NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Canadä

University of Alberta

Agronomic, quality performance and stability of F_1 and BC_1F_1-derived doubled haploid lines from an interspecific backcross, *Brassica juncea* Czern and Coss. x *B. napus* L. x *B. juncea*.

James Gichuru Gethi

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirement for the degree of Master of Science

in

Plant Breeding

Department of Plant Science

Edmonton, Alberta, Canada
Spring 1996

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-612-10712-4

Canada

University of Alberta

Library Release Form

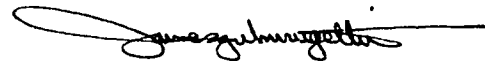**Name of Author:** James Gichuru Gethi

Title of Thesis: Agronomic, quality performance and stability of F_1 and BC_1F_1-derived doubled haploid lines from an interspecific backcross *Brassica juncea* Czern and Coss. x *B. napus* L. x *B. juncea.*

Degree: Master of Science

Year this Degree granted: 1996

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly, or scientific research purposes only.

The author reserves all the other rights in association with the copyright in the thesis, and except hereinbefore provided, neither the thesis nor any substantial portion thereof may be printed or otherwise reproduced in any material form whatever without the author's prior written permission.

C/O John Gatu Nderitu
P.O. Box 221,
Nyeri, Kenya

Date: March 28th 1996
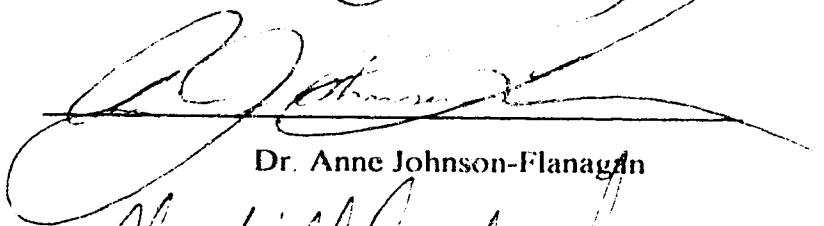
University of Alberta

Faculty of Graduate Studies and Research
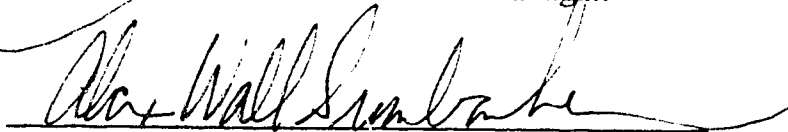
The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Agronomic, quality performance and stability of F_1 and BC_1F_1-derived doubled haploid lines from an interspecific backcross *Brassica juncea* Czern and Coss. x *B. napus* L. x *B. juncea*" in partial fulfillment of the requirements for the degree of Master of Science in Plant Breeding.

Dr. Gary R. Stringam

Dr. Anne Johnson-Flanagin

Dr. Alan W. Grombacher

Date: 15 March 1996

Abstract

Introgression of traits in a (*B. juncea* x *B. napus*) x *B. juncea* cross was studied using the doubled haploid method. Characterization of F_1 and BC_1F_1-derived DH lines using RAPDs indicated genetic transfer had occurred between the species. Stability evaluation through male and female fertility and meiotic chromosome configuration for both F_1 and BC_1F_1-derived DH lines was $II_{11.7}+III_{0-0.7}+I_{3.5-10}$ and $II_{14.7}+III_{0-0.6}+I_{1-3.9}$ indicating increased stability of BC_1F_1-derived DH lines. No morphological abnormalities were observed, although the F_1-derived DH lines were more vegetative than parent lines. Preliminary data showed yield performance of some of the BC_1F_1-derived DH lines to be superior to the parents. Oil content as high as 46.3% and total glucosinolate content of one of the BC_1F_1-derived DH lines was reduced to 31.4 μmg^{-1}. Linolenic acid levels could not be altered appreciably although other fatty acid profiles were modified. Resistance to *Leptosphaeria maculans* and *Alternaria brassicae* was modified with some lines showing improved resistance to both diseases.

Dedication

To my late father, Josphat Gethi Matheri whose sacrifices throughout the years made this possible.

Acknowledgements

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter 1
Literature Review

1.1. Introduction

The family Cruciferae (Syn. Brassicaceae) comprises some of the most important domesticated flowering plants. The cruciferae flower consists of four sepals and four petals shaped like a cross. The flower contains six stamens (Prakash and Hinata 1980), of which two are placed higher than the other four. The *Brassica* inflorescence is a corymbiform raceme with indeterminate flowering. Flowering begins at the lowest bud on the main branch. The flowers are regular, bisexual and hypogynous with four free petals in two whorls, median and transverse. The ovary is syncarpus and develops into siliqua with two carpels separated by a false septum.

The diploid rape species *B. rapa* L. (2n=20) appears to have had the widest distribution from the Atlantic islands in the west to the eastern shores of China and Korea and from Northern Norway to the Sahara region and Northern India. *Brassica napus* L. probably evolved only relatively recently in southern Europe from interspecific crosses of *B. oleracea* L. (2n=18) and *B. rapa* (2n=20) (Robbelen 1987). *B. juncea* (L.) Czern and Coss (2n=36), the brown or oriental mustard is assumed to have arisen from the middle east by hybridization between different genotypes of *B. rapa* and *B. nigra* L. Koch 2n=16). *B. carinata* A. Braun (2n=34) which includes the Abyssinian mustard originated from northeast Africa (Robbelen 1987).

Brassica crops, which have a broad range of diversity, are mainly found in the temperate zones of the world. These crops are important as oil plants for production of margarine, cooking oil and lubricants (*B. napus, B. rapa* and *B. juncea*) as fodder (*B. oleracea, B. napus* and *B. rapa*) and as vegetables (*B. oleracea*). Other crucifer plants are used in the manufacture of dyes, and are sources of industrial products such as soap and lamp oils (Stern 1982). The cruciferae also contain agriculturally important wild and weedy species including *Arabidopsis thaliana* which serves as a model for study of *Brassica* species, stink weed (*Thlapsis arvanse*), shepherds purse (*Capsella bursapastoris* L.), flix weed (*Descurainia sophia* L. Webb), ball mustard (*Neslia paniculata* L. Desv.), wormseed mustard (*Erysimum cheirathoides* L.), hares ear

1

mustard (*Erucastrum gallicum* [wild] O.E. Schultz), and common pepper grass (*Lepidium campestris* L. R. Br) (Canola Council of Canada 1992). *B. napus* and *B. rapa* are both well established oilseed crops of the temperate zones whereas *B. juncea* and *B. carinata* have been grown in other areas of the world as sources of oil and condiments.

Rapeseed was cultivated in India as early as 2000 B.C. and was introduced to Japan from China about 35 B.C. (Rapeseed Association of Canada 1970). The Greek and Roman writings of 200-500 BC indicate that *Brassica* oilseeds were used as medicinal remedies for stomach and skin diseases (Downey and Robbelen 1989, Prakash and Hinata 1980). Extensive use of rapeseed oil began after development of steam engines when it was found that rapeseed oil clings better to water and steam washed surfaces than any other lubricant.

Prior to World War II, rapeseed had been grown in Canada in small research trials at experimental farms and stations. The need for Canadian rapeseed production arose from a critical shortage of rapeseed oil needed for lubricating steam engines of the rapidly increasing number of naval and merchant ships that followed the World War II blockade of European and Asian sources of rapeseed oil in the early 1940's. A Polish immigrant brought some seed from Poland and started growing a small amount of rapeseed in Canada in 1936. Because of the origin of this farmer and his seed, the species came to be known as 'Polish rapeseed'. This species was later determined to be *B. rapa* (syn *B. campestris*). The shortfall in production of the Polish rape for planting seed led to importation of rapeseed from U.S. seed companies. Since this seed was originally secured from Argentina, (Bell 1984, Canola Council of Canada 1995a) this type of rapeseed came to be known as 'Argentine rapeseed'.

The first edible rapeseed oil extracted in Canada was in 1956-57. As early as 1956, the nutritional aspects of rapeseed oil were questioned concerning the high eicosenoic and erucic fatty acids. In addition to erucic acid, it was found that the protein meal fraction of rapeseed contained sharp tasting anti-nutritive glucosinolates. These deficiencies led to the development of greatly improved cultivars now known as canola. Canola is presently used as a generic term to describe *B. napus* and *B. rapa*

2

varieties yielding oil of less than 2% erucic acid and 30 μmg^{-1} aliphatic glucosinolates of air dried oil free meal. New canola definitions have been proposed to take effect in 1997, where canola seed will be required to contain no more than 1% erucic acid and less than 18 μmg^{-1} of total glucosinolates, and a meal containing less than 30 μmg^{-1} total glucosinolates in air dried oil free meal (Canola Council of Canada 1995b). The first low erucic acid cultivar 'Oro' was produced in 1968 through transfer of traits from the European cultivar 'Liho', into adapted Canadian germplasm. The low erucic acid cultivars (LEAR) subsequently developed in Canada included *B. napus* 'Midas', and *B. rapa* 'Torch' and 'Span'. The first double low variety 'Tower' with reduced erucic acid and glucosinolate levels was developed in 1974 through trait transfers from Polish *B. napus* variety 'Bronowski' which was found to contain about one-tenth of the glucosinolate content of standard adapted Canadian germplasm at that time (Canola Council of Canada 1979). This *B. napus* variety was the first to meet specific quality requirements of canola.

Rapeseed oil has been used for edible and non-edible purposes. Rapeseed oil with over 50% erucic acid is used for cold steel rolling and in jet engines. This oil is also used in the production of erucamide, a slip agent used in the plastics industry that prevents plastic sheets and moldings from sticking together or sticking in extrusion machines. This represents just a small proportion of the total Brassica crop, and is grown on contract.

From 1971 to 1981, the world rapeseed/canola production increased by 30% while the Canadian production fluctuated from 12% to 51% in 1976 and 1978. This averaged to 32% over a nine year period (Canola Council of Canada 1982), but fell to 18.9% of the world's production in 1985 (Canola Council of Canada 1987). From 1982 to 1992 Canada's production of canola/rapeseed averaged 3.439 million tonnes, second only to China and India (Canada Grains Council 1993).

Canola is now the largest oilseed crop in Canada, representing two thirds of the total oilseed production (Polon 1994). In 1993, over 10 million acres (4 million hectares) were seeded to canola in Canada with a seed production of 5 million tons (Canola Council of Canada 1995a). In 1994, a record 14.375 million acres (5.817

million hectares) was seeded to canola (Canola Council of Canada 1995a). According to the council, canola production in the prairie provinces of Canada was highest in Alberta (3,063,800 tons), Saskatchewan (2,313,300 tons) and Manitoba (884,500 tons). Both Ontario (45,400 tons) and British Columbia (43,100 tons) had a smaller share of the 1994 production.

Exports of canola seed to Japan account for approximately 40% of Canada's seed production. Canada is the highest per capita consumer of canola in the world with canola oil comprising 42% of margarine, 56% of shortenings and 80% of salad oil (Polon 1994 and Canola Council of Canada 1995a). A gradual shift to canola can be expected in the world production of rape seed oil, because of its nutritional advantages over other vegetable oils (Canola Council of Canada 1982) and the granting of "Generally Recognized as Safe" status granted to canola oil by the Food and Drug Administration of the U.S.A. government in 1985 (National Archives and Records 1985).

Canola is produced under a wide range of environments from the northern short season Peace river region in Alberta to the long season southern regions of Manitoba (Canola Council of Canada 1979). The need to develop well adapted cultivars for these specific environments is recognized by growers and researchers (Alberta Agriculture 1987).

B. juncea (Indian or Oriental mustard) is grown on limited acreage in many countries for production of condiment mustard, and in western Canada occupies 50,000 hectares annually (Woods et al. 1991). Although *B. juncea* is agronomically well adapted to areas traditionally used for growing canola quality *B. napus* and *B. rapa*, the presence of high levels of glucosinolates (approx. 200μm g^{-1} of oil free meal) and of erucic acid (approx. 25%) makes the seed unsuitable for edible oil processing in western countries (Woods et al. 1991).

Canadian condiment mustard cultivars of *B. juncea* are well adapted to dry land agriculture on the Canadian prairies. They are higher yielding than *B. napus* and *B. rapa* canola cultivars (Rapeseed Association of Canada 1970, Rakow 1995), resistant to blackleg caused by *Leptosphaeria maculans* syn. *Phoma lingam*, (Kirk and

Hurslstone 1983) early maturing, drought tolerant and resistant to seed shattering (Woods et al. 1991, Love et al. 1991) Woods et al. (1991) reported instances where plots of *B. napus* were abandoned as a result of drought and shattering whereas *B. juncea* plots were not affected. *B. juncea* generally matures earlier and shows more vigour in the seedling stage. In tests conducted in western Canada, *B. juncea* on average yielded more than *B. napus* (Westar) and *B. rapa* under irrigation. *B. juncea* retained 38.7% of its yield under dry land conditions as compared to *B. napus* 'Westar' and *B. rapa* 'Tobin' which retained 18.7% and 20.7% of their dry yield respectively in co-operative trials done over a three year period in Western Canada (Alberta Agriculture 1987). This is particularly important in areas where the growing season is short. If this trait could be introgressed into other varieties; this would reduce the risks associated with hail and frost especially in short growing season areas. Canola production could expand to drier southern regions if canola quality *B. juncea* with the desirable characteristics could be obtained. As a result of this potential, research aimed at developing canola quality *B. juncea* has been initiated especially to improve oil and reduce glucosinolate contents. Other major goals include increasing canola oil and protein contents while improving seed yield. Oil quality characteristics like fatty acid composition have been identified as research priority issues (Canola Council of Canada 1995a).

1.2. Parent and donor line development

The genus *Brassica* is remarkable for its diversity of form. Cultivated varieties represent only a portion of the abundant variability that occurs in this genus (Kianian and Quiros 1992). The common *Brassica* species *B. rapa, B. nigra*, and *B. oleracea* have x=10, x=8 and x=9 chromosomes respectively. These have been assigned the genome designation A, B and C (U 1935, Robbelen 1960, Mizushima 1980). Therefore, their genomes are referred to as AA, BB and CC. Experiments involving interspecific hybrids and pairing behaviour during meiosis established two groups of three species each; monogenomic diploids consisting of *B. nigra* (2n=16), *B. oleracea* (2n=18) and *B. rapa* (2n=20) and digenomic alloploids comprising *B. carinata*

5

(2n=34), *B. juncea* (2n=36) and *B. napus* (2n=38) (Prakash and Hinata 1980). *B. juncea* (2n=36, AABB) is a natural amphidiploid which combines the genomes of *B. rapa* (AA) and *B. nigra* (BB). *B. napus* (2n=38, AACC) is another amphidiploid which combines the genomes of *B. rapa* (AA) and *B. oleracea* (CC). *B. carinata* (2n=34, BBCC) is formed through combination of the genomes of *B. nigra* (BB) and *B. oleracea* (CC) (Poehlman 1979). The cytogenetic relationships in the *Brassica* species were elucidated by U (1935) resulting in what is commonly referred to as U's triangle (Figure 1.1).

Pachytene chromosome analysis has shown that the monogenomic diploids are segmental alloploids arising from a common ancestor with a basic chromosome number of 6 denoted as A, B, C, D, E and F (Robbelen 1960, Venkateswarlu and Kamla 1971). *B. rapa*'s 10 chromosomes can be represented by AABCDDEFFF, *B. oleracea*'s 9 chromosomes by ABBCCDEEF and *B. nigra*'s 8 chromosomes by AABCDDEF.

Since different diploid species in the genus *Brassica* possesses useful agronomic traits that are absent in other diploid and amphidiploid species (Bijral et al. 1995), interspecifc crossing is an important way of introgressing new traits within *Brassica*. Resynthesis of amphidiploids from the diploid species has been used to develop cultivars (Olsson 1963). Artificial alloploids have been used by breeders for introduction of useful genes from diploids to alloploids (Prakash and Chopra 1990, and Lange et al. 1989). Autoploids have been developed from the *Brassica* species through doubling of chromosomes by the use of colchicine (Olsson 1963). The tetraploids of the diploid species developed at Svalof, Sweden for forage were vegetatively well developed than the diploid and amphidiploid species. A tetraploid from *B. rapa* was marketed in Sweden in 1953 under the name of 'Sirius'. In dry matter yields, it surpassed the then current diploid varieties by as much as 22% (Olsson 1963). Tetraploids from the diploid species yielded fewer well developed seeds than their corresponding diploids in the same study. Tetraploids from amphidiploid species were characterized by reduced vigour and fertility. Tetraploids from *B. napus* had 6.1 seeds per pod as compared to 22.2 seeds per pod in the natural amphidiploid. In *B. juncea*,

6

the tetraploids had 4.9 seeds per pod as compared to the natural amphidiploids with 15.6 seeds per pod (Olsson 1963).

Allosyndesis and autosyndesis has played an important role in improvement of *Brassica* species. Morinaga (1929) and Sasaoka (1930) reported that bivalent pairing in hybrids between *B. juncea* and *B. napus* (AABC) was confined to pairing between A genome chromosomes (10II) with B and C genome chromosomes remaining unpaired, i.e. autosyndetic pairing was apparent and that allosyndetic pairing was absent.

In a more recent study, Prakash and Chopra (1990) demonstrated that allosyndetic pairing occurred between the B and C genome in 9.3% of the meiotic cells studied and autosyndetic pairing occurred in 90.7% of the PMCs studied. This demonstrates that it should be possible to transfer traits between the B and C genomes. Other studies by Busso et al. (1987), showed that allosyndetic pairing occurred between A and C-genome chromosomes, more than between B genome chromosomes and those from A and C genomes. This could be as a result of diploid species having different centres of origin. The A and C-genome species originated from geographically closer regions than B-genome species, which were more isolated, resulting in more structural differences (Prakash and Hinata 1980).

Introgression of alien genes is often hampered by the lack of pairing between two well differentiated genomes (Ranga 1988). In the majority of interspecific hybridizations in *Brassica*, hybrids are usually formed if the species with the highest basic chromosome number are used as the female parent (Quazi 1988, Mohapatra and Bajaj 1987, Ayotte et al. 1987). However, in interspecific crosses between *B. juncea* and *B. napus* where *B. juncea* has fewer chromosomes than *B. napus* more hybrid seeds were produced in comparison to when *B. napus* is used as a female parent (Sabharwal and Bechyne 1991). It has been difficult to obtain interspecific hybrid seeds when *B. napus* was used as a female parent because of low cross ability (Meng 1987). In a cross between *B. napus* and *B. juncea* the pollen tubes of *B. juncea* were unable to penetrate the pappillar cells of *B. napus* because of a heavy deposition of callose after pollination (Meng and Yi 1988). This problem however may be circumvented by physically wounding the stigma of *B. napus* during crossing procedure.

7

Figure. 1.1.   Interrelationships between the diploid and amphidiploid *Brassica* species. After U (1935).
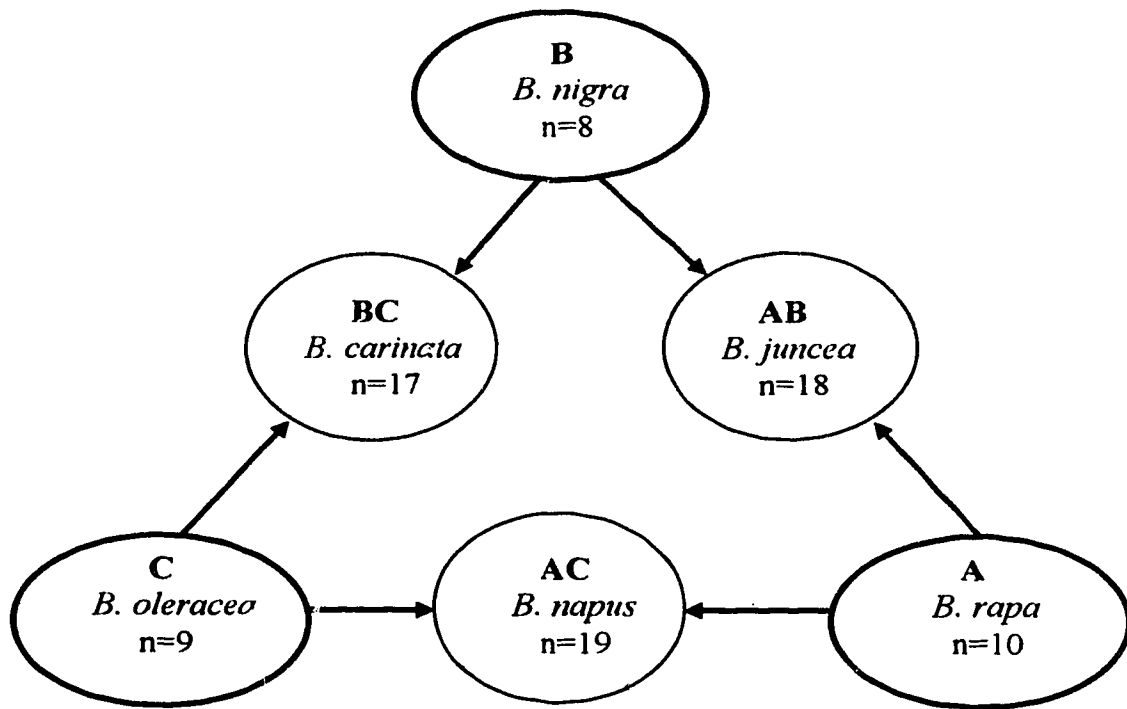
Intergenomic recombination between *B. oleracea* (CC) and *B. rapa* (AA) chromosomes has been demonstrated in the generation of *B. rapa - oleracea* addition lines developed from natural *B. napus* (Quiros et al. 1987). In the process of developing addition lines between *B. rapa* and *B. alboglabra*, Chen et al. (1992) suggested that alien chromosomes from *B. alboglabra* may not remain intact because of possible intra and intergenomic recombination, allowing the possibility of chromatin exchange between genomes. Chromosome substitutions have been known to occur spontaneously in interspecific crosses (Khush, 1973). Banga (1988) postulated that genetic activity of substituted chromosomes from the B genome of *B. juncea* by C chromosomes from the C genome of *B. napus* is complementary. This indicates that there are homoeologous regions in some chromosomes belonging to the B and C genomes of both *B. juncea* and *B. napus* where pairing is possible. Such substitutions are only possible if the substituted chromosome is phylogenetically related to the replaced chromosome. During anaphase II and telophase II, euploid and near euploid gametes occur at low frequencies in partially fertile F_1 hybrids between *B. napus* and *B. oleracea* (Ayotte et al. 1988).

Environment greatly affects cross ability, and of particular importance is the condition of the parents. In an interspecific cross between maize and sorghum, collapsing of embryos occurred very early at high temperatures (James 1978). The differences in developmental rates contribute to the failure in hybrid development in interspecific crosses (Forster and Dale 1983). In addition to hybrid production, alien pollination in interspecific crosses sometimes results in stimulation of development of unreduced or reduced egg cells giving rise to maternal and haploid plants respectively (Olsson 1960).

