B. rapa* (**AA**), was used successfully by Scarth et al. (1992), Growers (1982), Johnston (1974), and Lammerink (1970) as a bridge for trait transfer between these species. Similarly, evaluation of agronomic performance and quality characteristics (presented in Chapter 4) of the *B. napus* X *B. rapa* interspecific DH lines produced for this study revealed that many traits appear to be effected by this interspecific cross.

Quiros et al. (1991) utilized RAPD markers to develop genome-specific markers for the **A, B,** and **C** genomes of the *Brassica* species. Using 47 arbitrary primers, Quiros et al. (1991) found considerable variation within the *Brassica* species studied. Among the markers, Quiros et al. (1991) identified genome-specific bands, present in both the diploid and derived amphidiploid species, species-specific bands, present in the two accessions of a single species, and accession-specific bands, present in only one of the two accessions of the same species.

By applying nine of the primers utilized by Quiros et al. (1991), nineteen differences identified (Tabie 5.2.), appeared as bands in the *B. rapa* parent (93-1005-3), interspecific DH_1 parent (95-376), and backcross *B. rapa* parent (95-440-2). These differences may have been species specific as they were present in both *B. rapa* parents, and absent in the *B. napus* parent and therefore may represent slight differences between the **A** genome of the *B. rapa* parents, and the **A** genome of the *B. napus* parent. The high frequency of these differences inherited by the modified backcross DH progeny can be explained by the fact that the two parents used to generate the progeny both contained the marker. A 0.9 Kbp marker revealed by primer OPA 12, which was present in both parents used to produce the 95-1061-B population, segregated in the DH progeny. This marker may reveal a recombination event or mutation event, which resulted in loss of a priming site in these progeny. Non-homologous recombination was evident within many of the DH progeny. Quadrivalent chromosome configurations, which result from non-homologous recombination, were identified

in most of the DH lines through cytological analysis (Chapter 2). The non-homologous recombination observed might be responsible for modifying primer binding sites within some of the DH progeny evaluated. The presence of a similar profile in the DH_1 parent 95-376 to that of both *B. rapa* parents, and high frequency of these markers in the modified backcross progeny suggests that there has been genetic material transferred from the *B. rapa* parents to the interspecific DH progeny. Thiagarajah et al. (1994) was able to demonstrate a similar result from interspecific crosses between *B. juncea* and *B. napus.*

A marker, revealed by primer OPA 18 also appeared to be species specific as it appeared in both *B. rapa* parents. The high frequency of the marker within the modified backcross DH progeny suggests that the marker is highly heritable.

It is difficult to determine if the two markers that appeared in the *B. napus* parent 94-98 and DH_1 parent 95-376, were genome specific or species specific differences as there were no other *B. napus* lines with which to compare them. The presence of the markers in the DH_1 parent and segregation of these markers in the modified backcross DH progeny suggests that there has been genetic material transferred from the *B. napus* parent to the interspecific DH_1 parent, and from the interspecific DH_1 parent to the modified backcross DH progeny.

The remaining four polymorphic differences appear to be accession specific. Quiros et al., (1991) concluded that accession specific-specific bands, displayed by only one of the two accessions of the same species, represent intraspecific variability. Therefore, two markers revealed by OPA 3 and 12,

present in the one *B. rapa* parent and not the other despite the fact that these are sister plants, may be due to slight differences between the two plants. Although these plants are assumed to be homozygous, they were derived through seven generations of self-pollinating in a pedigree system and therefore there is a slight chance of variability. According to the equation put forward by Allard (1960), after 7 generations of self-pollinating, 99.2% of the individuals should be homozygous. The markers revealed by OPA 18, and primer OPA 17, may also reflect slight differences and similarities between single plants as they do not appear to be species specific. Although they appeared at different frequencies in the DH population, it is apparent that these markers are heritable to different degrees through this interspecific cross and therefore support the hypothesis that genetic material can be transferred between species during interspecific crosses.

Chi-square analysis revealed P values less than 0.05 for primers OPA 12-1.0Kbp and OPA 18-1.7Kbp. This indicates that the RAPD markers, revealed by these two primers are not segregating in a 1:1 ratio as would be expected in a DH population. The *Brassica* species are known to contain a significant amount of duplicated and rearranged genetic material within their genomes as they evolved from a common ancestor (Downey and Röbbelen, 1989; Langercrantz and Lydiate, 1996). Duplication within the genomes of these species may have contributed to the altered segregation ratios.