The crosses between species produce partially sterile F_1 plants (Yarnell 1956). Islam et al. (1981) reported duplication and elimination of both wheat and barley chromosomes because of mitotic disturbances in the F_1. This seems to agree with the observation that there was a gradual and erratic elimination of alien chromosomes throughout the life of the hybrid from a maize and sorghum cross (James 1978). Often in association with chromosome elimination, there is a change in chromosome size of one genome due the presence of chromosome bridges and fragments in hybrid cells leading to chromosome breakage (Gupta and Gupta 1973). Progenies from interspecific crosses are usually weak

and slow growing. Hybrid inviability often prevents any utilization of genome combinations usually as a result of poor seed set and agronomic characteristics (Sprague 1982).

In naturally occurring amphidiploids the constituent genomes have undergone sufficient genetic differentiation and tend to lose the ability to pair allosyndetically (Heyn 1977). Genetic suppression of homoeologous chromosome pairing was suggested by Prakash (1974). Rajan and Hardas' (1963), Attia et al. (1987) and Attia and Robbelen (1986) showed that the *Brassica* genomes from different species are not completely homologous. In hybrids obtained from digenomic hexaploids of *B. rapa*, (varieties toria, brown yellow, and Burma sarson) gave a range of meiotic configurations from IV $_{0-7}$ II $_{13-26}$ and I $_{20-10}$ with fertility ranging from 3-40% where an even chromosome pairing would be expected since they all come from the same genome. As a result of similarities between the *Brassica* genomes Sacristan and Gerdemann (1986) were able to transfer agronomically valuable genes for disease resistance from *B. nigra* (BB) into *B. napus* (AACC) by interspecific crossing with *B. juncea* (AABB) suggesting an interaction between the A and B *Brassica* genomes. Attia and Robbelen (1986) reported a higher degree of meiotic pairing in the synthesized AACC amphidiploid than in AABB and BBCC amphidiploids, from which they concluded that A and C genomes are more closely related to each other than to the B genome. A high number of bivalents were observed in plants with one A and one C genome (Tai and Ikonen 1988). Tai and Ikonen (1988) suggested that chromosomes of tetraploid plants obtained through doubling of dihaploids compete between homologous and homoeologous chromosomes. The studies conducted by Attia and Robbelen (1986), Busso et al. (1987) and Attia et al. (1987) revealed that at meiosis homoeologous pairing in *Brassica* is more common between A and C genomes as compared to the B genome. The presence of multivalent associations at metaphase I provided a mechanism for recombination between various genomes (Ayotte et al. 1988), and showed that it would be possible to transfer traits from one genome to the other. This degree of homology between genomes greatly influences the potential use of different germplasm sources to improve *Brassica* crops through interspecific crosses.

Genes transferred from closely related diploid species result in progressive reduction in resistance to rust, both at tetraploid and hexaploid levels (Knott 1978). The resistance to leaf rust and stem rust from *Agropyron elongatum* (2n=70) to wheat was not, however, overcome by rust races when transferred to hexaploid wheat. Such transfers are often found to be associated with undesirable genes. In *Brassica*, Paulmann and Robbelen (1988) reported introduction of restorer genes from *Raphanus sativus*, while Sacristan and Gerdemann (1986) transferred genes for resistance against *Phoma lingam* from *B. juncea* into rapeseed. Resistance to *Plasmodiophora brassicae* was transferred from *B. rapa* to *B. napus* (Lammerink 1970, Johnstone 1974) and from *B. oleracea* to *B. napus* (Yamagishi et al. 1980). Different workers have reported success in transferring traits from *B. juncea* to other *Brassica* species. Roy (1984) reported complete transfer of high degree of blackleg resistance to *B. napus* from *B. juncea* in an interspecific cross. This line was later found to be an addition line that lost the blackleg resistance in subsequent generations (Stringam, Personal communication). It has also been possible to develop a low linolenic acid line from a *B. juncea* and a *B. napus* cross (Roy and Tarr 1986).

In general both male and female fertility increase from backcross 1 (BC₁) to backcross 2 (BC₂) in interspecific crosses (McGrath and Quiros 1990). This results from a decrease in chromosome number or the effect of an additional backcross in removing some chromosome abnormalities. In the *B. juncea* and *B. rapa* cross reported by Olsson in 1960 seed sizes in the F₁ were smaller than in their parents, but the reciprocal cross resulted in seeds of similar size. Pollen fertility in the resulting interspecific hybrids was poor in all the material, and ranged from 19-26%. From the above examples, it can be inferred that gene transfer between various genomes is possible (Sjodin and Glimelius 1989).

1.3. Genetic characterization using RAPDs

Identification of individual cultivars is essential now that breeders rights have been introduced in many countries. Previous identification methods have tended to rely on agronomic or botanical characteristics of individual plants which is expensive and labour intensive (Adams et al. 1989). Also, phenology and morphological characteristics such as plant growth period (Halligan et al. 1991) and seed shape

(Barker et al. 1992) may not be sufficiently different to differentiate cultivars (Mailer et al. 1994). In *Brassica*, specialized differentiating tests have been developed based on seed or plant composition such as fatty acids and glucosinolate content (Heaney and Fenwick 1980). The utility of phenotypic assays may be reduced by environmental effects or by complex inheritance of multigenic traits (Rafalski et al. 1991).

Molecular markers, such as random amplified polymorphic DNAs (RAPDs) and restriction fragment length polymorphisms (RFLPs), provide an efficient method of estimating genetic relationships among genotypes (Thormann et al. 1994). Markers provide a means of ensuring that desirable traits are indeed recovered in selected progeny while ensuring that important alleles from the adapted cultivar are again recovered (Edwards 1992).

It has been proposed that linkage drag, that is, retention of chromosomal fragments near the gene of interest is more persistent than retention of unlinked fragments in advanced progeny. It is likely that chromosomal fragments unrelated to the gene of interest derived from the foreign genome may also be retained and contribute genomic material to desirable phenotypes (Durham and Korban 1994). When unique markers are identified that appear to be associated with genomic regions of interest, segregation analysis must be performed to determine their location in relation to the gene of interest (Durham and Korban 1994). This assumes that closely related DNA will have in common a number of sequences depending on the degree of sequence divergence or convergence between them (Upholt 1977).

The development of polymerase chain reaction (PCR) to amplify DNA and the use of randomly amplified polymorphic DNA (RAPD) or arbitrary primed polymorphic chain reaction (AP-PCR) has resulted in a potentially useful tool for cultivar or line discrimination (Aitken et al. 1994, Williams et al. 1990, Welsh and McClelland 1991). RAPDs have been used for mapping inbred lines in soybean (Williams et al. 1990) and mouse (Welsh et al. 1991), and in construction of genomic maps in diploid species (Fritsch et al. 1993).

Using a set of *Brassica napus* genotypes, Hallden et al. (1994) detected highly similar genetic distances using Restriction Fragment Length Polymorphisms (RFLPs)

and RAPDs, thereby demonstrating the close relationship between the genotypes. Polymorphisms can be detected in fragments containing highly repeated sequences using RAPDs and this provides markers that can be used in regions of the genome previously inaccessible using RFLPs (Michelmore et al. 1991, Williams et al. 1990). In interspecific comparisons in the genus *Glycine* (Williams et al. 1992) and within *Brassica* (Thorman and Osborn 1992), the presence of a RAPD band in both genotypes indicated a high level of sequence homology in their respective DNAs.

RAPD markers provide information at many loci (Rafalski et al. 1991, Welsh et al. 1991, Williams et al. 1990). Nearly all RAPD markers are dominant (Williams et al. 1990). The dominant nature of the markers is offset by the large number of polymorphisms generated by a single primer using AP-PCR (Welsh et al. 1991). It is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous or homozygous with dominant RAPD markers. Since the majority of RAPD markers are expressed in a dominant manner it is necessary for a single primer to generate at least one polymorphic amplification product from each parent when being used to identify hybrids (Baird et al. 1992).

DNA markers by themselves have no measurable effect on the phenotype and the environment does not affect their expression (Tanhuanpaa et al. 1994). The ease of assaying RAPDs make them an attractive method for obtaining intraspecific genetic information (Thormann et al. 1994). This makes it a more reliable and efficient marker system than phenotypic based systems. RAPD markers have advantages over RFLP markers since no initial cloning and characterization of the genome is necessary and only a limited amount of DNA is required (Hallden et al. 1994). Taylor (1993) using PCR assay to detect seed contaminated with *Leptosphaeria maculans* determined that PCR requires less than one half the time required using the current RFLP methods. With PCR, larger samples could be assayed with greater reliability without even planting the seeds. RAPDs are also more practical and simpler to use than RFLPs in germplasm characterization and organization. Since no radio isotope labeling is involved, RAPDs are considered safer than RFLPs. Coupled with backcross introgression and bulked segregant analysis, identification of markers that are tightly

linked to genes of interest is enhanced (Michelmore et al. 1991, Miklas et al. 1993, Haley et al. 1993). This makes them useful to selection systems. RAPD assay has been used to develop genetic maps targeting genetic markers and in segregant analysis in population genetics (Tingey and Tufo 1993).

RAPDs can provide an efficient assay for polymorphism (Williams et al. 1990, Quiros et al. 1991) if distinguishing between heterozygotes and homozygotes is not required (Welsh et al. 1991). They can also be used to tag specific chromosomes and identify gene transfers. As few as three random primers provided enough information to distinguish between cultivars of broccoli and cauliflower (Hu and Quiros 1991).

Studies comparing species of *Brassica, Raphanus* and *Sinapis* genera concluded that RAPD markers might be useful for taxonomic studies (Demeke et al. 1992, Tanhuanpaa et al. 1993). Less than 55 markers could distinguish among 45 *B. oleracea* cultivars involving cauliflower and broccoli (dos Santos et al. 1994). Hu and Quiros (1991) used 40 RAPD markers to distinguish between 26 broccoli and cauliflower cultivars. Demeke et al. (1992) separated 5 cabbage and 4 cauliflower genotypes with 122 RAPDs and 13 primers. With only six primers, Mailer et al. (1994) discriminated 24 of 26 cultivars of *B. napus*. Occasionally, one primer can distinguish a wide range of genotypes. In a study of 13 cocoa (*Theobroma*) clones Wilde et al. (1992) separated all of them with a single primer, identifying unique markers for each of the 13 clones. In apple, DNA amplification finger printing was used in paternity analysis, where the parent contributing the pollen was unknown. Only 5 arbitrary primers and RFLP analysis were required to single out a paternal parent among six putative parents (Harada et al. 1993). Cornique and Marecier (1994) used RAPDs to compare and successfully identify tissue and offshoot derived plants of date palm cultivars.

The PCR strategy was used to isolate alleles in self incompatible *B. napus* introgressed from *B. campestris* and found to show high levels of homology to alleles in *B. oleracea* isolated from *B. rapa* (Goring et al. 1992). RAPDs were used to document and confirm gene introgression in apple. A marker was identified linked to a fragment from *M. floribunda* clone 821 genome introgressed into the cultivated scab

resistant apple (Durham and Korban 1994). Linked RAPDs can be used to screen germplasm for disease resistance genes and monitor their introgression into other genotypes (Miklas et al. 1993). In conjunction with near isogenic lines, RAPDs have been used to identify DNA sequences that are linked to *Pseudomonas* resistance genes in tomatoes (Martin et al. 1991) and rust resistance in beans (Haley et al. 1993, Miklas et al. 1993). The transfer of genetic material from one species to another is the basis of introducing new traits into already established cultivars.

1.4.   Pollen and female fertility

Pollen viability describes the ability of pollen to complete post pollination events and to effect fertilization (Shivana and Rangaswamy 1992). The viability of pollen is determined by both genetic and cytological factors. These factors include among them genic control, pollen sterility genes, pollen killer genes, gametocidal genes, chromosomal aberrations, non-homology of genomes, and cytoplasmic abnormalities. According to Iwanami et al. (1988), F_1 hybrids between different species produce pollen of reduced fertility because of lack of pairing during meiosis resulting from differences in chromosome homology. This lack of homology results in unbalanced or abnormal chromosome distribution.

Several stain based methods have been used to assess pollen fertility. These stains assess the contents of the pollen grain with varying degrees of specificity for different constituents. The methods leave the exine unstained but stain the pollen grain contents. There are methods for starch and other polysaccharides like callose for example Periodic acid-Schiff Procedure and Aniline blue which utilizes I+K as active ingredients. Other methods for chromatin and RNA and various defined cytoplasmic constituents utilizes acetocarmine, acetic orcein and similar stains like acridine orange (Heslop-Harrison et al. 1984). In these methods, viable pollen grains are specifically stained depending on stain used, leaving the inviable grains unstained. The presence of high viability levels in pollen would suggest a high probability of seed setting as a result of effective pollination. However, this is not always true because sometimes there are problems associated with the maternal parent (Meng and Zhou 1988).

15

1.5.   Cytology

Cytological observations in many species provide information that can be used for classification (Gomez-Campo 1980). Analysis of meiotic configurations and differential chromatin condensation at diakinesis can provide specific information on morphological features of paired or unpaired chromosomes as size differences are more pronounced at this stage (Cheng et al. 1994). Chromosome banding, although difficult due to the small size of the chromosomes has been used to characterize *Brassica* chromosomes (Wang et al. 1989, and Olin-Fatih and Heenen 1992). Therefore, chromosome behaviour at meiosis could be used as a measure of the degree of pairing between chromosomes from various species particularly the interaction between the various genomes composing the species. Since chromosome pairing is thought to occur between A and C genomes (Sacristan and Gendermann 1986), any deviation from 10 bivalents and 17 univalents at meiosis would indicate the relative stability of the cross, and the degree of pairing present between the B and C genomes.

1.6.   Agronomic Performance

1.6.1.   Yield components

The Brassica species are widely cultivated in Europe and Canada, as well as Australia, South America, and China. *B. juncea* is grown mostly on the South Asian subcontinent (Kjellstrom 1993). Breeding efforts have been directed to improving production potential as well as the stability of these crops (Prakash 1980). High seed yield in *B. napus* has most commonly been associated with the production of a large number of pods per plant or per unit area in diverse environments such as United Kingdom (Allen and Morgan 1972) and western Australia (Thurling 1974b).

Dry matter accumulation, growth rate and development area indices of different plant parts in *B. napus* have been investigated by Inanaga and Kumura (1974), Thurling (1974a), Allen and Morgan (1975), Tayo and Morgan (1975), Clarke and Simpson (1978), Kasa and Kondra (1986), Augustinussen (1987), Mendham et al. (1990), Morrison et al. (1990), and Kjellstrom (1993). Relationships between seed yield and

16

growth characteristics have been studied by Degenhardt and Kondra (1984), Thurling (1974b), Chay and Thurling (1989) and Varshney et al. (1991). Other yield components, such as number of racemes per plant, vegetative yield, harvest index and 1000 seed weight, have also been associated with seed yield (Campbell and Kondra 1978, Ramanujam and Rai 1963, Thurling 1974a, 1974b and Zuberi and Ahmed 1973). In *B. juncea* growth and yield have been analyzed by Roy et al. (1981), Chauhan and Bhargava (1984), and Bhargava and Tomar (1988). In Canada, growth and yield have been studied by Woods et al. (1991).

1.6.2. Phenotypic characteristics

Tall plants have been associated with high yields (Campbell and Kondra 1978, Thurling 1974a) and significant heterosis for harvest index (Varshney et al. 1990). Degenhardt and Kondra (1984) however, found a negative correlation between plant height and seed yield per plot, suggesting shorter plants may be more desirable than tall plants. In *B. napus*, growth prior to anthesis has been found to have a greater influence on seed yield than post anthesis growth (Thurling 1974a). In short season environments, the period between sowing and flowering is an important determinant of yield; with the time of flowering being highly heritable and under control of relatively few major genes (Thurling 1993). Flowering time appears to have a potential as a selection criteria for yield in early generations.

According to Kjellstrom (1993), *B. juncea* starts to flower earlier but with a shorter flowering period than *B. napus*. Thurling (1974b) observed that yield was significantly correlated with time to 50% anthesis. The days to flowering are important because more than 75% of the pods retained at maturity are formed from flowers opening within fourteen days of anthesis (Tayo and Morgan 1975) and were found mainly on the terminal raceme and on the basal and middle regions of the axillary inflorescence.

Pods are important as photosynthetic organs (Clarke and Simpson 1978, Chapman et al. 1984, Major et al. 1978). The contribution of assimilates from pods to rapidly developing seeds are nearly equal to the assimilates from leaves (Brar and Thies

17

1977) with leaves exerting an early effect in seed yield by influencing development of sink capacity in the number of pods and seeds (Clarke and Simpson 1978). Pod number was found to be correlated with seed yield in two of the three *B. napus* cultivars studied by Campbell and Kondra (1978). The harvest index is a relatively simple character to measure although it is a manifestation of complex photosynthetic, translocatory and storage processes associated with seed development (Thurling 1993). Harvest index is often highly correlated with grain yield (Varshney et al. 1990, Donald and Hamblin 1976, Clarke and Simpson 1978 and Thurling 1974b) and can be used as a selection criteria (Bhatt 1976, 1977, Varshney et al. 1990, Rosielle and Frey 1977) although it is suggested that this will not be effective in all situations (Donald and Hamblin 1976, Varshney et al. 1990).

1.7. Quantitative Traits

1.7.1. Oil

Rapeseed/canola contains approximately 40% oil and yields a meal containing about 38-43% protein. Hulls comprise about 16.5-18.7% of the seed weight equivalent to about 27-30% of that of the de-fatted meal (Shahidi 1990). The unique polyunsaturated fatty acid composition of canola oil differentiates it from other oils. Canola oil has a higher oleic acid (C18:1) (about 55%) and a lower linoleic acid (C18:2) (about 26%) than most of the other vegetable oils. Canola oil has a high content of linolenic acid (C18:3) (8-12%) compared to vegetable oils such as soybean, sunflower olive and corn, which have 8.0, 0.2, 0.8 and 0.7% respectively (Sheppard et al. 1978).

The low level of palmitic acid and other saturated fatty acids (< 5%) is considered nutritionally desirable but an increase of short chain fatty acids such as palmitic acids could improve the suitability of canola oil for manufacture of margarines and shortenings. The high unsaturated fatty acid content in canola, especially C18:3 influences oil stability and quality. Fresh canola oil is odourless, bland and light coloured. Its quality deteriorates in storage as a result of autoxidation. Although chemical and physical measurements are useful in determining the oxidative stability of

oils, sensory evaluation of the odour and flavour quality of vegetable oils is considered to be the ultimate method of assessing oil quality (Warner and Frankel, 1985).

A reduction in linolenate levels in canola oils can effectively retard oil autoxidation and flavour deterioration. The fatty acid content, especially the linolenate (C18:3) was genetically altered to produce low linolenate canola oils (Scarth et al. 1988). These low linolenate oils showed improved flavour quality and storage stability (Eskin et al. 1989) as a result of reduced oxidation of the oil. However, the reduction of linolenic acid in the oil might reduce the quality of the oil because it is one of the essential fatty acids.

B. juncea is characterized by the presence of high levels of erucic acid in its seed oil. Erucic acid was found to be under embryonic control (Downey and Harvey 1962) with genes present in A and B genomes (Kirk and Hurlstone 1983). In *B. napus*, erucic acid is controlled by at least two genes acting in an additive manner with embryonic control (Jack et al. 1994, Harvey and Downey 1964, Downey and Dorrell 1971, Chen et al. 1988a), with each gene located in the A and C genome (Anand and Downey 1981). In a study of a *B. carinata* and *B. napus* cross, Fernandez-Escobar et al. (1988) confirmed the presence of erucic acid controlling genes in each of the three *Brassica* genomes. Zero erucic acid and low glucosinolate *B. juncea* has been developed at Agriculture and Agri-Food Canada (Rakow 1995, Love et al. 1990b, 1991) and Australia (Kirk and Oram 1981) but unfortunately the fatty acid profiles of these lines is more unsaturated than canola cultivars of *B. napus* and *B. rapa* (Raney et al. 1995). This level of unsaturation, particularly increased linolenic acid levels, is usually associated with reduced stability (Eskin et al. 1989, Przybylski et al. 1993) producing oil of lower quality than canola.

The quality of *B. juncea* oil could be improved by introgressing the fatty acid profile from *B. napus*. Edible oil from *Brassicas* should contain no more than $30\mu m$ g^{-1} of aliphatic glucosinolates and no more than 2% erucic acid by mass (Vaisey Genser 1987).

1.7.2. Glucosinolates

Glucosinolate content is one of the major constraints associated with canola meal usage because of problems associated with long term animal feeding. More than 90 different glucosinolates are known in the genus *Brassica* (Fenwick et al. 1983, McDannel et al. 1988). Glucosinolates are sulphur containing glycosides that are found within *Brassica* and related genera. They consist of a glycone moiety and variable aglycone side chain. The major glucosinolates occurring in the six *Brassica* species in the U triangle (U 1935) are gluconapin, progoitrin and glucobrassicanapin forming the alkenyl group and 4-hydroxy-glucobrassicin and glucobrassicin forming the indole group (Kraling et al. 1990). These glucosinolates lack any sulphur containing functional group in the 3-5 carbon side chain (Gland et al. 1981). The aliphatic glucosinolates can be divided into propyl butyl and pentyl glucosinolates depending on the length of the side chain. The side chain is modified to produce alkenyl hydroxyalkenyl methylthioalkyl and methylsulphinylalkyl homologues.

The aliphatic glucosinolates give the characteristic flavour found in Brassica vegetables due to the presence of isothiocyanates. The aliphatic glucosinolate profile appears to be determined by simple genetic systems with two distinct sets of genes; one determining the side chain length and the other modifying the structure of the side chain regardless of length (Magrath et al. 1993). The alkenyl glucosinolates are controlled by at least three genes (Lein 1970). Incomplete dominance was found in 2-hydroxy 3-butenyl (Progoitrin) and 3-butenyl (Gluconapin) or overdominance in 4-pentenyl (Glucobrassicanapin) (Kondra and Stefansson 1970). The total aliphatic glucosinolate content of the seed of *B. napus* is determined by the genotype of the maternal parent rather than the genotype of the zygote (Kondra and Steffanson 1970, Love et al. 1990b) with nuclear rather than cytoplasmic gene control. Stringam and Thiagarajah (1995) suggested that 4 recessive genes are required for the expression of low alkenyl glucosinolate levels in *B. juncea*. Multiple genes (Love et al. 1990a) have also been suggested to control low glucosinolate values. In a study of F_1-derived DH lines, from a cross of low x high glucosinolate parents, 5-8 recessive alleles controlled complete absence of alkenyl glucosinolates in *B. juncea*. In the backcross, involvement of 6-9

recessive alleles was found (Stringam and Thiagarajah 1995). This implies that larger populations are required to recover the necessary alleles for low glucosinolates in backcross generations. The inheritance of glucosinolates is complex, but doubled haploidy cou. : be used in *B. juncea* to efficiently recover desirable genotypes controlled by multiple recessive genes (Stringam and Thiagarajah 1995). Of the two *B. juncea* lines identified as higher yielding than *B. napus* by Rakow (1995), the levels of alkenyl glucosinolates were lowered, but were still three times the levels observed in *B. napus* (Rakow and Raney 1995).

Environment, nutrition, (Sones et al. 1984) time of sowing, (Sang et al. 1986) and pod position on the plant (Kondra and Downey 1970) affect the level of glucosinolates in the seeds of *B. napus*. Biosynthesis of indolyl glucosinolates starts as early as the second day of germination whereas the biosynthesis of alkenyl glucosinolates starts between the fourth and sixth week of development (Bennert 1991). The level of glucosinolates in the cotyledons of *B. napus* reflect those present in the seeds suggesting lack of synthesis of glucosinolates within the cotyledons (Glen et al. 1990), however the enzyme responsible for the hydroxylation of butenyl glucosinolates was found active in the cotyledons. This enzyme may be responsible for synthesis of glucosinolate components in the cotyledons (Rossiter et al. 1990). Magrath and Mithen (1993) found that the rate of glucosinolate synthesis and degradation in the pod tissue is directly related to the amount of glucosinolates in the pod tissue. The levels of glucosinolates however decrease in the pods as the seeds develop, a factor attributed to their transport to the seed (De March et al. 1989). The level of aliphatic glucosinolates in leaves has not been reduced even in low glucosinolate varieties (Inglis et al. 1992).

Upon hydrolysis of glucosinolates by myrosinase at neutral pH, the glucose and sulphate are split off, freeing oxazolidinethione isothiocyanate which is goitrogenic (Bell 1984). Indolylglucosinolates occur in significant quantities but show little differences between low and high glucosinolate cultivars (McGregor 1978).

B. napus has a restricted and uniform aliphatic glucosinolate profile (Adams et al. 1985). It contains butenyl and pentenyl glucosinolates and their hydroxylated analogues. Low levels of methylsulphinyl pentyl glucosinolates are also found. In the seed, the major glucosinolates in *B. napus* are 2-hydroxy 3-butenyl 3-butenyl and 4-hydroxy 3-indolyl ethylglucosinolate. In the vegetative phase, most of the glucosinolates are located in the leaves with 3-butenyl glucosinolate accumulating in large quantities in the inflorescence during maturation (Clossais-Besnard and Larher 1991). The proportion of pentyl and propenyl glucosinolates in seeds was found proportional to the levels in the leaves, with the pentyls lower in the seeds than in the leaves (Magrath et al. 1993). Because of the presence of seed specific glucosinolates, it has been suggested that vegetative parts contain mainly precursors and that glucosinolate synthesis occurs in the seed (Clossais-Bernard and Larher 1991).

The evidence that meal contains goitrogens was first noted in rats (Kennedy and Purves 1941), then swine and poultry (Daun and McGregor 1991, Bell and Belzile 1965) and in growing bulls (Iwarsson et al. 1973). The problem results from hydroxyalkenyls which affect iodine metabolism thereby resulting in depressed growth, hemorrhaging liver related to the presence of epithionitriles and skeletal abnormalities in poultry. Aliphatic glucosinolates reduce meal palatability (Magrath et al. 1993).

Glucosinolate amounts do not appear to be directly involved in resistance to *L. maculans* but by the concentration on a fresh weight basis (Van den Berg and Rimmer 1991). The activity of glucosinolates depend on the type present in the leaves, for example 2- hydroxy 3-butenyl glucosinolate does not inhibit mycelial growth whereas indoles have an ED50 of 139 nmol ml^{-1} (Mithen et al. 1986). Glucosinolates may be involved in defense reactions against vertebrate and invertebrate pests (Mithen and Magrath 1992). Glucosinolates are thought to be rapidly metabolized to sustain plant growth and catabolized during the reproductive phase especially during flowering (Clossais-Bernard and Larher 1991).

1.7.3. Water stress tolerance

In *B. napus*, yield components such as pods per plant and seeds per pod are all significantly reduced by drought (Richards and Thurling 1978), with plants being most sensitive at flowering. Drought tolerant plants can use several mechanisms to adapt to water stress including reduction in water loss by increased water uptake, increased stomatal resistance and development of large or deep root systems (Parsons and Howe 1984). This adaptation is necessary because maintenance of growth and function depends on maintaining a relatively high water content in the protoplasm (Good and Maclagan 1993). Osmotic adjustment under drought has received considerable attention (Blum 1989, Cortes and Sinclair 1986, Cutler et al. 1977, Levitt 1985). Cell membranes are one of the first targets of plant stresses. Cell membrane stability plays a critical role in drought tolerance (Bewley 1979). Mechanisms that tend to promote drought tolerance by maintaining turgor include osmotic adjustment, an increase in cell wall elasticity or decrease in cell size (Turner 1979). The loss of membrane integrity after water stress results in an increase in the cell permeability This leads to an increase in ion efflux (El Hafid et al. 1989). The degree of solute leakage varies according to species and appear correlated with drought sensitivity of the species (Vasquez et al. 1990) . Krishnamani et al. (1984) observed that when leaf discs of soybean cultivars were dried under fluorescent light cultivar differences were found in leaf solute leakage.

The rate of injury to cell membranes by drought may be estimated through measurement of electrolyte leakage from the cells (Blum and Ebercon 1981). The osmoticum polyethylene glycol (PEG) causes injury to plant tissues and can be used to induce dehydration in tissues simulating water stress (Blum and Ebercon 1981) Evaluation of drought tolerance using this method in wheat and sorghum was found to be comparatively efficient in partitioning between cultivars (Blum and Ebercon 1981).

1.8.   Seed Colour

The seed colour in *Brassicas* is thought to arise from inability of seed colour gene(s) to achieve normal black seed pigmentation. Brown seeds arise as a result of

partial inability of seed colour gene(s) to synthesize the seed coat pigment (Chen et al. 1988b). Several authors have reported single gene controlling seed colour in *B. rapa* (Hawk 1982, Chauhan et al. 1995) and *B. carinata* (Getinet et al. 1987), two genes in *B. juncea* (Vera et al. 1979, Vera and Woods 1982) and *B. rapa* (Stringam 1980), three genes in oleiferous *Brassica* (Mohammad et al. 1942) and in *B. napus* (Shirzadegan 1986) and as many as five genes in *B. rapa* (Schwetka 1982). Genes controlling black or brown colour being either dominant, partially dominant or epistatic to the genes for yellow colour in *Brassica*. Yellow seeds are obtained only when the loci are in the homozygous recessive condition, with any other combination resulting in either brown or black seeds. Getinet et al. (1987) found all F_1 seed obtained from a *B. carinata* cross identical suggesting that seed colour is under the control of nuclear rather than cytoplasmic genes. In amphidiploid species, the individual genomes can function independently of each other to achieve a specific seed colouration, but intergenomic complementation is possible as a result of existing homology between the *Brassica* genomes (Chen et al. 1988b). The genes for the yellow colour are thought to be in the A and C genomes Chen et al. 1988b, Rashid et al. 1994). Presence of other factors has however been shown to play a role in seed colour expression. Stringam (1980) and Hawk (1982) reported difficulties in separation of phenotypic classes a prblem attributed to modifier genes. Shirzadegan (1986) observed differences between reciprocals of segregating yellow and black seeded forms of *B. napus*, a factor he attributed to cytoplasmic effects on seed colour.

Johnsson and Bengtsson (1970) reported that yellow seeded *B. rapa* had a lower percent seed coat (hull) than brown seeded types. Yellow seeds obtained from segregating *B. rapa* lines examined by Stringam et al. (1974) were found to contain less crude fibre, more protein and more oil than brown hulls. Bell and Shires (1982) and Abraham and Kotwal (1995) confirmed a lower fibre content in yellow seeds than in brown seeds. However, while the yellow seed coats had lower fibre contents, the embryos in these seeds had higher fibre content than brown seeds. These differences were also noted by Slominski and Campbell (1990). Yellow hulls were more digestible by pigs than brown hulls, while corresponding crude protein was more digestible in the

brown seed hulls, a factor attributed to higher crude protein, and low lignin and fibre in yellow seeded hulls (Bell and Shire 1982). Oil free meal from yellow seed coat contains more non-starch polysaccharides, more cellulose and slightly soluble and structural polysaccharides (Slominski and Campbell 1990). Yellow meal also contained more non starch neutral detergent matter but only one half as much lignin and polyphenols as brown meal (Bell 1993). The yellow seeded form of *B. juncea* produces more oil, a meal of low fibre and highly desirable light colour as compared with other *Brassica* species (Weiss 1983). The yellow seed trait should improve the quality of oil obtained from brown seeded canola quality *Brassicas* like *B. napus*. The protein content in *B. juncea* is higher than in canola although the difference is not significant (Woods et al 1991). This is an important quality because the cake would be desirable for animal feed industry.

1.9.   Disease resistance

1.9.1.   Blackleg Disease

Blackleg disease is a world wide problem of oleiferous *B. napus* and other cruciferous crops (Bokor et al. 1975). It has been reported in Australia (Mcgee 1977) and Europe (Gugel and Petrie 1992). The pathogen has been reported in 49 countries and on all continents except Antarctica (Punithalingam and Holliday 1972). In Germany, the introduction of low erucic and glucosinolate *B. napus* and *B. rapa* varieties have resulted in more interest in the disease because of its increased incidence (Kruger 1982, 1983). In Canada, the fungus is found in almost all areas where *B. napus* or *B. rapa* are grown. In Alberta, the disease is largely confined to areas east and south of Edmonton (Evans et al. 1992), and was absent from the Peace river region of Alberta and British Columbia (MacDonald 1992, Harrison and Loland 1991) until 1992 when virulent strains started appearing in these regions (Evans et al. 1993, cited by Lange 1993). The causal agent of blackleg, *Leptosphaeria maculans* (Desm.) Ces et de Not. is a fungus of the family ascomycetes, belonging to the order Pleosporales. Its anamorph is *Phoma lingam* (Tode ex Fr.) Desm., a deutromycetes of the order Sphaeropsidales (Agrios 1988). *Phoma lingam* was first described by Tode in 1871 as

25

a saprophyte of cabbage and was named *Sphaeria lingam* (Pound 1947). The disease is a serious yield limiting factor in all canola growing provinces (Petrie 1986, Van den Berg and Platford 1991, Gugel and Petrie 1992).

Leptosphaeria maculans infection symptoms vary from damping off and small leaf spots during the early phase of infection to severe stem cankers referred to as 'blackleg' during later stages of the disease (Gabrielson 1983). The leaf infection is characterized initially by biotrophic growth with completely necrophytic growth in later stages (Hammond et al. 1985). Resistance is characterized by the formation of a restrictive dark hypersensitive reaction around the wound observed ten days after inoculation (Ferreira et al. 1993). The *B. juncea* type of resistance to *L. maculans* is important from an agronomic point of view because it can be transferred through interspecific crossing to *B. napus* (Roy 1978, 1984). The hypersensitive type of resistance is associated with the B genome, and is thought to be conditioned by two nuclear genes with dominant recessive epistatic action (Rimmer and Van der Berg 1992). This type of resistance has been observed in *B. juncea, B. nigra* and *B. carinata* (Sjodin and Glimelius 1988).

Some resistance genes are thought to be located on the C genome (Sacristan and Gerdemann 1986) as different reactions are obtained in crosses between *B. napus* with either *B. juncea* or *B. carinata*. Several models of inheritance of blackleg resistance has been proposed, ranging from monogenic dominant (Stringam et al. 1992) to polygenic systems (Cargeeg and Thurling 1980) in the adult stage. In the seedling stage resistance is thought to be conditioned by a single dominant gene (Stringam et al. 1992). A large emphasis has been placed on the incorporation of blackleg resistance into *B. napus* since seed treatment is not an effective disease control in areas where *L. maculans* is endemic and foliar fungicides are not available (Lange 1993).

1.9.2. Blackspot Disease

Black spot disease is caused by *Alternaria brassicae* (Berk.) Sacc. and *Alternaria raphani* Goves and Skolko (Degenhardt et al. 1974). The fungi attack leaves, stems inflorescence and pods. They produce prominent spots or lesions that

may be entirely brown, black or greyish-white (Degenhardt et al. 1974, Conn and Tewari 1989). The fungus produces a phytotoxin called destruxin which produces the symptoms observed on the sensitive species' leaves and pods (Bains et al. 1993). The pathogen infects all aerial plant parts, reduces the photosynthetic area and accelerates senescence and defoliation (Bains and Tewari 1987). Early attack on pods results in small wrinkled seeds while attacks at later stages causes premature ripening accompanied by pod shattering (Grontoft and O'Connor 1990). Early maturing varieties are more severely affected than late varieties but disease severity at the same stage of ripening is similar in all varieties within a species (Grontoft and O'Connor 1990). Among the *Brassica* species studied by Rai and Kumar (1995), *B. carinata* was the least susceptible, followed by *B. napus, B. nigra, B. oleracea, B. juncea* and *B. rapa*. In a study on pollen selection for *A. brassicae* resistance between *B. napus, B. nigra, B. juncea* and *B. rapa*, a similar trend was found between the species. *B. napus'* pollen was the most insensitive and *B. nigra* the most sensitive (Shivanna and Sawhney 1993).

Crop losses as a result of infection by *Alternaria* species results in 5-30% yield loss in western Canada (Conn et al. 1990, Tewari 1991). Artificially inoculated field crops resulted in yield losses of as high as 63% in *B. rapa* cv Span and up to 42% in *B. napus* cv Zephyr (Degenhardt et al. 1974). In a survey conducted in central Alberta, 46% of the fields growing *B. rapa* and 26% growing *B. napus* had *Alternaria* black spot (Conn and Tewari 1993). In a survey in Europe reported by Kennedy and Graham (1995) heavy infestations resulted in yield losses of as high as 50%. Infection by the fungus not only reduces yield, but also results in a reduction in seed oil content and meal protein content in *B. napus* and *B. rapa* varieties (Degenhardt et al. 1974). The severity of the disease is increased by applying excess amount of nitrogen and phosphorus. The application of potassium reduced the disease severity by inhibiting conidial germination (Sharma and Kolte 1994).

Sources of resistance to black spot include wild and allied species of *Brassica* such as *Camelina sativa* and *Sinapis alba* (Shivanna and Sawhney 1993). In crosses in which one parent was resistant and the other highly susceptible like *B. carinata* x *B.*

27

juncea, the F_1 plants were resistant while the cross between *B. juncea* x *B. napus* resulted in F_1 plants of moderate resistance (Rai and Kumar 1995) showing the possibility of transferring the resistance to the fungus to the susceptible interspecific cross plants.

1.2. Objectives

The overall objective of this Thesis was to study introgression of traits from *B. napus* to *B. juncea*,and determine their agronomic performance and stability in a new genetic background.

References

Abraham A. and S.A. Kotwal 1995. Yellow seeded Trombay mustard varieties with increased oil and protein content. Cruciferae Newsletter No. 17 pp58-59.

Adams H., J.G. Vaughan and G.R. Fenwick 1985. The limited chemotaxonomic value of glucosinolates for cultivar identification in *Brassica napus* L. var. oleifera (Metzg.). Z. Pflanzenzuchtg 95: 97-102.

Adams H., J.G. Vaughan and G.R. Fenwick 1989. The use of glucosinolates for cultivar identification in Swede, *Brassica napus* L. var napobrassica (L.) oleifera (Metzg.). Z. Pflanzenzuchtg 95: 97-102.

Agrios G.N. 1988. Plant Pathology. 3rd Edition, Academic Press, San Diego pp271-272.

Aitken S.A., N.A. Tinker, D.E. Mather and M.G. Fortin 1994. A method for detecting DNA polymorphism in large populations. Genome 37: 506-508.

Alberta Agriculture Project 83-0093 1987. Farming for the future, Acceleration of canola variety development for the Peace river and southern Alberta regions p.56-62.

Allen E.J. and D.G. Morgan 1972. A quantitative analysis of the effects of nitrogen on the growth, development and yield of oilseed rape. J. Agric. Sci. Camb 78: 315-324.

Allen E.J. and D.G. Morgan 1975. A quantitative comparison of the growth, development and yield of different varieties of oilseed rape. J. Agric. Sci Camb 85: 159-174.

Anand I.J. and R.K. Downey 1981. A study of erucic acid alleles in digenomic rapeseed (*Brassica napus* L.). Can. J. Plant Sci. 61: 199-203.

Attia T. and G. Robbelen 1986. Cytogenetic relationship within cultivated *Brassica* analyzed in amphihaploids from the three diploid ancestors. Can. J. Genet Cytol. 323-329.

Attia T., C. Busso and G. Robbelen 1987. Digenomic triploids for an assessment of chromosome relationships in the cultivated diploid *Brassica* species. Genome 29: 326-330.

Augustinussen E. 1987. The influence of nitrogen fertilizing on growth and development and yield of different varieties of oilseed rape. Tidskr. Planeavl. 91: 33-44.

Ayotte R., P.M. Harney and V. Souza Machado 1987. The transfer of triazine resistance from *Brassica napus* L. to *B. oleracea* L. I. Production of F₁ hybrids through embryo rescue. Euphytica 36: 615-624.

Ayotte R., P.M. Harney and V. Souza Machado 1988. The transfer of triazine resistance from *Brassica napus* L. to *B. oleracea* L. II. Morphology, fertility and cytology of the F₁ hybrid. Euphytica 37: 189-197.

Bains P.S., J.P. Tewari and W.A. Ayer 1993. A note on phytotoxicity of homodestruxin B- a compound produced by *Alternaria brassicae*. Phytoprotection 74: 157-160.

Bains P.S. and J.P. Tewari 1987. Purification, chemical characterization and host specificity of the toxin produced by *Alternaria brassicae*. Physiological and Molecular Plant Pathology 30: 259-271.

Baird E., S. Cooper-Bland, R. Waugh, M. DeMaine and W. Powell 1992. Molecular characterization of inter and intra-specific somatic hybrids of potato using randomly amplified polymorphic DNA (RAPD) markers. Mol Gen Genet 233: 469-475.

Banga S.S. 1988. C-genome chromosome substitution lines in *B. juncea* (L.) Coss. Genetica 77: 81-84.

Barker T.A., T.A. Vuori, M.R. Hegedus and D.G. Myers 1992. The use of ray parameters for the discrimination of Australian wheat varieties. Pl Var and Seeds 5: 35-45.

Bell J.M. 1984. Nutrients and Toxicants in rapeseed meal: A review. Journal of Animal Science, 58(4): 996-1010.

Bell J.M. 1993. Factors affecting the Nutritional value of canola meal: A review. Can. J. Anim. Sci. 73: 679-697.

Bell J.M. and A. Shires 1982. Composition and digestibility by pigs of hull fractions from rapeseed cultivars with yellow or brown seed coats. Can. J. Anim. Sci. 62: 557-565.

Bell J.M. and R. Belzile 1965. Goitrogenic properties: Rapeseed meal for livestock and poultry - A review, Canada Department of Agriculture Publication No. 1257 Ottawa Ontario.p45-60.

Bennert H. 1991. Variation in glucosinolate content and glucosinolate pattern in the development of rape. In: Changes in levels of glucosinolates in leaves of oilseed rape (*Brassica napus*) during growth, Rapeseed in a changing world: Proceedings Vol. 6: 1702-1707.

Bewley J. D 1979. Physiological aspects of desiccation tolerance. Ann. Rev. Plant physiol. 30: 195-238.

Bhargava S.C. and D.P.S. Tomar 1988. Physiological contribution for increased yields in rapeseed-mustard. Proc. int. Cong. Plant Physiology. Feb 15-20. New Delhi, India. p352-360.

Bhatt G.M. 1976. Variation of harvest index in several wheat crosses. Euphytica 25: 41-50.

Bhatt G.M. 1977. Response to two way selection for harvest index in two wheat (*Triticum aestivum* L.) crosses. Aust J. Agric Res 28: 29-36.

Bijral J.S., T.R. Sharma B.B. Gupta and K. Singh 1995. Interspecific hybrids of *Brassica maurorum* with *Brassica* crops and their cytology. Cruciferae Newsletter No. 17: 18-19.

Blum A. 1989. Osmotic adjustment and growth of barley genotypes under drought stress. Crop Sci. 26:993-998.

Blum A. and A. Ebercon 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat, Crop Sc. 21: 43-47.

Bokor A., M.J. Barbetti, A.G.P. Brown, G.C. MacNish and P.M.C.R. Wood 1975. Blackleg of rapeseed. J. Agric. West. Austr. 16: 7-10.

Brar G. and W. Thies 1977. Contribution of leaves, stem, siliqua and seeds to dry matter accumulation in ripening seeds of rapeseed (*Brassica napus* L.). Z. Pflanzenphysiol 82: 1-13.

Busso C., T. Attia and G. Robbelen 1987. Trigenomic combinations for the analysis of meiotic control in the cultivated *Brassica* species. Genome 29: 331-333.

Campbell D.C. and Z.P. Kondra 1978. Relationships among growth patterns, yield components and yield of rapeseed. Can. J. Plant Sci. 58: 87-93.

Canada Grains Council 1993. Canadian grain industry statistical handbook, Winnipeg Manitoba. pp53.

Canola Council of Canada 1979. Canola. Canada's rapeseed crop. Publication no. 56 p 9.

Canola Council of Canada 1982. Canola. Canada's rapeseed crop, Publication no. 60 p.5-9.

Canola Council of Canada 1987. Canola oil. Properties and performance, Publication no.60 p.5.

Canola Council of Canada 1992. Canola growers manual pp 201-211.

Canola Council of Canada 1995a. Canada's Canola. Winnipeg Manitoba.

Canola Council of Canada 1995b. Canola Council's policy positions. Canola digest. Vol. 29(6): 2.

Cargeeg L.A. and N. Thurling 1980. Contributions of host pathogen interactions to the expression of blackleg disease in of spring seed rape (*Brassica napus* L.)

31

caused by *Leptosphaeria maculans* (Desm.) Ces. et de Not Euphytica 29: 465-476.

Chapman J.F., R.W. Daniels and D.H. Scarisbrick 1984. Field studies on ^{14}C assimilate fixation and movement in oilseed rape (*Brassica napus*). J. Agric. Sci Camb 102: 23-31.

Chauhan Y.S. and S.C. Bhargava 1984. Physiological analysis of growth and yield variation of rapeseed and mustard. J. Agric. Sci. Camb 103: 249-252.