Although this study was not designed to map traits within these crosses, polymorphic differences, which segregated in the DH population were observed and therefore tested for association with traits of interest. Correlation analysis

revealed several statistically significant associations between the RAPD markers and agronomic traits measured in Chapter 4. By examining the square root of the R^2 value, it is apparent that these correlations could account for 34 to 53% of the variation in these traits. Although the markers and traits were not highly correlated, a more complete characterization of this population with more of the primers may reveal markers that are more strongly correlated with traits of agronomic importance.

The introgression of genetic material in doubled haploid progeny derived through interspecific crosses between *B. napus* and *B. rapa* has been demonstrated using genome specific primers reported by Quiros et al. (1991). The amount of polymorphism revealed by the 9 primers utilized for this preliminary analysis, suggests that there is potential for finding markers associated with traits of interest.

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Chapter 6

Discussion and Conclusions.

6.1. Summary:

The objective of this study was to evaluate the potential for introgressing traits of agronomic importance, between *B. napus* and *B. rapa* from interspecific crosses. Microspore culture was utilized to accelerate the in-breeding process by rapidly "fixing" desirable genotypes, and eliminate fertility problems caused by unpaired chromosomes, which are known to arise in interspecific plants (Thiagaragah et al., 1994; Stringam et al., 1995). Chromosome number, disease resistance, agronomic field performance, oil and protein content and quality within the interspecific DH lines were determined to evaluate the success of trait introgression. Genome-specific RAPD markers were utilized to demonstrate that exchange of genetic material occurred during the introgression process.

A doubled haploid line, derived from an interspecific cross between *B. napus* and *B. rapa*, was utilized in additional crosses with four other *B. rapa* lines, in a modified backcross scheme. One interspecific cross was more embryogenic than the other three. Although a few lines from the other 3 crosses were analyzed, the conclusions regarding the success of this study are based on the 95-1061-B DH population. Since crosses where *B. rapa* was used as the female parent were unsuccessful, and no *B. rapa* individuals were recovered during this study, the introgression of traits in one direction only, from *B. rapa* to *B. napus*

could be evaluated. From a review of previous work conducted on interspecific crosses between the *Brassica* species, Quazi (1988) reported that there may be a genotype or variety influence on seed set in these crosses. Meng and Lu (1993) reported that endosperm failed to form properly in developing seeds from *B. napus* by *B. juncea* interspecific crosses. Embryo rescue techniques have been shown to improve the chances of obtaining plants from seeds in which endosperm does not develop properly (Chen et al., 1988; Quazi, 1988). Future work on *B. rapa* by *B. napus* crosses should focus on embryo rescue to improve the survival of the interspecific F_1 progeny produced when *B. rapa* is the female parent.

Cytological analysis revealed that despite having two crosses with *B. rapa*, the interspecific DH progeny all contained 38 chromosomes during meiosis I. These chromosomes formed 15 to 19 bivalents as well as 1 to 2 quadrivalents, 2 univalents, and occasionally a hexavalent or chain of 6 chromosomes. Little is known of the response of interspecific *Brassica* species to the doubled haploid breeding technique. Although the mechanism by which the interspecific progeny maintained a *B. napus* chromosome number is unknown, it appears that there is a maternal influence on embryogenesis. An objective of the present study was not to prove that this type of influence on microspore culture exists. Producing more interspecific progeny using a number of different *B. napus* and *B. rapa* parents, and employing embryo rescue to improve the success of reciprocal crosses, may provide further evidence of a maternal influence on chromosome number in interspecific progeny obtained through microspore culture.

Quadrivalent chromosome associations were observed in most of the DH lines analyzed for chromosome. Quadrivalents are known to result from non-homologous recombination in the F_1 of interspecific crosses. Application of the doubled haploid technique was expected to stabilize the recombined chromosomes by making them homozygous. The presence of quadrivalents in the DH lines suggests that non-homologous associations and recombination have taken place after the microspore culture process. Prakash (1974) suggested that there might be a system that evolved in the amphidiploid *Brassica* species which suppresses non-homologous pairing, as seen in wheat. Prakash and Hinata (1980) also suggested that the genomes of the naturally occurring amphidiploids might have undergone significant genetic differentiation during their evolution, which prevents non-homologous association. Suppression of a genetic system that prevents non-homologous pairing, or loss of differentiation between **A** and **C** genomes in the interspecific progeny may be responsible for the increase in non-homologous pairing and therefore increased quadrivalent associations observed.