Chauhan Y.S., K. Kumar, R. Bhajan and K.N. Maurya 1995. Inheritance of seed coat colour and its use in determining natural cross pollination in yellow sarson. Cruciferae Newsletter No. 17 pp60-61.

Chay P. and N. Thurling 1989. Variation in pod length in spring rape (*Brassica napus*) and its effect on seed yield and yield components. J. Agric. Sci. Camb 113: 139-149.

Chen B.Y., V. Simonsen, C. Lanner-Herrera and W.K. Heneen 1992. A *Brassica campestris-alboglabra* addition line and its use for gene transfer and generation of trisomics. Theor Appl Genet 84: 592-599.

Chen B.Y., W.K. Heneen and R. Johnsson 1988a. Independent inheritance of erucic acid content and flower colour in the C-Genome of *Brassica napus* L. Plant Breeding 100: 147-149.

Chen B.Y., W.K. Heneen and R. Johnsson 1988b. Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breeding 101(1): 52-59.

Cheng B.F., W.K. Heneen and B.Y. Chen 1994. Meiotic studies on a *Brassica campestris-alboglabra* monosomic addition line and derived *B. campestris* primary trisomics. Genome 37: 584-589.

Clarke J.M. and G.M. Simpson 1978. Growth analysis of *Brassica napus* cv. Tower. Can. J. Plant Sci. 58: 587-595.

Clossais-Besnard N. and F. Larher 1991. Glucosinolates accumulation pattern during the development of single low and double low oilseed rape (*Brassica napus* L.)

In: Changes in levels of glucosinolates in leaves of oilseed rape (*Brassica napus*) during growth, Rapeseed in a changing world: Proceedings Vol. 6: 1714-1717.

Conn K.L. and J.P. Tewari 1989. Interactions of *Alternaria brassicae* conidia with leaf epicuticular wax of canola. Mycological Rasearch 93(2): 240-242.

Conn K.L., J.P. Tewari and R.P. Awasthi 1990. A disease assessment key for Alternaria black spot in rapeseed and mustard. Canadian Plant Disease Survey 70(1): 19-22.

Conn K.L. and J.P. Tewari 1993. Survey of *Alternaria* blackspot and sclerotinia stem rot of canola in central Alberta in 1992. Canadian Plant Disease Survey 73(1): 83-85.

Corniquel B. and L. Mercier 1994. Date palm (Phoenix dactylifera L.) cultivar identification by RFLP and RAPD. Plant Science 101: 163-172.

Cortes P.M. and T.R. Sinclair 1986. Water relations of field grown soybeans under drought Crop Sci. 26: 993-998.

Cutler J.M., D.W. Rains and R.S. Loomis 1977. The importance of cell size in the water relations of plants. Physiologia Plantarum 40: 255-260.

Daun J.K. and D.I. McGregor 1991. Glucosinolates in seeds and residues In: Analysis of oilseeds, fats and fatty foods Ed. J.B. Rossell and J.L.R. Pritchard, Elsevier Science Publishers Ltd. p185-211.

De March G., D.I. McGregor and G. Seguin-Shwartz 1989. Glucosinolate content of maturing pods and seeds of high and low glucosinolate summer rape. Can. J. Plant Sci. 69: 929-932.

Degenhardt D.F. and Z.P. Kondra 1984. Relationships between seed yield and growth characters, yield components, seed quality of summer-type oilseed rape (*Brassica napus* L.). Euphytica 33: 885-889.

Degenhardt K.J., W.P. Skoropad and Z.P. Kondra 1974. Effects of *Alternaria* blackspot on yield, oil content and protein content of rapeseed. Can. J. Plant Sci. 54: 795-799.

33

Demeke T., R.P. Adams and R. Chibbar 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. Theor. Appl. Genet. 84: 990-994.

Donald C.M. and J. Hamblin 1976. The biological yield and harvest index of cereals as agronomic and plant breeding criteria. Advances in agronomy 28: 361-405.

dos Santos J.B., J. Nienhuis, P. Skroch, J. Tivang M.K. Slocum 1994. Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theor Appl Genet 87: 909-915.

Downey R.K. and B.L. Harvey 1962. Methods of breeding for oil quality in rape. Can. J. Plant Sci 43: 271-275.

Downey R.K. and D.G. Dorrell 1971. Genetic control of fatty acid composition in oilseed crops. Proc. Flax Inst. Nov. 1971. ed. Fargo N.D.

Downey R.K. and G. Robbelen 1989. *Brassica* species In: Oilcrops of the world. Their Breeding and Utilization. McGraw Hill. pp 339-362.

Durham R.E. and S.S. Korban 1994. Evidence of gene introgression in apple using RAPD markers. Euphytica 79: 109-114.

Edwards M. 1992. Use of molecular markers in the evaluation and introgression of genetic diversity for quantitative traits. Field crops Research 29: 241-260.

El Hafid L., A.T. P. Thi, Y. Zuily-Fodil and J.B. Vieira da Silva 1989. Enzymatic breakdown of polar lipid in cotton leaves under water stress. I Degradation of monogalactosyl-diacylglycerol, Plant Physiology and Biochemistry 27: 495-502.

Eskin N.A.M., M. Vaisey-Genser, S. Durance-Todd and R. Przybylski 1989. Stability of low linolenic acid canola oil to frying temperatures. Journal of American Oil Chemists' Society 66: 1081-1084.

Evans I.R., P.D. Kharbanda, L. Harrison and D. Kaminsky 1992. Blackleg of canola survey in Alberta 1991. Can. Plant Dis. Surv. 72:73.

Fenwick G.R., R.K. Heaney and W.J. Mullin 1983. Glucosinolates and their breakdown products in food plants. CRC Critical Rev. Food Sci. Nutr. 18: 123-   201.

Fernandez-Escobar J., J. Dominguez, A. Martin and J.M. Fernandez-Martinez 1988.
Genetics of the erucic acid content in interspecific hybrids of Ethiopian Mustard
(*Brassica carinata* Braun) and Rapeseed (*B. napus* L.). Plant Breeding 100:
310-315.

Ferreira M.E., J.S. Dias, A. Mengistu and P.H. Williams 1993. Screening of
Portuguese cole landraces (*Brassica oleracea* L.) with *Leptosphaeria maculans*
and *Xanthomonas campestris* pv. campestris. Euphytica 65: 219-227.

Forster B. P. and J. E. Dale 1983. Effects of parental embryo and endosperm mitotic cycle
times on development of hybrids between barley and rye. Ann. Bot. 52: 613-620.

Fritsch P., M.A. Hanson, C.D. Spore, P.E. Pack and L.H. Rieseberg 1993. Constancy
of RAPD Primer Amplification Strength among distantly relaxed Taxa of
flowering plants. Plant Molecular Biology Reporter 11(1): 10-20.

Gabrielson R.L. 1983. Blackleg disease of crucifers caused by *Leptosphaeria*
maculans (*Phoma lingam*) and its control. Seed Science and Technology. 11:
749-780.

Getinet A., G. Rakow and R.K. Downey 1987. Seed colour inheritance in *Brassica*
carinata A. Braun, Cultivar S-67. Plant breeding 99: 80-82.

Gland A., G. Robbelen and W. Thies 1981. Variation of Alkenyl glucosinolates in seed
of *Brassica* species. Z. Pflanzenzuchtg 87: 96-110.

Glen D.M., H. Jones and J.K. Fieldsend 1990. Damage to oilseed rape seedlings by the
field slug *Deroceros reticulutum* in relation to glucosinolate concentration.
Ann. Appl. Biol. 117: 197-207.

Gomez-Campo C. 1980. Morphology and morpho-taxonomy of the tribe *Brassiceae*.
In S. Tsunoda, K. Hinata and C. Gomez-Campo (Eds.), pp 3-31, *Brassica*
crops and wild allies: Biology and Breeding. Japan Sci. Soc. Press, Tokyo.

Good A.G. and J.L. Maclagan 1993. Effects of drought stress on the water relations in
Brassica species. Can. J. Plant Sci. 73: 525-529.

Goring D.R., P. Banks, W.D. Beversdorf and S.J. Rothstein 1992. Use of the
polymerase chain reaction to isolate an S-locus glycoprotein cDNA

introgressed from *Brassica campestris* into *B. napus* ssp. oleifera. Mol Gen Genet 234: 185-192.

Grontoft M. and D. O'Connor 1990. Green house method for testing of resistance of young Brassica plants to *Alternaria brassicae*. Plant Breeding 105: 160-164.

Gugel R.K. and G.A. Petrie 1992. History , occurrence, impact and control of blackleg of rapeseed. Can. J. Plant Pathol. 14: 36-45.

Gupta S.B. and P. Gupta 1973. Selective somatic elimination of *Nicotiana glutinosa* chromosomes in the F_1 hybrids of *N. suaveolens* and *N. glutinosa*. Genetics 73: 605-612.

Haley S.D., P.N. Miklas, J.R. Stavely, J. Byrum, and J.D. Kelly 1993. Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor Appl Genet 86: 505-512.

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

Halligan E.A., M.B. Forde and I.J. Warrington 1991. Discrimination of rye grasses by heading date under various combinations of vernalization and day length. Pl. Var. and seeds 4: 115-123.

Hammond K.E., B.G. Lewis and T.M. Musa 1985. A systemic pathway in the infection of oilseed rape plants by *Leptosphaeria maculans*. Plant Pathol. 34: 557-565.

Harada T., K. Matsukawa, T. Sato, R. Ishikawa, M. Niizeki, K. Saito 1993. DNA-RAPDs detect genetic variation and paternity in *Malus*. Euphytica 65: 87-91.

Harrison L.M. and J. Loland 1991. Canola disease survey in the Peace river region in 1990. Can. Plant Dis. Surv. 71:100.

Harvey B.L. and R.K. Downey 1964. The inheritance of erucic acid content in rapeseed (*Brassica napus*). Can. J. Plant Sci. 44: 104-111.

Hawk J.A. 1982. Single gene control of seed colour and hypocotyl colour in turnip rape. Can. J. Plant Sci. 62: 331-334.

Heaney R.K. and G.R. Fenwick 1980. The glucosinolate content of *Brassica* vegetables. A Chemotaxonomic Approach to cultivar identification. J Sci Food Agric 31: 794-801.

Heslop-Harrison J., Y. Heslop-Harrison and R.K. Shivana 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. Theor Appl Genet 67: 367-375.

Heyn F.W. 1977. Analysis of unreduced gametes in the *Brassiceae* by crosses between species and ploidy levels. Z. Pflanzenzuchtg 78: 13-30.

Hu J. and C.F. Quiros 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Reports 10: 505-511.

Inanaga S. and A. Kumura 1974. Studies on matter production of rape plant (*Brassica napus* L.) I. Changes with growth in rates of photosynthesis and respiration of rape plant population. Proc. Crop Sci. Soc. Japan 43(2): 261-266.

Inglis I.R., J.T. Wadsworth, A.N. Meyer and C.J. Feare 1992. Vertebrate damage to OO and O varieties of oilseed rape in relation to SMCO and glucosinolate concentration in the leaves. Crop Protection 11: 64-68.

Islam A.K.R., K.W. Shepherd and D.H.B. Sparrow 1981. Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46: 161-174.

Iwanami Y., T. Sasakuma and Y. Yamada 1988. *In* Pollen: Illustrations and scanning electromicrographs. Springer- Verlag New York.

Iwarsson K., L. Ekman, B.R. Everitt, H. Figueiras and P.O. Nilsson. 1973. The effect of feeding rapeseed meal on thyroid function and morphology in growing bulls. Acta Vet. Scand. 14:1

Jack B., A.P. Brown, J.B. Davis and D. Erickson 1994. Selection for fatty acid composition of *Brassica napus* using microspore culture. Cruciferae Newsletter 16: 102-103.

James J. 1978. New maize x Tripsum hybrids for maize improvements, Euphytica 28: 239-247.

Johnsson R. and L. Bengtsson 1970. Yellow-seeded rape and turnip rape. I. Influence of breeding for yellow seeds upon yield quality properties. Sver. Utsaedesfoeren. Tidskr. 2-3: 149-155.

Johnstone T.D. 1974. Transfer of disease resistance from *B. campestris* L. to rape (*Brassica napus* L.). Euphytica 23: 681-683.

Kasa G.R. and Z.P. Kondra 1986. Growth analysis of spring-type oilseed rape. Field Crops Res. 14: 361-370.

Kennedy R. and A.M. Graham 1995. Infection oilseed rape by *Alternaria brassicae* under varying conditions of temperature and wetness. Rapeseed today and tomorrow. Proceedings: Vol. 2: 601-603. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Kennedy T.H. and H.D. Purves 1941. Studies on experimental goitre. I. The effects of *Brassica* seed diets on rats. Brit. J. Exp. Pathol. 22: 241.

Khush G.S. 1973. Cytogenetics of aneuploids. Academic press New York pp300.

Kianian S.F. and C.F. Quiros 1992. Trait inheritance fertility and genomic relationships of some n=9 *Brassica* species. Genetic Resources and crop Evolution 39: 165-175.

Kirk J.T.O. and C.J. Hurlstone 1983. Variation and inheritance of erucic acid content in *Brassica juncea*. Z. Pflanzenzuchtg. 90: 331-338.

Kirk J.T.O. and R.N. Oram 1981. Isolation of erucic acid free lines of *Brassica juncea*: Indian mustard now a potential oilseed in Australia. The journal of the Australian Institute of Agricultural Science 47: 51-52.

Kjellstrom C. 1993. Comparative growth analysis of *Brassica juncea* under Swedish conditions. Can. J. Plant Sci. 73: 795-801.

Knott D. R. 1978. Transfer of genes for rust resistance to wheat from related species. Proceedings: 5th International wheat genetics symposium, New Delhi p.358-364.

Kondra Z.P. and B.R. Stefansson 1970. Inheritance of the major glucosinolates of rapeseed (*Brassica napus* L.) meal. Can. J. Plant Sci. 50: 643-647.

Kondra Z.P. and R.K. Downey 1970. The glucosinolate content of rapeseed (*Brassica napus* L. and *B. campestris* L.) meal as influenced by pod position on the plant. Crop Sci. 10: 54-56.

Kraling K., G. Robbelen, W. Thies, M. Hermann and M.R. Ahmadi 1990. Variation of seed glucosinolates in lines of *Brassica napus*. Plant Breeding 105: 33-39.

Krishnamani M.R.S., J.H. Yopp and O. Myers 1984. Leaf Solute leakage as a drought tolerance indicator in soybean, Phyton 44: 43-49.

Kruger W. 1982. Die Wurzelhals und Stengelfaule des Rapses, verursacht durch *Phoma lingam* (Stat. gen *Leptosphaeria maculans*), eine schwer bekampfbare Krankheight. Zeitschrift fur Planzenkrankheiten and Pflanzenschutz (Journal of Plant Diseases and Protection) 89: 489-507.

Kruger W. 1983. Bekampfung von Rapskrankheiten. I. Verringerung des Befalls mit *Phoma lingam* bei Raps und Methoden zue Bestimmung der Anfalligkeit von Sorten. Phytopathol. Z. 108: 106-113 (Summary in English).

Lammerink J. 1970. Interspecific transfer of club root resistance from *Brassica campestris* L. to *B. napus* L. New Zeal J. Agric Res 13: 105-110.

Lange R.M. 1993. Strain variability of *Leptosphaeria maculans*. Msc. Thesis, University of Alberta Canada.

Lange W., H. Toxopeus, J.H. Lubberts, O. Dolstra and J. L. Harrewijn 1989. The development of Raparadish (x Brassicaraphanus, 2n=38), a new crop in agriculture. Euphytica 40: 1-14.

Lein K.A. 1970. Qualitative Bestimmungs-methoden fur Samenglucosinolate in Brassica - Arten Und ihre Anwendung in der Zuchtung von glucosinolatarmem Raps. Z. Pflanzenzuchtg 63:137-145.

Levitt J. 1985. Relationship of dehydration rate to drought avoidance, dehydration tolerance and dehydration avoidance of cabbage leaves, and to their acclimation during drought -induced water stress. Plant, Cell and Environment 8:287-296.

Love H.K., G Rakow, J.P. Raney and R.K. Downey 1990a. Genetic control of 2-Propenyl and 3-Butenyl glucosinolate synthesis in mustard. Can. J. Plant Sci. 70: 425-429.

Love H.K., G. Rakow, J.P. Raney and R.K. Downey 1990b. Development of low glucosinolate mustard. Can. J. Plant Sci. 70: 419-424.

Love H.K., G. Rakow, J.P. Raney and R.K. Downey 1991. Breeding improvements towards canola quality *Brassica juncea*. Proceedings of the Eighth International Rapeseed Congress, Saskatoon 1: 164-169.

McGrath J.M. and C.F. Quiros 1990. Generation of alien chromosome addition lines from synthetic *Brassica napus*: morphology cytology fertility and chromosome transmission. Genome 33: 374-383.

MacDonald L.S. 1992. 1991 Canola disease survey in British Columbia. Can Plant Dis. Surv. 72: 74.

Magrath R. and R. Mithen 1993. Maternal effects on the expression of individual aliphatic glucosinolates in seeds and seedlings of *Brassica napus*. Plant Breeding 111: 249-252.

Magrath R., C. Herron, A. Giamoustaris and R. Mithen 1993. The inheritance of aliphatic glucosinolates in *Brassica napus*. Plant Breeding 111: 55-72.

Mailer R.J., R. Scarth and B. Fritensky 1994. Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theor Appl Genet 87: 697-704.

Major D.J., J.B. Bole and W.A. Charnetski 1978. $^{14}CO_2$ assimilation by stems, leaves and pods of rape plants. Can. J. Plant Sci. 58: 783-787.

Martin G.B., J.G.K. Williams and S.D. Tanksley 1991. Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenenic lines. Proc. Natl. Acad. Sci USA 88: 2336-2340.

McDanell R., A.E.M. McLean, A.B. Hanley, R.K. Heaney and G.R. Fenwick 1988. Chemical and biological properties of indole glucosinolates (Glucobrassicins): A Review. FdChem. Toxic 26(1): 59-70.

McGee D.C. 1977. Blackleg (*Leptosphaeria maculans* (Desm.) Ces. et de Not.) of rapeseed in Victoria: Sources of infection and relationships between inoculum , environmental factors and disease severity. Austr. J. Agric. Res. 28:53-62.

McGregor D.I. 1978. Thiocyanate ion, A hydrolysis product of glucosinolates from rape and mustard seed. Can. J. Plant Sci. 58: 795-800.

Mendham N.J., J. Russell and N.K. Russel 1990. Response to sowing time of three contrasting Australian cultivars of oilseed rape (*Brassica napus*). J. Agric. Sci. Camb 114: 275-283.

Meng J. 1987. Study advances on distant hybridization incompatibility in *Brassica*. Oil crops of China 4: 71-77.

Meng J. and G. Yi 1988. Studies on the embryology of reciprocal crosses between *Brassica napus* and *B. juncea*. Sci Agric Sin 21: 46-50.

Meng J. and J. Zhou 1988. The interspecific compatibility of *Brassica napus* x *B. oleracea*. II. The influence of maternal genotypes on the pollen-pistil interaction. J. Huazhong Agric Univ 7: 111-117.

Michelmore R.W., I. Paran and R.V. Kesseli 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci, USA 88: 9828-9832.

Miklas P.N., J.R. Stavely and J.D. Kelly 1993. Identification and potential use of a molecular marker for rust resistance in common bean. Theor Appl Genet 85: 745-749.

Mithen R.F. and G. Magrath 1992. Glucosinolates and resistance to *Leptosphaeria maculans* in wild and cultivated *Brassica* species. Plant Breeding 108: 60-68.

Mithen R.F., B.G. Lewis and G.R. Fenwick 1986. In vitro activity of glucosinolates and their products against *Leptosphaeria maculans*. Trans. Br. Mycol. soc. 87: 433-440.

Mizushima U. 1980. Genome analysis in Brassica and allied genera. In: Tsunada S, Hinata K, Gomez-Campo C (eds) Brassica crops and wild allies, Japanese Sci Soc Tokyo, pp 89-108.

Mohammad A., S.M. Sikka and M.A. Aziz 1942. Inheritance of seed colour in some oleiferous *Brassicae*. Ind. J. Genet. Plant Breeding 2: 112-127.

Mohapatra D. and Y.P.S. Bajaj 1987. Interspecific hybridization in *Brassica juncea* x *Brassica hirta* using embryo rescue. Euphytica 36: 321-326.

Morinaga T. 1929. Interspecific hybridization in Brassica. III. The cytology of F_1 hybrids of *B. cernua* and *B. napella*. Journal of department of agriculture. Ky␣␣␣␣ Imperial University 2: 199-206.

Morrison M. ..., P.B.E. McVetty and R. Scarth 1990. Effect of altering plant density on growth characteristics of summer rape. Can. J. Plant Sci. 70: 139-149.

National Archives and Records Administration 1985. Food and Drugs. Code of Federal Regulations, Title 21: 184.155.

Olin-Fatih M. and W.K. Heneen 1992. C-banded karyotypes of *Brassica campestris*, *B. oleracea* and *B. napus*. Genome 35: 583-589.

Olsson G. 1960. Species crosses within the genus *Brassica* I, Artificial *Brassica juncea* Coss. Hereditas 46: 171-223.

Olsson G. 1963. Induced Polyploids in *Brassica*. In: Recent plant Breeding Research, Svalof 1946-1961. Ed. W.M. Myers. John Wiley and Sons. London, New York pp 179-192.

Parsons I.R. and T.K. Howe 1984. Effects of water stress on water relations of *Phaseolus vulgaris* and drought resistant *Phaseolus acutifolius*. Physiol. Plant 60: 197-202.

Paulmann W. and G. Robbelen 1988. Effective transfer of cytoplasmic male sterility from radish (*Raphanus sativus* L.) to rape (*Brassica napus* L.). Plant Breeding 100: 299-309.

Petrie G.A. 1986. Blackleg and other diseases of canola in Saskatchewan in 1984 and 1985. Can. Plant Dis. Surv. 6: 51-53.

Poehlman J.M. 1979. Genetics and Plant Breeding: Variation in chromosome number. In: Breeding field Crops. 2nd Edition, Avi Publishing Co. West Port Connecticut pp 62-63.

Polon M. 1994. The healthy balance. Proceedings: Oilseeds for food and factory conference Jan. 25th and Feb. 8th 1994. Semudo/BASF Cambridge & Ipswich.

Pound G.S. 1947. Variability in *Phoma lingam*. J. Agric. Res. 75:113-133.

Prakash S. 1974. Non homologous meiotic pairing in the A and B genomes of
   Brassica: its breeding significance in the production of variable amphidiploids
   Genet Res 21: 133-137.

Prakash S. 1980. Cruciferous oilseeds in India. In: *Brassica* crops and wild Allies,
   Biology and Breeding (ed. S. Tsunoda, K. Hinata and C. Gomez Campo),
   p151-163. Tokyo: Japan Scientific Soc. Press.