Another aberrant chromosome behavior, observed in some of the DH lines, was bridge / fragment configurations. Bridge / fragment associations, which result from inversions (Burnham, 1962), are not expected to appear in the DH progeny. An inversion loop, seen when the inverted segment on an affected chromosome pairs with its homologue on an unaffected chromosome, should not occur in DH lines because the doubling process produces chromosomes identical. The fact that there are bridge / fragment associations in the DH lines

indicates that inversions have occurred after the doubling process. The most noticeable effect of bridges is a reduction in pollen viability. Because anaphase I and II were not found for every line, the frequency and phenotypic effects of inversions in this population were not fully characterized. The presence of both quadrivalents and bridge / fragment associations makes the cytological stability of some of the interspecific DH lines questionable.

The discovery that all the interspecific DH lines contained a *B. napus* chromosome complement lead to the hypothesis that *B. napus* type disease resistance may have been maintained in the interspecific progeny. White rust resistance was observed in all the DH lines. The fact that all the DH progeny were resistant to white rust, and all contained a full complement of **C** genome chromosomes, may be evidence that at least one of the 3 genes known to govern resistance to white rust race 7 in *B. napus* is on the **C** genome. Although blackleg resistance in the *B. napus* parent, is believed to behave as a single dominant trait, the genetics of resistance in the interspecifc DH progeny appears more complex. A continuous range of mean disease severity index (DSI) scores was recorded in the DH progeny. Recombination between the **A** genomes of *B. napus* and *B. rapa*, and non-homologous recombination between the **A** and **C** genomes, observed as quadrivalents in the cytological analysis may have contributed to the range of disease resistance observed.

Morphologically, the interspecific lines appeared more like the *B. napus* parent, with some *B. rapa* influence. Leaf waxiness and absence of hairs, which tend to be associated with *B. napus*, were present in all backcross DH progeny.

Leaf shape, the position of the cluster of buds with respect to the last opened flowers, and the flower type were strongly influenced by the *B. napus* parent, but *B. rapa* types did appear at very low frequencies.

The fatty acid profiles of the DH lines did not fit either a typical *B. napus* or *B. rapa* type profile. A high proportion of linolenic acid, observed in the profiles might have been the result of the cold climatic conditions late in the growing season. Precociously germinated seed may also have influenced the results. Further evaluation of multiple locations is required to determine the true nature of the fatty acid profiles within the DH lines. A high proportion of saturated fatty acids within the DH progeny raises some concern over the usefulness of these crosses for reducing saturate levels in *B. napus*. It must be considered that this is only one cross and therefore broad conclusions about improving every trait simultaneously can not be made. Whether linolenic acid and total saturate levels are truly high in these progeny, will dictate whether these crosses affect the quality of the oil produced by the seed.

Average glucosinolate content within the 95-1061-B population was reduced below that of the *B. napus* and *B. rapa* parents. Analysis of individual glucosinolates revealed that this reduction was brought about by a decrease in the total alkenyl glucosinolates. The interspecific parent (95-376) was found to be segregating for total glucosinolate content. Inadvertently selecting a low glucosinolate plant from 95-376, for use in the second cross, may have contributed to the reduction in glucosinolate content in the DH lines. Based on

the progeny derived from this cross, it appears that glucosinolate content can be reduced below the levels seen in either parent species.

The 95-1061-B interspecific cross appears to have produced lines that begin flowering earlier than the *B. napus* parent. However, prolonged flowering due to spacing problems, and an early snowfall prevented accurate measurement of maturity. Plants that reach first flower earlier also tend to mature earlier (Stringam GR, personal communication), however it is of interest to determine whether improvements to the number of days to reach maturity have been made. Further agronomic evaluation will determine if early maturity has been transferred from *B. rapa* along with shorter time to reach first flowering.