Prakash S. and K. Hinata 1980. Taxonomy, cytogenetics and origin of crop *Brassicas*,
   a review. Opera Bot. 55: 1-57.

Prakash S. and V.L. Chopra 1990. Reconstruction of allopolyploid *Brassicas*
   through non-homologous recombination: Introgression of resistance to pod
   shatter in *Brassica napus*. Genet Res. Camb. 56: 1-2.

Przybylski R., L.J. Malcomson, N.A.M. Eskin, S. Durance-Tod, J. Mickle and R. Carr
1993. Stability of low linolenic acid canola oil to accelerated storage at 60 °C.
   Libensmittel-Wissenschaft und-Technologie. Food Science and Technology 26:
   205-209.

Punithalingam E. and P. Holliday 1972. *Leptosphaeria maculans*. C.M.I. descriptions
   of pathogenic fungi and bacteria. Set 34 No. 331.

Quazi M.H. 1988. Interspecific hybrids between *Brassica napus* L. and *Brassica*
   oleracea L. developed by embryo culture. Theor Appl Genet 75: 309-318.

Quiros C.F., J. Hu, P. This, A.M. Chevre and M. Delseny 1991. Development and
   chromosomal localization of genome-specific markers by polymerase chain
   reaction in *Brassica*. Theor Appl Genet 82: 627-632.

Quiros C.F., O. Ochoa, S.F. Kianian and D. Douches 1987. Analysis of the *Brassica*
   oleracea genome by the generation of *B. campestris-oleracea* chromosome
   addition lines characterization by isozyme and rDNA genes. Theor Appl Genet 74:
   758-766.

Rafalski J. A., S.V. Tingey and J.G.K. Williams 1991. RAPD markers - a new
   technology for genetic mapping and plant breeding. AgBiotech News and
   Information 3:(4) 645-648.

Rai B. and H. Kumar 1995. Reaction of different oilseed Brassica species and some of their hybrids to infection of *Alternaria* leaf blight disease. Cruciferae Newsletter No. 17: 84-85.

Rajan S.S. and M.W. Hardas 1963. Autosyndetic and allosyndetic pairing in *Brassiceae*. Indian Journal of Genetics and Plant breeding 24: (1) 15-21.

Rakow G. 1995. Developments in the breeding of edible oil in other Brassica species. Rapeseed today and tomorrow. Proceedings: Vol. 2: 401-406. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Rakow G. and J.P. Raney 1995. Field Performance of canola quality *Brassica juncea*. Rapeseed today and tomorrow. Proceedings: Vol. 2: 428-430. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Ramanujam S. and B. Rai 1963. Analysis of yield components in *B. campestris* var. Yellow sarson. Indian J. of Genetics and plant breeding 23: 312-319.

Raney P., G. Rakow and T. Olson 1995. Development of zero erucic, low linolenic *Brassica juncea* utilizing interspecific crossing. Rapeseed today and tomorrow. Proceedings: Vol. 2: 413-415. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Rapeseed Association of Canada 1970. Rapeseed. Canada's 'Cinderella' crop. Publication No. 8 pp. 1.

Rashid A., G. Rakow and R.K. Downey 1994. Development of Yellow seeded *Brassica napus* through interspecific crosses. Plant Breeding 112: 127-134.

Richards R.A. and N. Thurling 1978. Variation between and within species of rapeseed (*Brassica campestris* and *B. napus*) in response to drought stress. 1 Sensitivity at different stages of development. Aust. J. Agric. Res 29:469-477.

Rimmer S.R. and C.G.J. Van den Berg 1992. Resistance of oilseed Brassica spp. to blackleg caused by *Leptosphaeria maculans*. Can. J. Plant Pathol. 14: 56-66.

Robbelen G. 1960. Beitraege zur Analyse des *Brassica* Genomes. Chromosoma 11: 205-228.

Robbelen G. 1987. Breeding hybrid varieties of Rape. In: Hybrid seed production of selected cereal oil and vegetable crops. FAO Plant Production and Protection

Paper 82. eds Walter Feistritzer and Fenwick Kelly. Rome, Food and Agricultural Organization of the United Nations.

Rossiele A.A. and K.J. Frey 1977. Inheritance of harvest index and related traits in oats. Crop Sci. 17-23-28.

Rossiter J.T., D.C. James and N. Atkins 1990. Biosynthesis of 2-Hydroxy-3-Butenyl glucosinolate and 3-Butenyl glucosinolate in *Brassica napus*. Phytochemistry 29(8) 2509-2512.

Roy N. N. 1978. A study of disease variation in the populations of an interspecific cross of *Brassica juncea* L. and *B. napus* L. Euphytica 27: 145-149.

Roy N. N. 1984. Interspecific transfer of *B. juncea* type high blackleg resistance to *B. napus*. Euphytica 33: 295-303.

Roy N. N. and A. W. Tarr 1986. Development of near zero linolenic acid (18:3) of rapeseed *B. napus*. Z. Pflanzenzuchtg 96: 218-223.

Roy V., S. Maiti and B.N. Chatterjee 1981. Growth analysis and fertilizer response of 'Varuna' Indian mustard. Indian J. Agric. Sci. 51: 173-180.

Sabharwal P.S. and M. Bechyne 1991. Morphology of F_1 interspecific hybrids of cross, *Brassica napus* L. x *Brassica juncea* L. Genet. a Slecht 27. (2-3) 87-95.

Sacristan M.D. and M. Gerdemann 1986. Different behaviour of *Brassica juncea* and *B. carinata* as sources of *Phoma lingam* resistance in experiments of interspecific transfer to *Brassica napus*. Plant Breeding 97: 304-314.

Sang J.P., C.A. Bluett, B.R. Elliott and R.J.W. Truscott 1986. Effect of time of sowing on oil content, erucic acid and glucosinolate contents in rapeseed (*Brassica napus* L. Cv. Marnoo). Aust. J. Exp. Agric. 26: 607-611.

Sasaoka T. 1930. Karyological observations in different interspecific hybrids of Brassica. Japanese Journal of Genetics 6: 20-32.

Scarth R., P.B.E. McVetty, S.R. Rimmer and B.R. Stefansson 1988. Stellar low linolenic-high-linoleic acid summer rape. Can J. Plant Sci 68: 509-511.

Schwetka A. 1982. Inheritance of seed colour in turnip rape (*Brassica campestris* L.). Theor Appl Genet 62: 161-169.

Shahidi F. 1990. Rapeseed and canola: Global Production and Distribution. In: Canola and Rapeseed. Production, Chemistry Nutrition and Processing Technology. Van Nostrand Reinhold New York pp 3-14.

Sharma S.R. and S.J. Kolte 1994. Effect of soil-applied NPK fertilizers on severity of black spot disease (*Alternaria brassicae*) and yield of oilseed rape. Plant and Soil 167: 313-320.

Sheppard A.J., J.L. Iverson and J.L. Weihrauch 1978. Composition of selected dietary fats oils margarines and butter. Handbook of lipids research: Fatty acids and glycerides, ed. A. Kuksis New York Plenum press p341-379.

Shirzadegan M. 1986. Inheritance of seed color in *Brassica napus* L. Z. Pflanzenzuchtg 96: 140-146.

Shivanna K.R. and V.K. Sawhney 1993. Pollen selection for *Alternaria* resistance in oilseed brassicas: responses of pollen grains and leaves to a toxin of A. *brassicae*. Theor Appl Genet 86: 339-344.

Shivanna K.R. and N.S. Rangaswamy 1992. Pollen Biology: In A laboratory Manual. Springer, Berlin Heidelberg New York.

Sjodin C. and K. Glimelius 1989. *Brassica naponigra*, a somatic hybrid resistant to *Phoma lingam*. Theor Appl Genet 77: 651-656.

Slominski B.A. and L.D. Campbell 1990. Non starch polysaccharides of canola meal: Quantification, digestibility in poultry and potential benefit of dietary enzyme supplementation. J. Sci. Food Agric. 47: 175-184.

Sones K., R.K. Heaney and G.R. Fenwick 1984. Glucosinolates in *Brassica* vegetables. Analysis of twenty seven cauliflower cultivars (*Brassica oleracea* L. var. *botrytis* subvar. *cauliflora* DC). J. Sci. Food Agric. 35: 762-766.

Sprague J.J. 1982. Combining genomes by conventional means in plant improvement and somatic cell genetics. Eds Indra K. Vasil, W.R. Scowcroft and K.J. Frey. Academic Press inc. pp 119-128.

Stern K.R. 1982. Introductory Plant Biology 2nd ed. Wm. C. Brown Co. Dubuque, Iowa, 493 pp.

Stringam G.R. 1980. Inheritance of seed colour in turnip rape. Can. J. Plant Sci. 60: 331-335.

Stringam G.R. and M. R. Thiagarajah 1995. Inheritance of alkenyl glucosinolates in traditional and microspore-derived doubled haploid populations of *Brassica juncea* L. Czern and Coss. Rapeseed today and tomorrow. Proceedings: Vol. 2: 413-415. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Stringam G.R., D.I. McGregor and S.H. Pawlowski 1974. Chemical and morphological characteristics associated with seed coat color in rapeseed. In: Proc. 7th Int. Rapeseed Congress, Giessen, Germany pp 99-108.

Stringam G.R., V.K. Bansal M.R. Thiagarajah and J.P. Tewari 1992. Genetic analysis of blackleg (*Leptosphaeria maculans*) resistance in *Brassica napus* L. using the doubled haploid method. In: Abstract, Reproductive biology and Plant breeding. 13th Eucarpia Congress, Angers France pp213-214.

Tai W. and H. Ikonen 1988. Incomplete bivalent pairing in dihaploids of *Brassica napus* L. Genome 30: 450-457.

Tanhuanpaa P.K., H.J. Vilkki, J.P. Vilkki and S.K. Pulli 1993. Genetic polymorphism at RAPD loci in spring rape (*Brassica rapa* ssp. oleifera). Agric. Sci. Finl. 2: 303-310.

Tanhuanpaa P.K., J.P. Vilkki and H.J. Vilkki 1994. Segregation and linkage analysis of DNA markers in markers in microspore derived and F_2 populations of oilseed rape (*Brassica napus* L.). Euphytica 74: 59-65.

Taylor J.L. 1993. A simple sensitive, and rapid method for detecting seed contaminated with highly virulent *Leptosphaeria maculans*. Applied and environmental microbiology 59:(11) 3681-3685.

Tayo T.O. and D.G. Morgan 1975. Quantitative analysis of the growth, development and distribution of flowers and pods in oilseed rape (*Brassica napus* L.). J. Agric. Sci. Camb 85: 103-110.

Tewari J.P. 1991. Structural and biochemical bases of the blackspot disease of crucifers. Adv Struct Biol. 1: 325-349.

Thorman C.E. and T.C. Osborn 1992. Use of RAPD and RFLP markers for germplasm evaluation. In: Applications of RAPD technology to plant breeding. Crop Science Society of America, Madison, Wisconsin, pp 12-17.

Thorman C.E., M.E. Ferreira, L.E.A. Carmago, J.G. Tivang and T.C. Osborn 1994. Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor Appl Genet 88: 973-980.

Thurling N. 1974a. Morphological determinants of yield in rapeseed (*Brassica campestris* and *Brassica napus*). I. Growth and morphological characters. Aust J. Agric Res 25: 697-710.

Thurling N. 1974b. Morphological determinants of yield in rapeseed (*Brassica campestris* and *Brassica napus*). II. Yield components. Aust J. Agric Res 25: 711-721.

Thurling N. 1993. Physiological constraints and their genetic manipulation. Monor Theor App Genet. New York Springer-Verlag. 1993 Vol. 19: 45-66.

Tingey S.V. and J.P. del Tufo 1993. Genetic analysis with random amplified polymorphic DNA markers. 101: 349-352.

Turner N.C. 1979. Drought resistance and adaptation to water deficits in crop plants. In Stress Physiology in crop plants. (H. Mussel and R.C. Staples, Eds.), pp 181-194. Wiley interscience, New York.

U N. 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap J Bot 7: 389-452.

Upholt W.B. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. Nucleic acids Research: 4:(5) 1257-1265.

Vaisey-Genser M. 1987. Regulations related to canola oil in Canola oil properties and Performance, Canola council of Canada p.10-15.

Van den Berg C.G.J. and R.G. Platford 1991. Distribution, prevalence and incidence of canola diseases in 1990. Can. Plant Dis. Surv. 71:92-93.

Van den Berg C.G.J. and S.R. Rimmer 1991. Changes in levels of glucosinolates in leaves of oilseed rape (*Brassica napus*) during growth, Rapeseed in a changing world: Proceedings Vol. 6: 1697-1699.

Varshney S.K. B. Rai and B. Singh 1990. Analysis of harvest index in three cultivated species of *Brassica*. Indian J. Genet. 50(3): 289-295.

Vasquez T. A., Y. Zuily-Fodil, A.T. Pham Thi and J.B. Vieira da Silva 1990. Electrolyte and Pi leakage and soluble sugar content as physiological test for screening resistance to water stress in *Phaseolus* and Vigna species. Journal of Experimental Botany vol. 41 No. 228: 827-837.

Venkanteswarlu J. and T. Kamala 1971. Pachytene chromosome complements and genome analysis in *Brassica*. J. Indian Bot Soc 50A: 442-449.

Vera C.L. and D.L. Woods 1982. Isolation of independent gene pairs at two loci for seed coat colour in *Brassica juncea*. Can. J. Plant Sci 62: 47-50.

Vera C.L., D.L. Woods and R.K. Downey 1979. Inheritance of seed coat colour in *Brassica juncea*. Can. J. Plant Sci. 59: 635-637.

Wang X.H., P. Luo and J.J. Shu 1989. Giemsa N-banding pattern in cabbage and chinese kale. Euphytica 41: 17-21.

Warner K. and E.N. Frankel 1985. Flavour stability of soybean oil based on induction periods for the formation of volatile compounds by gas chromatography. J. Amer. Oil Chem. Soc. 62: 100-103.

Weiss E. A. 1983. Rapeseed: In; Tropical oilcrops, Longman p.161-216.

Welsh J. and M. McClelland 1991. Genomic finger printing using arbitrarily primed PCR and a matrix of pairwise combination of primers. Nucleic Acids Research 19:(19) 5272-5279.

Welsh J., R.J. Honeycutt, M. McClelland and B.W.S. Sobral 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). Theor Appl Genet 82: 473-476.

Wilde J., R. Waugh and W. Powell 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor Appl Genet 83: 871-877.

Williams J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: (22) 6531-6535.

Williams J.G.K., J.A. Rafalski and S.V. Tingey 1992. Genetic analysis using RAPD markers. Methods in Enzymology.

Woods D.L., J.J. Capcara and R.K. Downey 1991. The potential of mustard (*Brassica juncea* (L.) Coss.) as an edible oil crop on Canadian prairies. Can. J. Plant. Sci 71: 195-198.

Yamagishi H.T., T. Mochizuki and H. Yoshikawa 1980. Introduction of clubroot resistance to *Brassica oleracea* into 'Hakuran' *B. napus* a new head forming vegetable. Cruciferae Newslett 5: 36:-37.

Yarnell S.H. 1956. Cytogenetics of the vegetable crops II. Crucifers. The Botanical review 22: 81-166.

Zuberi M.I. and S.U. Ahmed 1973. Genetic study of yield and some of its components in *B. campestris* L. Crop Sci. 13: 13-15.

Chapter 2

Characterization of Breeding Materials Using Molecular Genetic Markers

2.1. Introduction

2.1.1. Interspecific crosses

Transfer of alien chromosomes or chromatin has often been used to introduce desirable agronomic traits in crops. In *Brassica*, cytological investigations have demonstrated relationships between the monogenomic diploid species *B. nigra* (BB) 2n=16, *B. oleracea* (CC) 2n=18 and *B. rapa* (AA) 2n=20 and the amphidiploid species *B. napus* (AACC) 2n=38, *B. carinata* (BBCC) 2n=34 and *B. juncea* (AABB) 2n=36 (U 1935). U demonstrated that *B. napus* is an allotetraploid derived from hybridization between *B. rapa* and *B. oleracea*, while *B. juncea* was derived from *B. rapa* and *B. nigra*. Studies of chromosome pairing and morphology at the pachytene stage of meiosis have clarified the interrelationships between the various genomes. Studies at the cytogenetic level have shown the close phylogenetic relationship between the A B and C genomes (Armstrong and Keller 1981, Attia and Robbelen 1986 and Busso et al. 1987). *B. napus* (AACC) and *B. juncea* (AABB) possess homology in the A genome, however, over a period of time they have differentiated to some extent resulting in only partial homology (Rajan and Hardas 1963, Heyn 1977).

Introgression of alien genes is often hampered by the lack of pairing between two well differentiated genomes (Banga 1988). Interspecific hybridization has been widely used to transfer single genes or small blocks of genes across interspecific boundaries. Successful transfers of traits such as triazine resistance from *B. napus* to *B. oleracea* (Ayotte et al. 1988), earliness from *B. rapa* and *B. juncea* to *B. napus* (Zaman 1989), blackleg resistance from *B. juncea* to *B. napus* (Roy 1983), shattering resistance from *B. juncea* to *B. napus* (Prakash and Chopra 1988, 1990), and blackleg resistance genes from *B. nigra* to *B. napus* by interspecific crossing with *B. juncea* have been reported (Sacristan and Gerdemann 1986). Paulmann and Robbelen (1988) reported introduction of restorer genes from *Raphanus sativus* to *B. napus*. Resistance to *Plasmodiophora brassicae* was transferred from *B. rapa* to *B. napus* (Lammerink 1970, Johnstone 1974) and from *B. oleracea* to *B. napus* (Yamagishi et al. 1980). Roy and Tarr (1986) reported successful development of a

low linolenic acid line from a *B. juncea* x *B. napus* cross. Intergenomic recombination between *B. oleracea* (CC) and *B. rapa* (AA) chromosomes has been demonstrated in a generation of *B. rapa* - *oleracea* addition lines developed from natural *B. napus* (Quiros et al. 1987). In the process of developing addition lines between *B. rapa* and *B. alboglabra*, Chen et al. (1992) suggested that alien chromosomes from *B. alboglabra* may not remain intact because of possible intra and intergenomic recombination allowing the possibility of chromosome exchange between genomes. In naturally occurring Brassica amphidiploids the constituent genomes have undergone sufficient genetic differentiation that they lose the ability to pair allosyndetically (Heyn 1977). Genetic suppression of homoeologous chromosome pairing was suggested by Prakash (1974). Attia and Robbelen (1986) reported a higher degree of meiotic pairing in the artificially synthesized AACC amphidiploid than in AABB and BBCC and concluded the A and C genomes are more closely related to each other than to the B genome. Some interaction occurs between the A and the B genomes as evidenced by work of Sacristan and Gerdemann (1986) involving *B. nigra*, *B. juncea* and *B. napus*. A high number of bivalents were observed between A and C genome chromosomes by Tai and Ikonen (1988). This suggested that chromosomes of allotetraploid plants obtained through doubling of dihaploids show pairing both in homologous and homoeologous chromosomes. The presence of multivalent associations at metaphase I provide a mechanism for recombination between the various genomes (Ayotte et al. 1988). The degree of homology between genomes greatly influences the potential use of different germplasm sources to improve *Brassica* crops through interspecific crosses. To stabilize the lines and remove the undesired traits, backcrossing is usually performed to the parent with the desired background. This also tends to increase the male and female fertility in subsequent backcross generations of the cross (McGrath and Quiros 1990).

2.1.2. Genetic characterization by RAPDs

Phenology and morphological characteristics may not be sufficiently different to differentiate cultivars (Mailer et al. 1994). Some of the methods used to differentiate cultivars have included plant growth period (Halligan et al. 1991) and seed shape

(Barker et al. 1992). In *Brassica*, specialized tests have been developed based on seed or plant composition such as fatty acids and glucosinolate content (Heaney and Fenwick 1980). Not only are these approaches time consuming, the efficiencies may be reduced by environmental effects or by complex inheritance of multigenic traits (Rafalski et al. 1991).

Molecular markers provide a means of ensuring that desirable traits are indeed recovered in selected progeny and ensuring that important alleles from the adapted cultivar are again recovered (Edwards 1992). When unique markers are identified that appear to be associated with genomic regions of interest, segregation analysis must be performed to determine their location in relation to the gene of interest (Durham and Korban 1994).

The development of polymerase chain reaction (PCR) to amplify DNA and the use of randomly amplified polymorphic DNA (RAPDs) or arbitrary primed polymerase chain reaction (AP-PCR) has resulted in a potentially useful tool for cultivar or line discrimination (Aitken et al. 1994, Williams et al. 1990, Welsh and McClelland 1991). The DNA markers per se have no measurable effect on the phenotype, and the environment does not affect their evaluation (Tanhuanpaa et al. 1994). The information, therefore, generated through DNA markers is independent of environmental conditions.

RAPDs provides an efficient assay for polymorphism, allowing rapid identification and isolation of chromosome specific DNA fragments (Williams et al. 1990, Quiros et al. 1991) if distinguishing between heterozygotes and homozygotes is not required (Welsh et al. 1991). They can also be used to tag specific chromosomes and identify gene transfers. RAPD markers provide information at many loci (Rafalski et al. 1991, Welsh et al. 1991) although large differences are found across primers and species (Williams et al. 1990).

The dominant nature of the markers is offset by the large number of polymorphisms generated by a single primer using AP-PCR (Welsh et al. 1991). It is also not possible to distinguish with dominant RAPD markers whether a DNA segment is amplified from a locus that is heterozygous or homozygous. Since the majority of

RAPD markers are expressed in a dominant manner it is necessary for a single primer to generate at least one polymorphic amplification product from each parent when being used to identify hybrids (Baird et al. 1992). Coupled with backcross introgression and bulked segregant analysis, identification of markers that are tightly linked to genes of interest is enhanced (Michelmore et al. 1991, Miklas et al. 1993, Haley et al. 1993). This results in production of markers useful in selection systems.

Studies comparing species in *Brassica, Raphanus* and *Sinapis* genera concluded that RAPD markers might be useful for taxonomic studies (Demeke et al. 1992, Tanhuanpaa et al. 1993). As few as three random primers generated enough information to distinguish between cultivars of broccoli and cauliflower (Hu and Quiros 1991). Less than 55 markers could distinguish among 45 *B. oleracea* cultivars involving cauliflower and broccoli (dos Santos et al. 1994) which compared well with 40 RAPD markers used by Hu and Quiros (1991) to distinguish between 26 broccoli and cauliflower cultivars. Demeke et al. (1992) separated 5 cabbage and 4 cauliflower genotypes with 122 RAPDs and 13 primers. With only six primers, Mailer et al. (1994) discriminated 24 of 26 cultivars of *B. napus*. At times one primer can distinguish a wide range of genotypes. In 13 cocoa (*Theobroma*) clones Wilde et al. (1992) could separate all genotypes using a single primer.