Total silique angle, within the 95-1061-B population was more lax than the angle seen in either parent species. The second angle, the degree that the silique is deflected up from parallel with the pedicel, which gives *B. rapa* its upright silique angle, was not as heritable as predicted in this cross. Although the overall trend within the population was towards a more lax silique angle, individual lines with total silique angles that were smaller than that observed in the *B. napus* parent were identified. It appears that selection for a larger second angle may be a more effective means of improving silique angle than would be selecting for smaller first angles.

Several agronomic characters, such as yield, could not be assessed accurately due to inconsistent stands and the fact that only one site year of data was collected. Preliminary analysis of yield components suggests that there is a potential to improve yield through interspecific crosses. Within the 95-1061-B

population, the average number of siliques on the main stem was higher than what is seen in the *B. napus* parent. Several lines with more seeds per silique than the *B. napus* parent were identified. Average carpel length was intermediate between the *B. napus* and *B. rapa* parent species, with several lines having longer carpels than either parent species. Average 1000-seed weight within this population was higher than that seen in either the *B. napus* or *B. rapa* parent. There appears to be potential for combining more siliques on the main stem, with more seeds per silique and a larger seed size. Further testing will determine if yields in the DH lines from this interspecific cross are high yielding.

Seed protein content appears quite stable, at a level intermediate between the *B. napus* and *B. rapa* parents. However, oil content was quite variable, and may be a reflection of the precocious seed germination problem. Because of the precocious germination problem, further field evaluation is required in order to determine if acceptable levels of oil and protein content were maintained in the interspecific progeny.

A strong influence on seed color was observed in the 95-1061-B population. The absence of yellow seeded types despite two crosses to a yellow seeded type may be a reflection that seed color genes were present in the **C** genome. Yellow seeded species are known to contain less fiber in their meal and therefore a larger proportion of the seed is made up of oil and protein rich embryo (Stringam et al., 1974). This cross does not appear to be an effective means of increasing oil and protein content by transferring yellow seed color to *B. napus*.

RAPD primers revealed species and accession specific differences between the parents that were inherited by the DH progeny. Although a strong correlation between these differences and traits of interest could not be identified, the polymorphisms demonstrated that genetic material was introgressed from both species.

6.2. Conclusions:

The interspecific DH progeny produced for this study demonstrated the potential for introgressing many traits from both species. The discovery that the DH progeny all contained a *B. napus* chromosome number, is consistent with the fact that a strong *B. napus* influence was seen in many traits. The possibility that a maternal influence on microspore culture may have influenced chromosome in the DH population needs to be explored. If there is a maternal influence, using *B. rapa* as the female parent may produce DH progeny which all contain a *B. rapa* chromosome complement.

The observation that quadrivalent and bridge / fragment chromosome configurations were persisting in some of the DH progeny suggests that there is cytological instability within the population. Although lines appeared phenotypically stable, the long term effects of these aberrant chromosome associations on the genetic stability of these lines needs to be characterized if the lines are to be utilized as cultivars, or in future breeding efforts.

Difficulty in breeding *B. rapa* with high yield potential has lead to a focus on developing early maturing *B. napus*. It would be of interest to determine whether high yield and early maturity have been inherited along with disease resistance and shorter time required to reach flowering in the DH progeny derived from interspecific crosses between *B. napus* and *B. rapa.* These determinations, however, await further study. The complex genetics and the maternal influence observed with many traits suggests that combining all the traits of interest may not be achieved through a single cross.

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Appendix 1: Leaf and Flower Morhpology Based on IBPGR Descriptors.

Leaf Characteristics							Flower Characteristics	
Line	Parent	Color	Shape	Margin	Hairiness	Clasp	Bud position	Flower type
95-439-2	*B. rapa*	glossy	ovate	D. dent.	apex	full	below	*B. rapa*
95-442-2	*B. rapa*	glossy	ovate	Dentate	apex	full	below	*B. rapa*
95-442-3	*B. rapa*	glossy	ovate	Dentate	apex	full	below	*B. rapa*
96-797-2	95-1060-B	waxy	ovate	D. dent.	none	50%	above	*B. napus*
96-1057-1	95-1060-B	waxy	elliptic	D. dent.	none	50%	above	*B. napus*
96-2015-4	95-1060-B	waxy	elliptic	Dentate	none	50%	above	*B. napus*
96-815	95-1062-B	waxy	elliptic	Dentate	none	75%	above	*B. napus*
96-1776-2	95-1062-B	waxy	ovate	D. dent.	none	75%	below	*B. napus*
Eclipse	Eclipse	glossy	ovate	Dentate	apex	full	below	*B. rapa*
Quantum	Quantum	waxy	orbicular	Dentate	none	75%	above	*B. napus*
D. dent. = Doubly dentate								

Appendix 2: Lsmeans for Fatty Acid Profiles (% Total Profile).

Line	Parent	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Erucic	Saturates
95-439-2	*B. rapa*	3.39	1.60	57.32	23.41	11.68	0.05	5.93
95-442-2	*B. rapa*	3.50	1.51	55.44	24.06	12.72	0.05	5.98
95-442-3	*B. rapa*	3.08	1.95	58.05	22.91	10.85	0.03	6.39
96-797-2	95-1060-B	5.20	1.71	52.07	21.03	16.88	0.07	8.07
96-1057-1	95-1060-B	4.06	1.42	52.81	23.81	14.70	0.02	6.64
96-2015-4	95-1060-B	4.25	1.75	52.63	22.34	15.95	0.08	7.12
96-815	95-1062-B	4.48	1.74	51.15	22.37	16.79	0.08	7.67
96-1776-2	95-1062-B	4.42	1.82	50.12	24.91	15.54	0.07	7.49
Eclipse	Eclipse	3.19	1.43	55.98	24.11	12.70	0.05	5.49
Quantum	Quantum	3.65	1.70	59.95	19.89	11.24	0.13	6.53

Appendix 3: Lsmeans for Glucosinolate Content of Air Dried Oil Free Meal (umol/gram).

Line	Parent	T. Akenyl	T. Indoyl	T. Gluc.	Butanyl	OHIndoyl	OHButanyl
95-439-2	*B. rapa*	3.5	9.8	13.2	0.4	9.3	3.0
95-442-2	*B. rapa*	6.8	9.6	16.4	1.5	9.1	4.5
95-442-3	*B. rapa*	5.2	9.6	14.8	1.6	9.3	3.0
96-797-2	95-1060-B	12.4	7.0	19.4	0.9	6.7	11.0
96-1057-1	95-1060-B	3.5	8.8	12.3	0.7	8.3	2.6
96-2015-4	95-1060-B	35.2	8.1	43.3	0.8	7.8	33.6
96-815	95-1062-B	34.8	7.1	41.9	2.3	6.8	31.6
96-1776-2	95-1062-B	18.3	8.6	26.9	2.0	8.0	15.7
Eclipse	Eclipse	4.9	10.0	15.0	1.7	9.7	2.3
Quantum	Quantum	7.1	11.2	18.3	2.6	10.5	3.4

Appendix 4: Agronomic Traits.

Line	Parent	Days to Flower	Silique Angle Degrees	Angle 1 Degrees	Angle 2 Degrees	Length main (cm)	Height (cm)
95-439-2	*B. rapa*	41.2	48	69	22	41.1	77.1
95-442-2	*B. rapa*	38.0	41	69	28	45.8	83.6
95-442-3	*B. rapa*	38.4	58	74	16	50.2	85.4
96-797-2	95-1060-B	41.4	50	57	7	56.6	84.2
96-1057-1	95-1060-B	50.5	43	59	17	35.9	71.6
96-2015-4	95-1060-B	43.1	51	60	9	53.7	97.0
96-815	95-1062-B	48.9	50	63	12	50.4	94.6
96-1776-2	95-1062-B	43.4	59	76	17	50.9	87.3
Eclipse	Eclipse	38.9	52	72	20	45.5	77.7
Quantum	Quantum	45.0	61	76	15	59.1	108.6

Appendix 5: Yield Components.