2.2. Objectives

The objectives of this study were to develop DH lines from an interspecific cross (*B. juncea* x *B. napus*) x *B. junea* through microspore culture, and genetically characterize the developed lines with RAPDs.

2.3. Materials and Methods

2.3.1. Development of breeding lines

Two breeding populations were maintained throughout the study. The breeding lines were developed through a series of crosses between *B. juncea* and *B. napus*. The breeding scheme is shown in Figure 2.1. The *B. juncea* parent was developed by crossing breeding lines 91-5350-2 x 91-5467-1 from the University of Alberta canola breeding program. The

other *B. juncea* parent was developed by crossing breeding lines 91-5350-1 x 91-5467-1.

This latter parent was crossed to a *B. napus* breeding line 91-5174-1 obtained through spontaneous diploidy during microspore culture of immature pollen. The F_1 plants obtained were backcrossed to the *B. juncea* parent developed earlier, to produce the donor parent population 94-1. Concurrently, another cross was made between the *B. juncea* breeding line 91-5349-2 and the *B. napus* line 91-5174-1. The F_1 obtained was backcrossed to an open pollinated form of the *B. juncea* parent 91-5349-2 to obtain donor parent population 94-2. The two donor line populations were seeded in 5 inch pots using soil free mixture (Stringam 1971) and placed in growth cabinets maintained at 18/10 °C day/night temperature and 16 hour photoperiod. Photosynthetic photon flux density at plant level was 425-450 $\mu Em^{-2}m^{-1}$. Plants were fertilized with 20-20-20 fertilizer solution of NPK twice weekly. At the first true leaf stage, the plants were thinned to one per pot. Insects were controlled both by manual removal from the plant and through chemical sprays. Buds at the uni-nucleate stage were picked from plants of the two breeding populations as they matured. Buds at the appropriate developmental stage for microspore harvest were selected when petals extended about half to two thirds the length of the anther. The harvest period was prolonged by removing open flowers and by occasionally cutting back to allow the re-growth of the plant.

2.3.1.1. Microspore culture

The protocol used for the microspore culture was as described by Coventry et al. (1988); and modified for *B. juncea* (Thiagarajah and Stringam 1990). Buds were harvested shortly before or at first flower stage from main racemes and lateral branches, wrapped in aluminum foil and stored at 4°C for four days. Buds at the appropriate developmental stage were selected and placed in steel cages, then dipped in 7% Calcium hypochlorite and surface sterilized for 15 minutes. The buds were then rinsed three times for 5 minutes each with cold sterile double distilled water and transferred to a chilled sterile mortar and pestle. They were then gently crushed in B5 wash media (13% w/v sucrose) to release microspores. The suspension consisting of microspores and other plant debris was swirled gently and poured through two layers

of nested sterile filters (Nytex-63µm top and, 44µm bottom) (B & SH Thompson) placed in a sterilized plastic funnel and washed with cold B5 culture medium. The microspores were then collected in sterile 50ml falcon tubes (Fisher) and centrifuged at 900-1000 rpm for 5-8 minutes. The solution was decanted under sterile conditions and microspores resuspended in B5 medium. The process was repeated four times and the microspores re-suspended in NLN media (Lichter 1985).

The concentration of the microspores was altered by adjusting the volume of the medium to approximately 1ml bud^{-1} utilized. Approximately 10ml of the suspension was decanted into sterile 60x15mm disposable petri dishes (Fisher cat No. 8-757-13) and the dishes wrapped in a double layer of paraffin film. The plates were incubated at 35°C overnight in darkness and then transferred to 30°C in similar conditions. The plates were incubated for 14-21 days and then placed on a shaker at 60-70 rpm for 4-7 days depending on the size of the embryos. The embryos were then transferred (10 embryos per plate) to solid B5 media (0.8% agar containing 0.15mg/l of filter sterilized gibberellic acid (GA_3) from Sigma). The plates containing small undeveloped embryos were then sealed and returned to the shaker until the embryos had grown sufficiently for transfer to solid media. The plates were then sealed and maintained at 4°C in an 8 hour photoperiod regime for 7 days, and then transferred to a culture room in a 12/12 hour photoperiod with a photon flux density of 30 $µEm^{-2}s^{-1}$ at room temperature.

When the embryos had germinated and developed true leaves and fine roots, they were transferred to autoclaved soil free mixture (Stringam 1971) and covered with plastic caps to conserve moisture and reduce stress. The plants were then placed in growth chambers and watered daily. The caps were removed when the plants became established and were allowed to grow until flowering.

Figure 2.1. Development of the breeding population scheme

Breeding line 1

91-5350-2 x 91-5467-1
(B. juncea)

91-5350-1 x 91-5467-1   91-5174-1
(B. juncea)   *(B. juncea)*   *(B. napus)*

93-433-1   X   93-419-5
B. juncea   *B. napus*

F₁
93-700-2
(interspecific)

X   93-702-1
(B. juncea)

94-1

Breeding line 2

91-5349-2
(B. juncea)

92-1773-1   91-5174-1   93-435-1 (OP)
DH₁ seed   SD blk Res

93-435-1   X   93-419-5
B. juncea   *B. napus*

F₁
93-701-3   X   93-703-2
(interspecific)   *B. juncea*
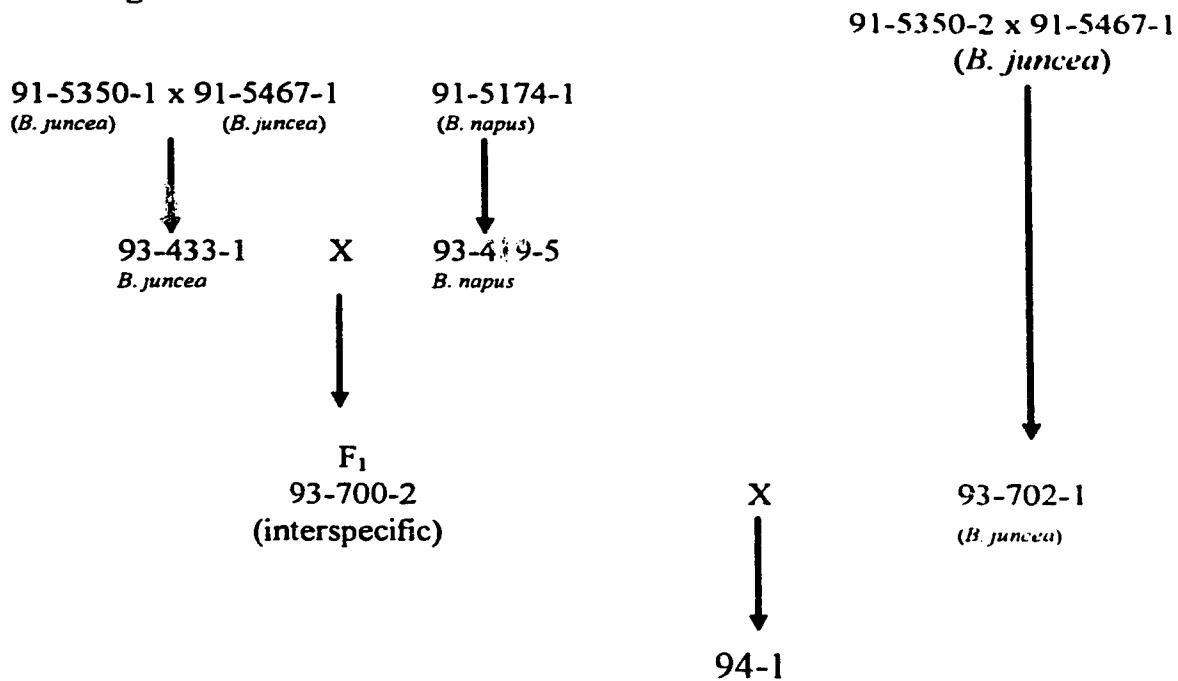
94-2
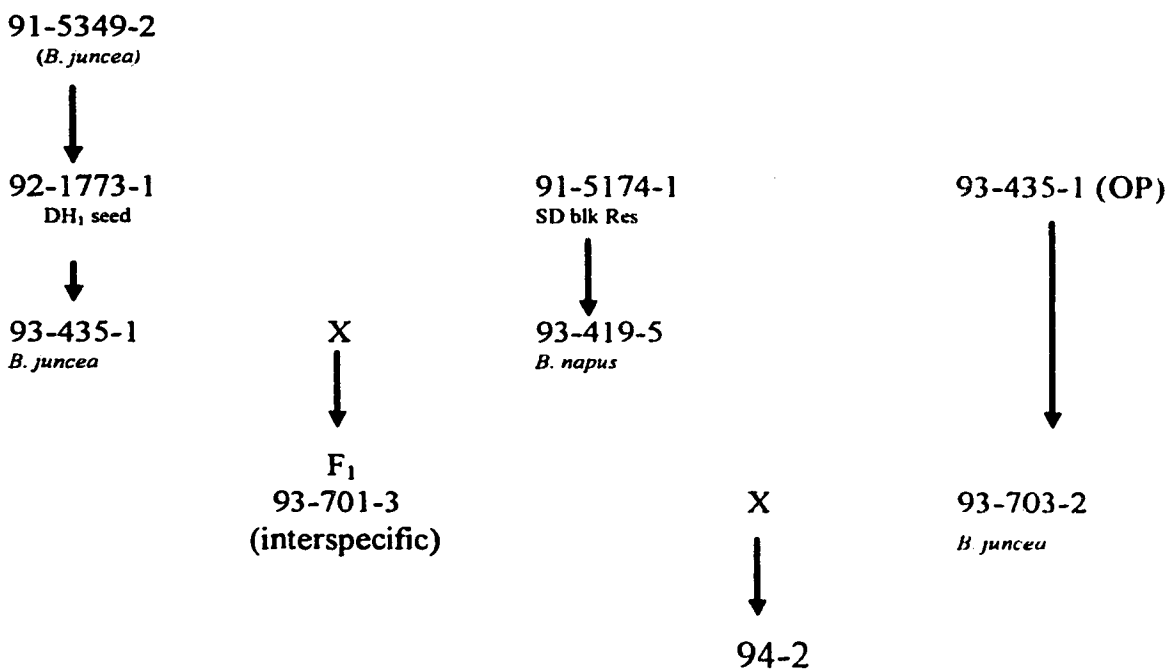
2.3.1.2. Colchicine treatment

Plant morphology was examined to identify spontaneous diploids and haploid plants. The spontaneous diploids, which were identified by their larger flowers and copius production of pollen, were self-pollinated. The roots of the haploid plants were washed to remove all potting media and immersed in a colchicine solution (3.4g l^{-1}) for one and a half hours (Coventry et al. 1988), and exposed to bright light to improve transpiration and speed the uptake of the colchicine solution. The plants were then transplanted into fresh 5 inch pots and allowed to regenerate. At flowering, the plants were bagged and the bags agitated periodically to ensure the development of self-pollinated seed.

2.3.2. Genetic characterization by use of RAPDs

2.3.2.1. Plant material

Seeds from *B. juncea*, *B. napus*, F_1 and BC_1F_1-derived DH plants were planted in five inch pots and grown as earlier described. Prior to leaf harvesting, plants were kept in the dark for 48 hours to reduce photosynthetic activity. This procedure reduced the amount of polysaccharides present in the leaf tissues which interferes with the analysis (Delaporta et al., 1983). The tender young leaves were harvested from three week old plants. Samples were collected from actively growing plants, wrapped in aluminum foil, and stored in liquid nitrogen (Delseny et al. 1990) until DNA was extracted.

2.3.2.2. DNA Extraction

The DNA was extracted using the protocol described by Delaporta et al. (1983) with slight modification outlined below. The leaf tissue was ground in a chilled mortar and pestle under liquid nitrogen. This prevented degradation of the sample upon thawing. Eight ml of extraction buffer, consisting of: 100 mM Tris pH=8, 50 mM EDTA pH=8, 500 mM NaCl and 10 mM β-Mercaptoethanol (added fresh) were added to the ground tissue and transferred to a sterile Oakridge tube. One ml of 20% sodium dodecyl sulphate (SDS) was added followed by vigorous shaking. One-hundred-fifty μg/ml of Proteinase K (112 μl of 20mg/ml) was added to the macerated tissue and the

mixture incubated for one hour at 55 °C. Five ml of 5M potassium acetate (KoAc) was added, mixed thoroughly, and the entire mixture then placed on ice for twenty minutes. The mixture was then centrifuged at 25,000 xg (16,500 rpm) using a Beckman Model J-21B centrifuge and the supernatant filtered through a single layer of Miracloth (Sigma) to remove floating debris. An equal volume of phenol:chloroform (24:1 Chloroform:Isoamyl alcohol) was added, mixed thoroughly and then centrifuged for 5 minutes at 3,500 rpm using a Hitachi Himac model CT5 DL clinical centrifuge. An additional chloroform extraction completed the extraction process. The DNA was precipitated by adding 0.6 vol. isopropanol (2-propanol) and centrifuging the cloudy mixture for five minutes at 3,500 rpm. The DNA pellet was placed into a 1.5 ml tube and rinsed twice with 70% ethanol. The DNA pellet was then resuspended in 500 µl TE pH=8 containing 10 mM Tris and 1 mM EDTA. The DNA solution was treated with 5µl of RNAse for one hour at 37 °C followed by a phenol:chloroform extraction. Sequential chloroform extractions were done until no white interface remained (approx. 3 times). An equal volume of 5M NaCl was added and mixed, and the DNA precipitated by adding an equal volume of 95% ethanol. If necessary, the DNA solution was centrifuged at 13,000 rpm using a Canlab Biofuge A centrifuge before a pellet was obtained. The DNA pellet was washed three times with 70% ethanol and vacuum dried using a Savant Speedvac concentrator. The dry pellet was then dissolved in a minimum amount of TE pH 8 (10mM Tris, 1mM EDTA) and stored at -20 °C.

2.3.2.3. Quantification

Twenty µl of DNA were added to 2,000 µl of sterile de-ionized water and optical density (OD) determined at 260 nm with of a Cary 219-0018 UV spectrophotometer. Optical density (OD) readings were also taken at 230nm (proteins) and 280nm (polysaccharides) to determine the level of contamination. An OD ratio reading of 1.7-2.2 was desired between DNA and the protein bands. The DNA concentration was calculated from the formula:

$$\text{DNA concentration } (\mu g/\mu l) = OD~(260nm) \times \text{dilution factor} \times \frac{50}{1000}$$

The original DNA solution was then diluted to a concentration of 5ng/μl. The diluted DNA was divided into 1.5ml aliquots and stored at -20 °C until required.

2.3.2.4. Amplification conditions

Amplification was completed according to Williams et al. (1990) with slight modifications. The reactions were carried out in volumes of 22.9μl containing 1.9μl of 25mM MgCl₂, 2.5μl of 10x Buffer (Promega), 0.5μl of 10mM dNTP (Pharmacia), 0.2μl of 1U Taq DNA polymerase (Promega), and 2.5μl of 2mM primer obtained by diluting stock primers ten times. The volume was made up to 22.9μl by adding sterile double distilled water. Of this solution only 20μl were used for amplification. The reaction mixture was placed in 0.5ml reaction tubes and overlaid with a drop of mineral oil to prevent evaporation of the mixture.

Amplification was performed in a Techne PHC 2 DNA Thermal Cycler programmed for 45 cycles of 1 minute at 94 °C, 1 minute at 35 °C and 2 minutes at 72 °C. An extra cycle at 94 °C, for 1 minute, 35 °C for 1 minute and at 72 °C for 10 minutes completed the amplification. The amplification products were separated by 1.4% agarose gel made by dissolving 0.42g of agar in 30ml of Tris acetate (TAE) buffer consisting of 0.04M Tris acetate with 0.001M EDTA. The buffer was made from a 50x stock solution. The agarose was dissolved by microwaving for 55 seconds on high. One and half μl of aqueous ethidium bromide (10 mg/ml) was added prior to gel casting in a mini gel mold (Styler Instruments). The PCR products were placed in reaction tubes taking care not to remove oil together with the products. Two μl of loading buffer consisting of 0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol in water were added to the reaction tube to act also as a tracking dye. An extra tube was included containing 2μl of 1 Kb ladder (GIBCO BRL), 2μl of loading buffer and 20μl of double distilled water. After loading, the gel was run at 80V for 1-2 hours, observed under UV light, and the results recorded on a 57 type Polaroid film.

2.3.2.5. Similarity Index

A similarity index was determined to compare the parents, F_1 and BC_1F_1-derived DH plants using the method of Nei and Li (1979). The calculations were based on presence or absence of specific markers in the parents F_1 and BC_1F_1-derived DH plants. The similarity index F, is a ratio of shared bands in two populations and total bands present in the population (Table 2.8 and 2.10).

$$F = \frac{2Nxy}{Nx + Ny}$$

Where Nx = No. of Bands present in population x

Ny = No. of bands present in population y

Nxy = No. of bands common to the two populations x and y

2.4.   Results

2.4.1. Donor line development

The donor plant population from 94-2 was more responsive with 50% of the population (3 plants) developing embryos. Donor plants had different embryogenic capabilities resulting in different plant populations (Table 2.2). The donor plant population from 94-1 had a 20% response (5 plants). The level of embryogenic capability was lower than in the 94-2 population. The F_1 plant population from which 94-2 was obtained on backcrossing produced doubled haploid plants on microspore culture. Most of the plants died from the effects of colchicine treatment as *B. juncea* is more sensitive to colchicine toxicity (Thiagarajah and Stringam 1993). The recovered doubled haploid plants were identified because of higher level of pollen production and larger flowers observed in the plants. The 94-1 derived DH population was severely affected resulting in only one double haploid line from donor 94-1. Ten lines were obtained from 94-2 derived population and six F_1-derived DH plant lines were obtained.

2.4.2. Phenotypic description of parent lines

B. napus leaves are dark green, glaucous, and lyrate. The lower leaves are partially clasping. The leaf lobes are acute, with a narrow elliptic lamina shape. The leaf margin dentition is undulate. The bracts have broad auricles, with coarse small teeth on

their margins. The inflorescence is an elongated raceme, borne terminally on the main stem as well as on the branches. The flowers are bright yellow although the colour may vary from orange to pale yellow. The flower petals tends to be wide apart giving an appearance of a wider flower. *B. juncea* contains light green leaves with a hairy surface. The leaf lobes are rounded, with a wide elliptic shape. The margin dentition contains sharp teeth. The bracts are elongate with small teeth on its margin. The flowers are pale yellow and show a more compact nature than *B. napus*. The morphotypes observed in the breeding lines were as shown in table 2.1.

Table 2.1. Morphological appearance of the breeding populations based on leaf structure and general flower appearance.

Breeding Population	Morphology of Plants	Number of Plants
94-1	*B. juncea* type	7
	Intermediate	6
	B. napus type	2
94-2	*B. juncea* type	3
	Intermediate	2
	B. napus type	1

The main advantage of using haploids is the rapid and theoretically complete homozygosity of the offspring resulting in a time saving of several years in cultivar development (Thiagarajah and Stringam 1993). It is also simpler to distinguish different classes within genetic populations because heterozygotes, usually common in early segregating populations, are absent (Choo et al. 1985).

DH populations have been used to study inheritance of traits such as fatty acids and glucosinolates in *B. napus* (Siebel and Pauls 1989a, 1989b, Stringam and Thiagarajah 1995), seed colour studies in canola type *B. napus* (Henderson and Pauls 1992), and seed colour and leaf hairiness in *B. juncea* (Thiagarajah and Stringam 1993).

Table 2.2. Response to microspore culture in F_1 and BC_1F_1 of the cross *B. juncea* x *B. napus*.

Type of Population	Plant identity	Recovered Plants after Colchicine treatment
F_1	93-701-3	8
BC_1F_1	94-1-2	2
	94-1-3	1
	94-1-5	14
	94-1-6	1
	94-1-8	1
BC_1F_1	94-2-1	4
	94-2-2	24
	94-2-3	8

The use of the doubled haploidy method of breeding did not differ significantly from traditional single seed descent (SSD) method of breeding (Thiagarajah and Stringam 1993). Jansen (1992) suggested a population of doubled haploid lines is nearly equivalent to a late generation of a single seed descent program, which in the case of unlinked genes, the minimum number of doubled haploids are only slightly smaller than the minimum number of single descent lines in the seventh to eleventh generation (Jansen and Jansen 1990).

2.4.3. Genetic characterization using RAPDs

Six primers out of twenty obtained from Operon Technologies were used to discriminate between 17 F_1 and BC_1F_1-derived DH lines, two *B. juncea* and one *B. napus* parents (Table 2.3). The primers used were single 10-mer oligonucleotides.

Only consistent bands were scored from the various primers used. Faint and non repetitive bands were not included in the analysis. The PCR was run at least twice per primer or group and the results from the multiple runs compared to determine the consistent bands. Polymorphisms was detected in eighty-six percent of the scorable RAPDs. Fourteen percent of the markers were monomorphic and appeared in all

plants. Higher numbers of markers were detected in the *B. juncea* parents than in the *B. napus* parent. The size of the amplification products ranged from 1.9 Kb to 0.3 Kb in the 29 scorable markers developed from the six primers (Table 2.4).

Primers OPA9, OPA16 and OPA18 produced six markers each, with primers OPA9 and OPA18 producing a monomorphic band at 0.3 Kb and 0.6 Kb respectively (Table 2.4, Figures 2.2 and 2.3). Primers OPA3 (Figure 2.6) and OPA14 (Figure 2.5) produced four markers each which were polymorphic. Monomorphic bands were produced by OPA9 (Figure 2.2). OPA20 and OPA18 produced 2 and 1 monomorhic markers of 1.2 and 0.8 Kb (Table 2.4) and 0.6 Kb (Figure 2.3) repectively. Monomorphic bands specific only to F_1 and BC_1F_1-derived DH plants were also produced. A 0.8 Kb band amplified by OPA3 (Table 2.4 and 2.7) was present in both *B. juncea* and *B. napus* but was only detected in BC_1F_1-derived DH lines. A monomorphic band of 0.87 Kb present only in the BC_1F_1-derived DH lines was detected by OPA18 (Table 2.4 and Figure 2.3).

Table 2.3.   Primers and their Sequences used for DNA amplification.