Line	Parent	Length Carpel (mm	Length Beak (mm)	# Siliques	Seed/ Silique	Seedwt (g)	DBS (mm)
95-439-2	*B. rapa*	39.5	14.9	37.1	14.0	2.89	3.5
95-442-2	*B. rapa*	38.7	12.3	42.7	12.2	3.45	3.6
95-442-3	*B. rapa*	38.8	17.9	38.8	10.6	3.14	4.8
96-797-2	95-1060-B	66.1	7.8	42.6	30.0	3.55	2.4
96-1057-1	95-1060-B	41.9	7.3	19.6	11.3	4.63	4.1
96-2015-4	95-1060-B	33.4	9.1	38.9	3.9	4.66	8.6
96-815	95-1062-B	38.2	8.5	61.3	3.2	3.96	14.3
96-1776-2	95-1062-B	55.2	9.9	44.0	13.6	5.13	4.3
Eclipse	Eclipse	44.8	15.4	38.6	22.1	2.87	2.1
Quantum	Quantum	72.2	8.3	43.8	34.1	3.90	2.1

Appendix 6: Oil and Protein Content, and Seed Color.

Line	Parent	% Oil	% Protein	Seed color
95-439-2	*B. rapa*	40.9	48.3	yellow
95-442-2	*B. rapa*	38.8	49.9	yellow
95-442-3	*B. rapa*	38.1	49.6	yellow
96-797-2	95-1060-B	37.8	46.4	black
96-1057-1	95-1060-B	34.6	48.3	brown
96-2015-4	95-1060-B	34.0	47.1	brown
96-815	95-1062-B	29.0	48.2	brown
96-1776-2	95-1062-B	30.6	49.6	brown
Eclipse	Eclipse	42.1	47.8	yellow
Quantum	Quantum	41.2	50.4	black

Appendix 7: R^2 and probability values for correlation between molecular markers and agronomic traits.

Trait		Primer Number and Size of Marker (Kbp)					
		OPA3-0.6	OPA3-1.4	OPA12-1.0	OPA17-0.6	OPA18-0.7	OPA18-1.7
angle 1	R^2	0.04	0.03	0.01	0.07	0.08	0.01
	Pr>P	0.2960	0.3780	0.5381	0.1514	0.1366	0.5716
angle 2	R^2	0.00	0.02	**0.24**	0.07	**0.13**	0.00
	Pr>P	0.9754	0.4299	**0.0049**	0.1463	**0.0528**	0.9488
Pod angle	R^2	0.02	0.05	**0.20**	0.00	**0.21**	0.00
	Pr>P	0.4496	0.2299	**0.0128**	0.9343	**0.0124**	0.7333
Days to Flower	R^2	0.03	0.06	0.00	0.06	0.03	0.00
	Pr>P	0.3204	0.1884	0.9085	0.1771	0.3725	0.7663
Palmitic Acid	R^2	0.04	0.00	0.00	0.02	0.03	0.04
	Pr>P	0.2914	0.7095	0.8548	0.4893	0.3291	0.2916
Stearic Acid	R^2	0.00	**0.12**	0.01	0.00	0.00	0.03
	Pr>P	0.9812	**0.0544**	0.6772	0.9687	0.9585	0.3816
Oleic Acid	R^2	0.01	0.02	0.00	0.00	0.00	**0.15**
	Pr>P	0.6712	0.4087	0.7732	0.7206	0.7841	**0.0333**
Linoleic Acid	R^2	0.00	0.01	0.06	0.01	0.02	**0.13**
	Pr>P	0.8453	0.5768	0.1721	0.6705	0.4402	**0.0517**
Linolenic Acid	R^2	0.01	0.06	0.07	0.00	0.01	0.04
	Pr>P	0.6528	0.1813	0.1589	0.8679	0.5977	0.2854
Erucic Acid	R^2	0.05	0.00	0.01	0.02	0.05	0.08
	Pr>P	0.2349	0.7020	0.6268	0.4574	0.2578	0.1219
Saturates	R^2	0.03	0.04	0.00	0.02	0.02	0.09
	Pr>P	0.3216	0.2469	0.9106	0.4696	0.4836	0.1145
T. Alkenyl	R^2	0.00	0.02	**0.20**	0.00	**0.21**	0.09
	Pr>P	0.7899	0.4302	**0.0104**	0.7409	**0.0110**	0.1133
T. Indoyl	R^2	0.00	0.02	0.00	0.05	0.01	0.02
	Pr>P	0.7296	0.3911	0.9857	0.2485	0.6046	0.4528
T. Gluc	R^2	0.00	0.03	**0.16**	0.00	**0.15**	0.05
	Pr>P	0.8797	0.3234	**0.0216**	0.9556	**0.0344**	0.2292
Butanyl	R^2	0.01	0.02	0.04	0.01	0.11	**0.32**
	Pr>P	0.6722	0.4855	0.2663	0.6069	0.0742	**0.0011**
OHIndoyl	R^2	0.00	0.02	0.00	0.03	0.01	0.02
	Pr>P	0.8017	0.3956	0.9281	0.3327	0.6581	0.4164
OHButanyl	R^2	0.00	0.02	**0.21**	0.00	**0.20**	0.05
	Pr>P	0.7846	0.4197	**0.0079**	0.7800	**0.0120**	0.2373
Length Main	R^2	0.00	0.07	0.12	0.00	0.02	0.01
	Pr>P	0.7187	0.1712	0.0653	0.7986	0.5051	0.6988

Appendix 7: continued

Trait		Primer Number and Size of Marker (Kbp)					
		OPA3-0.6	OPA3-1.4	OPA12-1.0	OPA17-0.6	OPA18-0.7	OPA18-1.7
Height	R^2	0.00	0.06	**0.17**	0.01	0.06	0.07
	Pr>P	0.7419	0.1838	**0.0221**	0.6105	0.2227	0.1595
# Siliques on Main	R^2	0.00	**0.16**	0.10	0.01	0.08	0.03
	Pr>P	0.8567	**0.0266**	0.0965	0.5550	0.1450	0.4042
Carpel Length	R^2	0.02	0.01	**0.28**	0.01	**0.18**	0.05
	Pr>P	0.4014	0.6397	**0.0024**	0.6708	**0.0229**	0.2528
Beak Length	R^2	0.08	**0.14**	0.01	0.01	0.00	**0.18**
	Pr>P	0.1160	**0.0415**	0.6103	0.6280	0.9962	**0.0210**
Seeds per Silique	R^2	0.00	0.01	**0.27**	0.01	**0.14**	0.11
	Pr>P	0.8202	0.6914	**0.0027**	0.6390	**0.0467**	0.0761
DBS	R^2	0.00	0.00	**0.19**	0.02	0.07	**0.14**
	Pr>P	0.8085	0.8371	**0.0133**	0.4530	0.1724	**0.0470**
% Oil	R^2	0.05	0.00	0.04	0.02	0.01	**0.22**
	Pr>P	0.2168	0.9585	0.2663	0.5038	0.6112	**0.0082**
% Protein	R^2	0.05	0.00	0.00	0.00	0.00	0.02
	Pr>P	0.2258	0.7221	0.9066	0.8691	0.9676	0.4902
1000 Seed Weight	R^2	0.04	0.06	0.07	0.07	**0.18**	0.05
	Pr>P	0.2793	0.1735	0.1467	0.1602	**0.0207**	0.2264
Seed Color	R^2	0.03	0.01	**0.13**	0.01	0.03	**0.18**
	Pr>P	0.3633	0.6046	**0.0430**	0.5327	0.3418	**0.0192**
Leaf Color	R^2	0.02	0.04	**0.14**	0.01	0.08	0.01
	Pr>P	0.4171	0.2945	**0.0350**	0.6323	0.1288	0.6626
Leaf Shape	R^2	0.01	0.05	0.00	0.04	0.00	0.01
	Pr>P	0.5121	0.2418	0.7421	0.3097	0.8223	0.6449
Leaf Margin	R^2	0.02	0.00	0.04	0.00	0.04	0.01
	Pr>P	0.4827	0.8185	0.2865	0.9520	0.2739	0.6079
Leaf Pubesc.	R^2	0.02	0.04	**0.14**	0.01	0.08	0.01
	Pr>P	0.4171	0.2945	**0.0350**	0.6323	0.1288	0.6626
Leaf Clasp	R^2	0.08	0.00	**0.13**	0.00	0.10	0.00
	Pr>P	0.1164	0.8185	**0.0388**	0.9520	0.0971	0.7399
Bud Posittion	R^2	0.01	0.03	0.09	0.03	0.00	0.02
	Pr>P	0.6948	0.3648	0.0923	0.3888	0.8988	0.4317
Flower Type	R^2	0.07	0.01	**0.16**	0.03	0.00	0.02
	Pr>P	0.1411	0.6348	**0.0253**	0.3888	0.8988	0.4317
Significant correlations are in bold text							