Primer	Sequence	No. of RAPDs
OPA3	5' GTT GCG ATC C 3'	4
OPA9	5' GGG TAA CGC C 3'	6
OPA14	5' TCT GTG CTG G 3'	4
OPA16	5' AGC CAG CGA A 3'	6
OPA18	5' AGG TGA CCG T 3'	6
OPA20	5' GTT GCG ATC C 3'	3
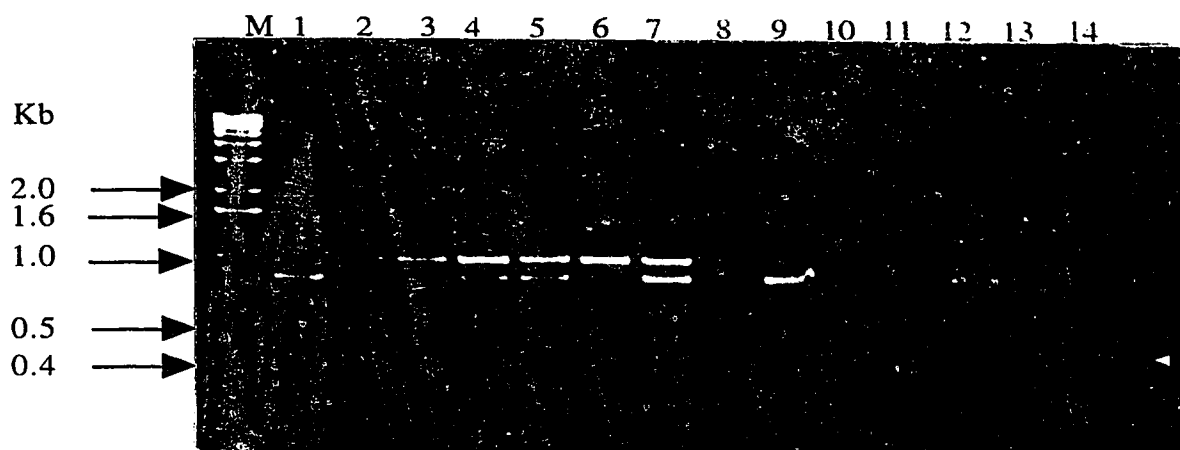
Figure 2.2. Genetic characterization of F_1 and BC_1F_1-derived DH plant:, using RAPD markers generated by Primer OPA9 (Operon Technology; Alameda California). Monomorphic band at 0.4 Kb present in all F_1 and BC_1F_1-derived plants is shown by an arrow. The 1.0 Kb and 0.8 Kb marker present in the *B. napus* and *B. juncea* are recovered both in the F_1 and BC_1F_1-derived plants. Lanes 1-2, *B. juncea*, and *B. napus*; Lanes 3-8, F_1-derived DH plants and Lanes 9-14, BC_1F_1-derived DH plants.

Table 2.4. Classification of RAPD markers developed between parents, F_1 and BC_1F_1-derived DH plants from 94-2-3.

Primer	Size (Kb)	B. j S1	B. n S3	F_1 S4	F_1 S5	F_1 S6	F_1 S7	F_1 S8	F_1 S9	BC_1F_1 S11	BC_1F_1 S14	BC_1F_1 S15	BC_1F_1 S16	BC_1F_1 S17	BC_1F_1 S18
OPA 16	1.9	1	1	1	1	1	0	0	0	1	0	0	0	0	0
	1.5	0	1	0	0	1	0	0	0	1	0	0	0	0	0
	1.4	0	1	1	1	1	0	0	0	1	0	0	0	0	0
	1	0	1	1	1	1	1	1	1	0	1	0	0	0	0
	0.95	1	0	0	1	0	0	0	0	1	0	1	1	1	1
	0.83	1	0	0	0	0	0	0	0	1	0	0	0	0	0
OPA9	1.6	1	0	1	1	1	1	1	1	1	1	1	1	1	0
	1.2	1	0	1	0	0	0	0	1	1	1	1	1	1	0
	1	0	1	1	1	1	1	1	0	1	0	0	0	0	0
	0.8	1	0	0	1	1	1	0	1	1	1	1	1	0	1
	0.4	1	0	0	0	1	1	0	0	1	1	0	1	1	0
	0.3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA14	1.5	1	0	1	1	1	1	1	1	1	1	1	1	1	1
	1.4	1	1	1	0	1	1	1	1	1	1	1	1	1	0
	0.9	1	1	1	1	0	1	1	1	1	1	0	0	0	0
	0.4	1	0	1	0	1	1	1	1	1	1	1	1	1	1
OPA20	1.5	1	0	1	1	1	0	1	1	1	0	1	1	1	0
	1.2	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.8	1	1	1	1	1	1	1	1	1	1	1	1	1	1
OPA3	1.8	1	0	1	1	1	1	1	1	0	1	1	0	1	1
	1	1	0	0	0	0	0	0	1	1	1	1	1	1	1
	0.9	0	1	0	0	0	0	0	0	1	1	1	1	1	1
	0.8	1	1	1	1	1	1	1	1	0	0	0	0	0	0
OPA18	1.05	1	0	1	1	1	0	1	1	1	1	1	0	1	1
	0.93	0	1	1	1	1	1	1	1	1	1	1	1	1	1
	0.87	1	1	1	0	1	1	1	1	1	1	1	1	1	1
	0.8	1	0	0	0	0	1	1	0	1	1	1	1	1	1
	0.7	1	0	0	0	0	1	1	0	1	1	1	1	0	0
	0.6	1	1	1	1	1	1	1	1	1	1	1	1	1	1

See Appendix 2 for sample abbreviations
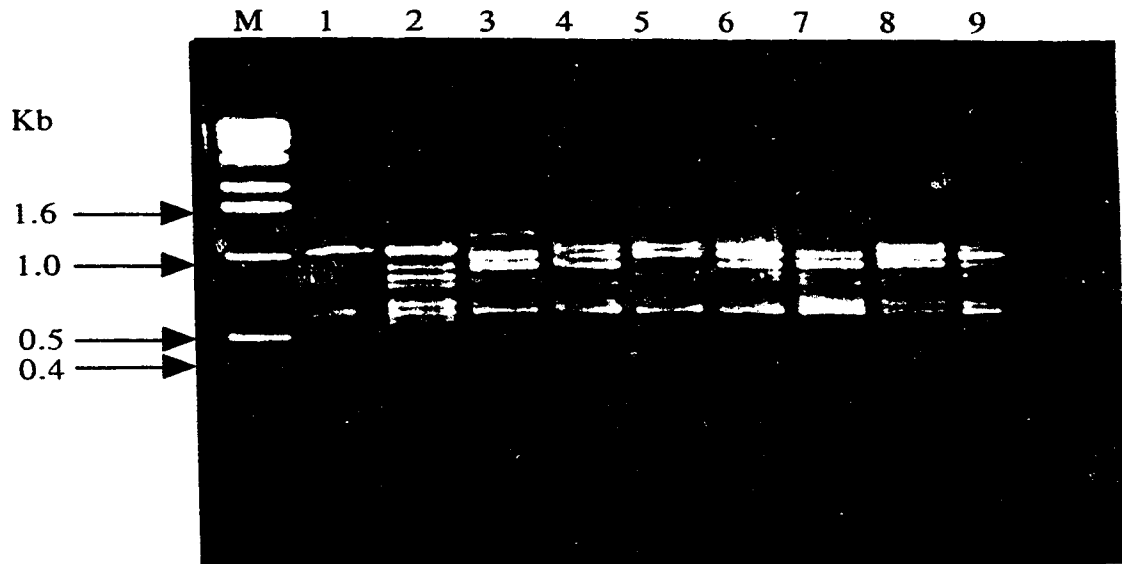
Figure 2.3. RAPD analysis of F₁-derived DH plants between *B. juncea* x *B. napus* using the random primer OPA18. Lanes 1-3, *B. juncea* parent 1 and 2, and *B. napus*; Lanes 4-9, F₁-derived DH plants. The 1.05 Kb marker from the *B. juncea* and 0.87 Kb from *B. napus* are found in the F₁-derived DH plants in lanes 4, 6, 8 and 9.

Table 2.5.   Classification of RAPD markers developed between parents, F_1 and BC_1F_1-derived DH plants from 94-2-1 and 94-2-2.

Primer	Size (Kb)	*B. j S1	*B. n S3	F_1 S4	F_1 S5	F_1 S6	F_1 S7	F_1 S8	F_1 S9	BC_1F_1 S12	BC_1F_1 S13	BC_1F_1 S19	BC_1F_1 S20
OPA9	1.6	1	0	1	1	1	1	1	1	0	0	0	0
	1.2	1	0	1	0	0	0	0	1	0	1	0	1
	1	0	1	1	1	1	1	1	0	0	0	0	0
	0.8	1	0	0	1	1	0	1	0	1	1	1	1
	0.4	1	0	0	0	1	1	0	0	1	1	1	1
	0.3	1	1	1	1	1	1	1	1	1	1	1	1
OPA16	1.9	1	1	1	1	1	0	0	0	0	0	0	0
	1.5	0	1	0	0	1	0	0	0	1	1	1	1
	1	0	1	1	1	1	1	1	1	0	0	0	0
	0.9	1	0	0	1	1	1	1	1	1	1	1	1
	0.6	1	1	0	1	1	1	1	1	0	0	1	1
	0.5	0	1	1	1	1	1	1	1	1	1	1	1
OPA18	1.05	1	0	1	1	1	0	1	1	1	1	1	1
	0.93	0	1	1	1	1	1	1	1	1	1	0	1
	0.87	1	1	1	1	0	1	1	1	1	1	1	1
	0.8	1	0	0	0	0	1	1	0	1	1	1	1
	0.7	1	0	0	0	0	1	1	0	1	1	1	1
	0.6	1	1	1	1	1	1	1	1	1	1	1	1

*B.j and B.n = *B. juncea* and *B. napus* respectively

2.5. Discussion

The *B. juncea* parent is an amphidiploid consisting of A and B genomes, whereas *B. napus* consists of A and C genomes. Since the parents share the A genome (U 1935), the shared bands observed in the gels could have resulted from the common genome A where DNA associated with A genome chromosomes from both *B. juncea* and *B. napus* could have homologous regions. Species specific bands were more abundant in *B. juncea* than in *B. napus*. In a previous study, Quiros et al. (1991) observed that the B genome had the highest amplification products, more than double the A genome and more than three times the C genome products. There were 14 amplification products detected only in *B. juncea* as compared to 6 observed in the *B. napus*. The number of markers observed in *B. juncea* and *B. napus* was in close agreement with observations of Quiros' et al. (1991).

OPA9 amplified a 1 Kb marker specific for the C-genome. B-genome specific markers were detected by OPA16 with 0.9 Kb and 1.4 Kb markers. In the study by Quiros et al. (1991) a 0.8 Kb A-genome specific marker was amplified by OPA9.

The 1000 bp C-genome specific marker detected by OPA9 was also detected in the F_1, but only in a single BC_1F_1-derived DH plant (Figure 2.2). This may have occurred as a result of pairing between C-genome chromosomes from *B. napus* and *B. juncea* B-genome chromosomes which on backcrossing dissociated and were lost after backcrossing in most of the BC_1F_1-derived DH lines. The distribution of the markers appeared random among the F_1 and BC_1F_1-derived DH lines. All markers observed in the parents were recovered either in the F_1 or BC_1F_1-derived DH plants.

Other than markers amplified by OPA3 (Figure 2.6), markers found in *B. napus* only were all detected in the F_1, showing that all the F_1 contained some genetic material from *B. napus*. As a result of backcrossing, the *B. napus* genetic material passed on to BC_1F_1-derived DH plants was reduced considerably.

The reduction of *B. napus* markers recovered from BC_1F_1-derived DH plants suggests loss of genetic material from *B. napus* in BC_1F_1-derived DH plants resulting from backcrossing to *B. juncea*. This loss supports the observations of reduction in chromosome numbers observed in the PMCs of F_1-derived DH plants discussed in

Chapter 3. The 1 Kb fragment amplified by OPA3 in the BC_1F_1-derived DH plants could be of *B. napus* origin arising through homeologous pairing of B- and C-genome chromosomes. The monomorphic bands detected by various primers were very likely from the A-genome chromosomes which are present in both parents. The influence of the C-genome chromosomes in the BC_1F_1-derived DH plants was diminished as a result of backcrossing, a fact shown by reduction in the number of *B. napus* markers in the BC_1F_1-derived DH plants. This suggests the desired *B. juncea* background was recovered and hopefully some of the *B. napus* markers observed in BC_1F_1-derived DH plants were expressed for the required traits.

The markers developed could be classified into species specific markers, present only in the parents *B. juncea* or *B. napus* (Table 2.6). Species-specific markers were detected by all six primers used in the study. Fourteen markers were detected only in the *B. juncea*, parents and only six in the *B. napus* parent.

Table 2.6. Species specific markers observed in the *B. juncea* and *B. napus* parents.

B. juncea	B. napus
OPA20 1500	OPA18 900
OPA18 1000	OPA16 1500
OPA18 800	OPA16 1400
OPA18 700	OPA16 1000
OPA16 900	OPA9 1000
OPA16 800	OPA3 900
OPA14 1500	
OPA14 400	
OPA9 1600	
OPA9 1200	
OPA9 800	
OPA9 400	
OPA3 1800	
OPA3 1000	
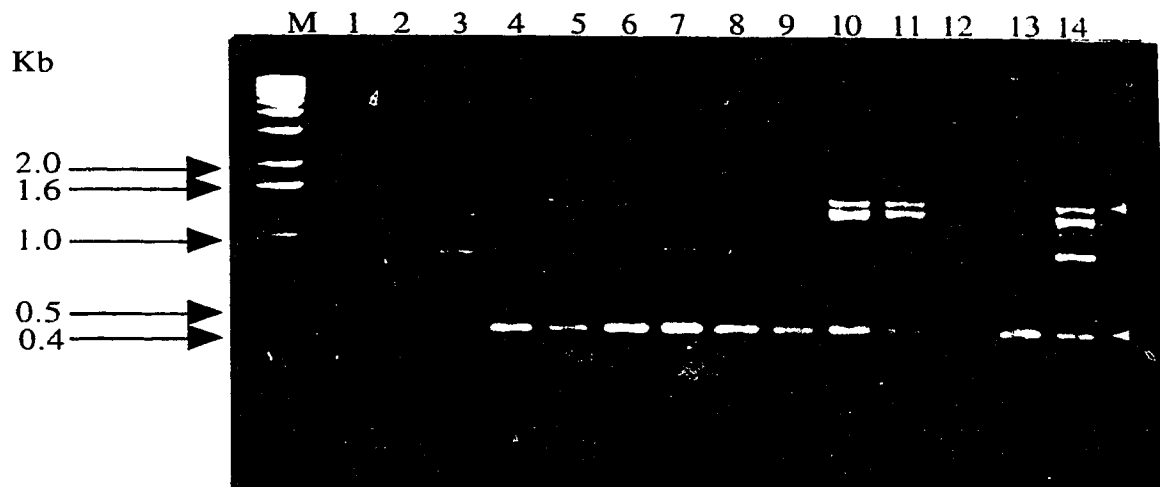
Figure 2.4. RAPD profiles of BC₁F₁-derived DH plants generated by Primer OPA14. Lanes 1-3 represent *B. juncea* parents 1 and 2, and *B. napus*. Lanes 4-14 represents BC₁F₁-derived plants. *B. juncea* specific markers 1.5 Kb and 0.4 Kb are shown by arrows.

71

Kb

2.0 ——
1.6 ——

1.0 ——

0.5 ——
0.4 ——

M 1 2 3 4 5 6 7 8 9
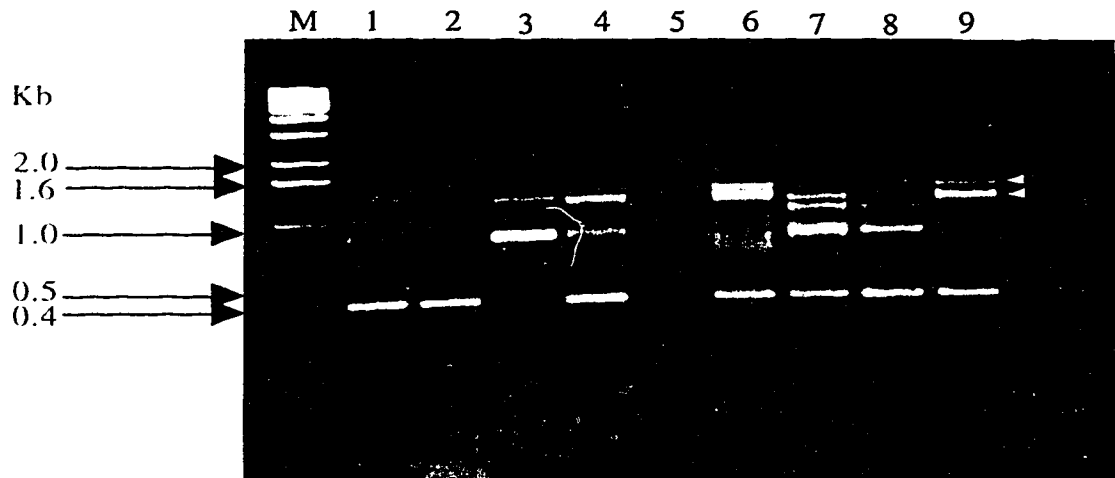
Figure 2.5. R \PD banding patterns of DNA amplification
products from F₁-derived DH plants. The Primer used was
OPA14. Lanes 1-3, *B. juncea* parents 1 and 2, and *B. napus*;
Lanes 4-9 represents F₁-derived DH plants. The B-genome
marker 1.5 Kb was found in all F₁-derived DH plants. The 1.4
Kb marker present in both parents is probably from the A
genome. It was detected in all the lanes except lane 5.

The number of markers detected in the BC_1F_1-derived DH plants increased by more than 1.5 times over the F_1 in *B. juncea*. This is probably as a result of extra *B. juncea* genetic material arising from the backcrossing, replacing some of the *B. napus* markers observed in the F_1-derived plants. Other than markers from primer OPA3, the *B. napus* marker bands from other primers detected in the F_1 were reduced by nearly 50% in the BC_1F_1-derived DH plants.

Table 2.7. Distribution of amplification bands from *B. juncea* and *B. napus* in DH plants derived from 94-2-3.

	Primer	F_1 derived DH	BC_1F_1 derived DH
B. juncea	OPA20	5	4
	OPA18	9	15
	OPA16	1	6
	OPA14	11	19
	OPA9	14	19
	OPA3	7	10
	Total	47	73
B. napus	OPA18	6	6
	OPA16	8	3
	OPA9	5	1
	OPA3	0	6
	Total	19	16
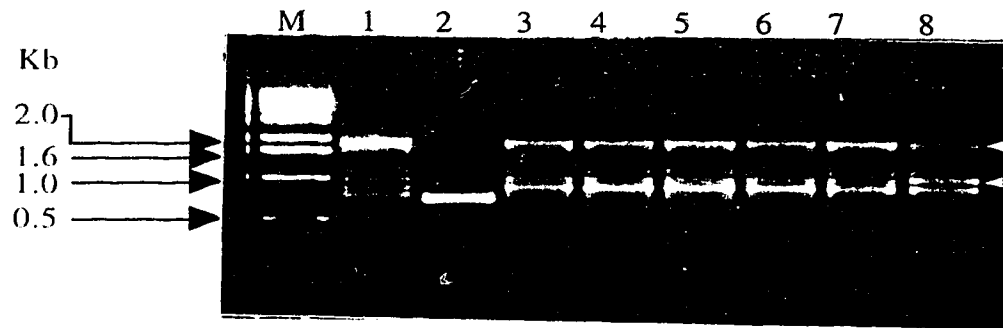
Figure 2.6. RAPD profile of six F_1-derived DH plants using primer OPA3. A 1.8 Kb marker from both *B. juncea* and *B. napus* was recovered in the F_1-derived DH plants. Lanes 1-2, *B. juncea* and *B. napus*; Lanes 3-8, F_1 -derived DH plants.

74

Table 2.8.   Similarity Index for parents, F_1 and BC_1F_1 DH plants derived from 94-2-3.

		B.j	B.n	F_1	F_1	F_1	F_1	F_1	F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1
		S1	S3	S4	S5	S6	S7	S8	S9	S11	S14	S15	S16	S17	S18
B.j	S1	1													
B.n	S3	0.42	1												
F_1	S4	0.67	0.65	1											
F_1	S5	0.61	.59	0.82	1										
F_1	S6	0.62	0.65	0.82	0.82	1									
F_1	S7	0.76	0.65	0.72	0.68	0.78	1								
F_1	S8	0.74	0.63	0.96	0.72	0.81	0.87	1							
F_1	S9	0.79	0.57	0.86	0.72	0.81	0.77	0.85	1						
BC_1F_1	S11	0.78	0.56	0.76	0.86	0.76	0.68	0.71	0.71	1					
BC_1F_1	S14	0.80	0.54	0.72	0.64	0.73	0.83	0.81	0.86	0.80	1				
BC_1F_1	S15	0.82	0.44	0.70	0.66	0.70	0.70	0.78	0.83	0.82	0.88	1			
BC_1F_1	S16	0.65	0.46	0.86	0.56	0.67	0.72	0.70	0.75	0.83	0.86	0.93	1		
BC_1F_1	S17	0.79	0.46	0.72	0.62	0.71	0.62	0.75	0.80	0.79	0.86	0.93	0.90	1	
BC_1F_1	S18	0.68	0.46	0.54	0.58	0.59	0.65	0.63	0.74	0.65	0.76	0.83	0.74	0.80	1

The plants were grouped into 8 comparison groups in an attempt to determine their similarities and hence the degree of genetic material transfer (Tables 2.8, 2.9, 2.10 and 2.11). The similarity indices were calculated using marker population bands obtained from primer amplification (Table 2.7). A total of 155 bands in the F_1-derived and BC_1F_1-derived lines were used. Because a common A-genome is shared between *B. juncea* and *B. napus*, a certain level of genetic similarity was expected. In this material, the similarity index was 0.42. The BC_1F_1-derived DH plants were more similar to the *B. juncea* parent than *B. napus* (0.80 and 0.508) as a result of backcrossing to *B. juncea*. The level of similarity between the *B. napus* parent and F_1, and BC_1F_1-derived DH plants was reduced from 0.67 to 0.508. As a result of backcrossing to the *B. juncea* parent, the BC_1F_1-derived DH plants were more similar (0.8) to the *B. juncea* parent than F_1 plants (0.730) (Table 2.9). The average number of similarities between group 2 and 4 was significantly different (p=0.05). Groups 6 and 7 were not significantly different.

Three primers were used to characterize the DH plants from donor plants 94-2-1 and 94-2-2. The BC_1F_1-derived DH plants that had more *B. napus*-like morphological characteristics had higher similarity score to *B. napus* than those that were more morphologically different such as DH plants from 94-2-3. The average similarity scores for groups from 94-2-1 and 94-2-2 donor line plants showed a similar trend to those from donor line 94-2-3 with slight variation in individual BC_1F_1-derived DH line similarity scores.
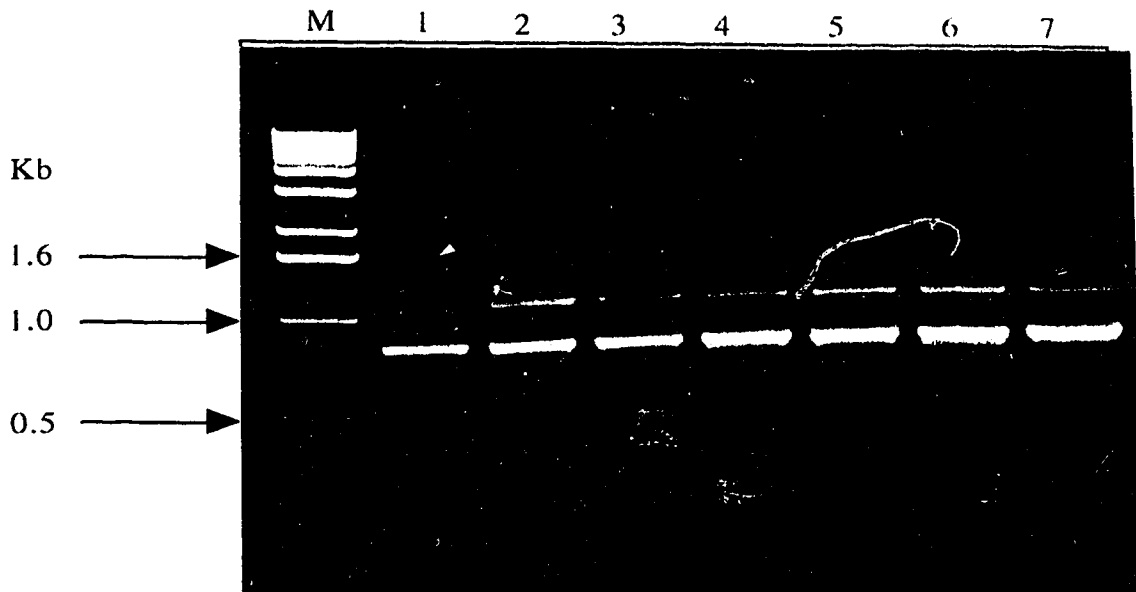
Figure 2.7. Comparison of seven BC₁F₁-derived DH plants using RAPD markers generated using Primer OPA20. The similarity between the plants is shown by the presence of 1.2 and 0.67 Kb in all the plants and a 0.55 Kb marker in all plants except BC₁F₁-derived DH plant in lane 1. The BC₁F₁-derived DH plants in lanes 3 and 7 did not contain a 1.5 Kb marker (shown by an arrow) present in all the other plants.

Table 2.9. Means and Standard Deviations of comparison groups for DH plants derived from 94-2-3.

Group	Plant comparisons	Mean Similarity Index	Std Deviation
1	*B. juncea* & F_1-derived	0.730	0.075
2	*B. napus* & BC_1F_1-derived	0.508	0.066
3	F_1 & BC_1F_1-derived	0.695	0.076
4	*B. napus* & F_1-derived	0.667	0.049
5	*B. juncea* & BC_1F_1-derived	0.800	0.060
6	Between BC_1F_1-derived	0.814	0.078
7	Between F_1-derived	0.798	0.059
8	*B. juncea* & *B. napus*	0.420	-

Table 2.10. Means and Standard Deviations of comparison groups for DH plants derived from 94-2-1 and 94-2-2.

Group	Plant comparisons	Mean Similarity Index	Std Dev.
1	*B. juncea* x F_1-derived	0.635	0.085
2	*B. napus* x BC_1F_1-derived	0.530	0.025
3	F_1 x BC_1F_1-derived	0.680	0.059
4	*B. napus* x F_1-derived	0.722	0.048
5	*B. juncea* x BC_1F_1-derived	0.810	0.081
6	Between BC_1F_1-derived	0.900	0.067
7	Between F_1-derived	0.787	0.071
8	*B. juncea* x *B. napus*	0.350	-

Table 2.11.   Similarity Index for parents, F_1 and BC_1F_1-DH plants derived from 94-2-1 and 94-2-2.

		B.j	B.n	F_1	F_1	F_1	F_1	F_1	F_1	BC_1F_1	BC_1F_1	BC_1F_1	BC_1F_1
		S1	S3	S4	S5	S6	S7	S8	S9	S12	S13	S19	S20
B.j	S1	1											
B.n	S3	0.43	1										
F_1	S4	0.31	0.92	1									
F_1	S5	0.38	0.88	0.8	1								
F_1	S6	0.71	0.88	0.67	0.75	1							
F_1	S7	0.71	0.75	0.88	0.89	0.95	1						
F_1	S8	0.57	0.86	0.77	0.88	0.82	0.95	1					
F_1	S9	0.53	0.67	0.71	0.82	0.78	0.88	0.8	1				
BC_1F_1	S12	0.71	0.43	0.46	0.63	0.71	0.75	0.57	0.67	1			
BC_1F_1	S13	0.8	0.4	0.57	0.59	0.67	0.53	0.75	0.93	0.93	1		
BC_1F_1	S19	0.8	0.53	0.43	0.71	0.78	0.82	0.67	0.75	0.88	0.88	1	
BC_1F_1	S20	0.88	0.5	0.53	0.53	0.67	0.74	0.63	0.67	0.82	0.75	0.94	1

References

Aitken S.A., N.A. Tinker, D.E. Mather and M.G. Fortin 1994. A method for detecting DNA polymorphism in large populations. Genome 37: 506-508

Armstrong K.C. and W.A. Keller 1981. Chromosome pairing in in haploids of *Brassica campestris*. Theor Appl Genet 59: 49-52.

Attia T and G. Robbelen 1986. Cytogenetic relationship within cultivated *Brassica* analyzed in amphidiploids from the three diploid ancestors. Can J Genet Cytol. 28 323-329.

Attia T., C. Busso and G. Robbelen 1987. Digenomic triploids for an assessment of chromosome relationships in the cultivated diploid *Brassica* species. Genome 29 326-330.

Ayotte R., P.M. Harney and V. souza Machado 1988. The transfer of triazine resistance from *Brassica napus* L. to *B. oleracea* L. II. Morphology, fertility and cytology of the F_1 hybrid. Euphytica 17: 189-197.

Baird E., S. Cooper-Bland, R. Waugh, M. DeMaine and W. Powell 1992. Molecular characterization of inter and intra-specific somatic hybrids of potato using randomly amplified polymorphic DNA (RAPD) markers. Mol Gen Genet 233 469-475.

Banga S.S. 1988. C-genome chromosome substitution lines in *B. juncea* (L.) Coss Genetica 77: 81-84.

Barker T.A., T.A. Vuori, M.R. Hegedus and D.G. Myers 1992. The use of ray parameters for the discrimination of Australian wheat varieties. Pl Var and Seeds 5: 35-45.

Busso C., T. Attia and G. Robbelen 1987. Trigenomic combinations for the analysis of meiotic control in the cultivated *Brassica* species. Genome 29: 331-333

Chen B.Y., V. Simonsen, C. Lanner-Herrera and W.K. Heneen 1992. A *Brassica campestris-alboglabra* addition line and its use for gene transfer and generation of trisomics. Theor Appl Genet 84: 592-599.

Choo T.M., E. Reinbergs and K.J. Kasha 1985. Use of haploids in breeding barley. Plant Breed Rev 3: 219-252.

Coventry J., L. Kott and W.D. Beversdorf 1988. Manual for microspore culture technique for *Brassica napus*. University of Guelph Department of Crop Science Technical Bulletin OAC Publication 0489.

Dellaporta S.L., J. Wood and J.B. Hicks 1983. A plant minipreparation: version II. Plant Mol. Biol. Rep.1: 19-21.

Delseny M., J.M. McGrath, P. This, A.M. Chevre and C.F. Quiros 1990. rDNA genes in diploid and amphidiploid Brassica and related species: organization, polymorphism and evolution. Genome 33: 733-734.

Demeke T., R.P. Adams and R. Chibbar 1992. Potential taxonomic use of random amplified polymorphic DNA (RAPD): a case study in *Brassica*. Theor Appl Genet 84: 990-994.

dos Santos J.B., J. Nienhuis, P. Skroch, J. Tivang and M.K. Slocum 1994. Comparison of RAPD and RFLP genetic markers in determining genetic similarities among *Brassica oleracea* L. genotypes. Theor Appl Genet 87: 909-915.

Durham R. E. and S. S. Korban 1994. Evidence of gene introgression in apple using RAPD markers. Euphytica 79: 109-114.

Edwards M. 1992. Use of molecular markers in the evaluation and introgression of genetic diversity for quantitative traits. Field crops Research 29: 241-260.

Haley S.D., P.N. Miklas, J.R. Stavely, J. Byrum, and J.D. Kelly 1993. Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor Appl Genet 86: 505-512.

Halligan E.A., M.B. Forde, and I.J. Warrington 1991. Discrimination of rye grasses by heading date under various combinations of vernalization and day length. Pl Var and seeds 4: 115-123.

Heaney R.K. and G. R. Fenwick 1980. The glucosinolate content of *Brassica* vegetables. A Chemotaxonomic Approach to cultivar identification. J Sci Food Agric 31: 794-801.

Henderson C.A.P. and K.P. Pauls 1992. The use of haploidy to develop plants that express several recessive traits using light seeded canola (*Brassica napus*) as an example. Theor Appl Genet 83: 476-479.

Heyn F. W. 1977 Analysis of unreduced gametes in the *Brassicae* by crosses between species and ploidy levels. Z Pflanzenzuchtg 78: 13-30.

Hu J. and C. F. Quiros 1991. Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Reports 10: 505-511.

Jansen R.C. 1992. On the selection for specific genes in doubled haploids. Heredity 69: 92-95.

Jansen R.C. and J. Jansen 1990. On the selection for specific genes by single seed descent. Euphytica 51: 131-140.

Johnstone T.D. 1974. Transfer of disease resistance from *B. campestris* L. to rape (*Brassica napus* L.). Euphytica 23: 681-683.

Lammerink J. 1970. Interspecific transfer of club root resistance from *Brassica campestris* L.to *B. napus* L. New Zeal J. Agric Res 13: 105-110.

Lichter R. 1985. From microspores to rape plants: A tentative way to low glucosinolate strains. In Sorenson H. (ed.). Advances in the production and utilization of cruciferous crops pp268-277. Martinus Nijhoff/ Dr. W. Junck Publishers, Dordrecht, Boston. Lancaster.

Mailer R.J., R. Scarth and B. Fritensky 1994. Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theor Appl Genet 87: 697-704.

McGrath J.M. and C.F. Quiros 1990. Generation of alien chromosome addition lines from synthetic *Brassica napus*: morphology cytology fertility and chromosome transmission. Genome 33: 374-383.

Michelmore R.W., I. Paran and R.V. Kesseli 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: A rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci, USA 88: 9828-9832.

Miklas P.N., J.R. Stavely and J.D. Kelly 1993. Identification and potential use of a molecular marker for rust resistance in common bean. Theor Appl Genet 85: 745-749.

Nei M. and W.H. Li 1979. Mathematical model for study of genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 74: 5267-5273.

Paulmann W. and G. Robbelen 1988. Effective transfer of cytoplasmic male sterility from radish (*Raphanus sativus* L.) to rape (*Brassica napus* L.). Plant Breeding 100: 299-309.

Prakash S. 1974. Probable basis of diploidization of *Brassica juncea* Coss . Can. J. Genet. Cytol. 16: 232-234.

Prakash S. and V.L. Chopra 1988. Introgression of resistance to shattering in *Brassica napus* from *Brassica juncea* through non homologousrecombination. Plant breeding 101: 167-168.

Prakash S. and V.L. Chopra 1990. Reconstruction of allopolyploid *Brassicas* through non-homologous recombination: Introgression of resistance to pod shatter in *Brassica napus*. Genet Res. Camb. 56: 1-2.

Quiros C.F., O. Ochoa, S.F. Kianian and D. Douches 1987. Analysis of the *Brassica oleracea* genome by the generation of *B. campestris-oleracea* chromosome addition lines characterization by isozyme and rDNA genes. Theor Appl Genet 74: 758-766.

Quiros C.F., J. Hu, P. This, A.M. Chevre and M. Delseny 1991. Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. Theor Appl Genet 82: 627-632.

Rafalski J. A., S.V. Tingey and J.G.K. Williams 1991. RAPD markers - a new technology for genetic mapping and plant breeding. AgBiotech News and Information 3:(4) 645-648.

Rajan S.S. and M.W. Hardas 1963. Autosyndetic and allosyndetic pairing in *Brassiceae*. Indian Journal of Genetics and Plant breeding 24(1): 15-21.

Roy N. N. 1983. Interspecific transfer of *B. juncea* type high blackleg resistance to *B. napus*. Euphytica 33: 295-303.

Roy N. N. and A. W. Tarr 1986. Development of near zero linolenic acid (18:3) of rapeseed *B. napus*. Z. Pflanzenzuchtg 96: 218-223.

Sacristan M.D. and M. Gerdemann 1986. Different behaviour of *Brassica juncea* and *B. carinata* as a source of *Phoma lingam* resistance in experiments of interspecific transfer to *B. napus*. Plant Breeding 97: 304-314.

Siebel J. and K.P. Pauls 1989a. Inheritance patterns of erucic acid content in populations of *Brassica napus* microspore derived spontaneous diploids. Theor Appl Genet 77: 489-494.

Siebel J. and K.P. Pauls 1989b. Alkenyl glucosinolate levels in adrogenic populations of *Brassica napus*. Plant Breed 103: 124-132.

Stringam G.R. and M. R. Thiagarajah 1995. Inheritance of Alkenyl glucosinolates in traditional and microspore-derived doubled haploid populations of *Brassica juncea* L. Czern and Coss. Rapeseed today and tomorrow. Proceedings Vol. 2 413-415. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995

Stringam G.R. 1971. Genetics of four hypocotyl mutants in *Brassica campestris* L. Heredity 62: 248-250.

Tai W. and H. Ikonen 1988. Incomplete bivalent pairing in dihaploids of *Brassica napus* L. Genome 30: 450-457.

Tanhuanpaa P.K., H.J. Vilkki, J.P. Vilkki and S.K. Pulli 1993. Genetic polymorphism at RAPD loci in spring rape (*Brassica rapa* ssp. oleifera). Agric. Sci. Finl. 2. 303-310.

Tanhuanpaa P.K., J.P. Vilkki and H.J. Vilkki 1994. Segregation and linkage analysis of DNA markers in markers in microspore derived and F2 populations of oilseed rape (*Brassica napus* L.). Euphytica 74: 59-65.

Thiagarajah M.R. and G.R. Stringam 1990. High frequency embryo induction from microspore culture of *Brassica juncea* L. Czern and Coss. Abstr. 7th Int. Congr. on plant Tiss. and Cell Cult., Amsterdam, p.190.

Thiagarajah M.R. and G.R. Stringam 1993. A comparison of genetic segregation in traditional and microspore derived populations of *B. juncea* L. Czern and Coss. Plant Breeding 111: 330-334.

U N. 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap J Bot 7: 389-452.

84

Welsh J. and M. McClelland 1991. Genomic finger printing using arbitrarily primed PCR and a matrix of pairwise combination of primers. Nucleic Acids Research 19:(19) 5272-5279.

Welsh J., R.J. Honeycutt, M. McClelland and B.W.S. Sobral 1991. Parentage determination in maize hybrids using the arbitrarily primed polymerase chain reaction (AP-PCR). Theor Appl Genet 82: 473-476.

Wilde J., R. Waugh and W. Powell 1992. Genetic fingerprinting of *Theobroma* clones using randomly amplified polymorphic DNA markers. Theor Appl Genet 83: 871-877.

Williams J.G.K., A.R. Kubelik, K.J.Livak, J.A. Rafalski and S.V. Tingey 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18: (22) 6531-6535.

Yamagishi H.T., T. Mochizuki and H. Yoshikawa 1980. Introduction of clubroot resistance to *Brassica oleracea* into 'Hakuran' *B. napus* a new head forming vegetable. Cruciferae Newslett 5: 36:-37.

Zaman M.W. 1989. Introgression in *Brassica napus* for adaptation to the growing conditions in Bangladesh. Theor Appl Genet 77: 721-728.

Chapter 3

Cytological Stability

3.1. Introduction

3.1.1. Cytology

Cytological observations in many species may be helpful for species classification (Gomez-Campo 1980). Analysis of meiotic configurations and differential chromatin condensation can elucidate morphological features of paired or unpaired chromosomes at diakinesis, since size differences are more pronounced at this stage (Cheng et al. 1994). Although chromosome banding is difficult in Brassica because of the small chromosome size (Olin-Fatih and Heenen 1992), banding has been used to characterize these chromosomes (Wang et al. 1989, Olin-Fatih and Heenen 1992). Chromosome behaviour during meiosis can be used as a measure of the degree of inter and intra genomic pairing between chromosomes in interspecific crosses. Since homoeologous chromosome pairing in Brassica is thought to occur between A and C genomes, 10 bivalents and 17 univalents are expected if complete non-pairing at meiosis occurs in B. juncea (AABB) x B. napus (AACC) (Sacristan and Gendermann 1986). Deviations would indicate the relative inter and intragenomic pairing in the cross. This would also indicate the degree of stability resulting from interaction between the B and C genomes.

3.1.2. Pollen and female fertility

Pollen viability refers to the ability of pollen to complete post pollination events and to effect fertilization (Shivana and Rangaswamy 1992). The viability of pollen is determined by several factors including genic control. For example, sterility genes, killer genes, gametocidal genes, chromosomal aberrations, non homology of genomes and cytoplasmic abnormalities are all under genetic control. According to Iwanami et al. (1988), F_1 hybrids between different species produce pollen of reduced fertility because of a lack of pairing during meiosis, resulting in unbalanced or abnormal chromosome distribution.

Several methods based on stains have been used to assess pollen viability. These differentially stain the constituents of the vegetative cell with varying degrees of specificity. The methods leave the exine unstained. Methods include polysaccharide staining as in the periodic acid-schiff procedure, aniline blue and chromatin and RNA staining methods involving acetocarmine, acetic orcein and acridine orange (Heslop-Harrison et al. 1984).

3.1.3. Reciprocal crosses between F_1 and parents

The presence of high viability levels in pollen would suggest a high probability of seed set from effective pollination. However, this is not always the case because occasionally the high pollen fertility is not reflected in high seed set, as few and shriveled seeds are obtained. On these occasions there are problems associated with fertilization both at the genetic and morphological level for example malformation of floral parts like stigma in the maternal parent resulting in poor seed set (Meng and Zhou 1988). Low stainability of pollen in the F_1 of interspecific crosses was reported by Struss et al. (1991), Lee and Namai (1992), and Chen et al. (1992) but effects of microspore culture and double haploidy on pollen stainability in F_1-derived DH plants has not been reported.

3.2. Materials and Methods

3.2.1. Cytology

The pollen mother cells were harvested from terminal buds of 21-30 day old plants grown in a growth chamber as described in Chapter 2. The buds were fixed in Newcomer's solution (Newcomer 1953) consisting of isopropanol, propionic acid, petroleum ether, acetone and dioxane in a ratio of 6:3:1:1. The solution was then mixed with pure powdered basic ferric acetate to a final concentration of 3% (w/v) to act as a mordant (Stringam 1970). The buds were allowed to stand in the fixative for 48 hours, transferred to 70% ethanol, and stored at 4 °C until required. The buds were then removed from the storage solution and blotted to remove excess solution. Starting from the largest to the smallest, individual anthers were dissected from each

bud and placed onto a clean glass slide. A drop of 2% acetocarmine was added and the anther macerated as quickly as possible with a rusted iron needle to aid in chromosome staining while avoiding excessive staining of the cytoplasm All debris were removed by fine forceps, and a cover slip placed gently over the drop. The underside of the slide was heated briefly over a steam jet. While still hot, the slide was inverted over a filter paper and pressed to express excess stain and flatten the cells. The slide was then observed first under low magnification wth a Phase Contrast microscope (Model BHS PM-10AD SP) to identify the proper cell stages for study Those slides with proper chromosome stages were temporary sealed using clear nail polish applied around the cover slip. Meiotic figures were photographed under an oil emersion objective using an attached camera (Olympus C-35AD) loaded with Technical Pan film (Kodak). Exposures were taken utilizing an Olympus Exposure control unit attached to the camera.

3.2.2. Pollen fertility

To determine the fertility of the pollen, samples were obtained from F_1-derived DH_2 , BC_1F_1 and parent plants. Five flowers from the main branch were harvested from each plant. Pollen grains from dehiscing anthers were brushed into culturing media (Shivana and Rangaswamy 1992) to prevent dehydration and to preserve the grains until staining was completed. Each flower was placed in a well containing about 200µl of media for analysis. After one hour, a drop of 2% acetocarmine was added into the pollen culture mixture and stirred. This procedure fixed and stained the pollen grains. By using a fine dropper, a drop of the pollen/culture solution was removed by suction and observed under low power of the microscope. Stained and germinated grains were counted as fertile and unstained as sterile.

3.2.3. Female fertility

To investigate whether the F_1-derived DH lines had both male and female gamete stability, reciprocal crosses were made between three F_1-derived DH lines and both *B. napus* and *B. juncea* parents and one non reciprocal cross between one F_1-

derived DH line and both parents. The two parents and F_1-derived DH plants were grown as previously described. Crosses were completed on buds borne on the main stem and occasionally on the primary branches. Buds were hand emasculated prior to anthesis and pollinated by brushing pollen from the selected donors on the stigma of the recipient. The exposed stigmas were then bagged to prevent contamination by foreign pollen. Pollinations were done consecutively until bud development ceased. The number of developed pods and the number of seeds obtained were determined. Fertility index (FI) was calculated by determining the number of seeds developed as compared to total seeds possible from the parents.

$$FI = \frac{SD}{BPxSPP}$$

where
SD=Seeds developed from the cross
BP=Buds pollinated per cross
SPP=Seeds per pod of open pollinated parent

3.3. Results

3.3.1. Cytology

Cytological observations were carried out on six F_1 and eleven BC_1F_1-derived DH_2 lines. A total of 343 pollen mother cell (PMC's) were studied among the lines; 123 from the F_1-derived DH lines and 220 from the BC_1F_1-derived DH lines. The PMC's were studied at various cell stages, however the majority of them were at diakinesis and anaphase I stages.

3.3.1.1. F_1-derived DH plants

There was considerable variation in the number of chromosomes between different cells even within the poles (Figure 3.5) probably resulting in gametes of unequal chromosome number. At diakinesis various chromosome arrangements were observed. Univalent numbers ranged from 3-14 in some F_1-derived DH plants (Figures 3.1, 3.3, and 3.4). Bodies were observed at the poles in the cells that could not be positively identified as chromosomes in some PMC's because of the small size

of chromosomes (Figure 3.5). In other PMC's, accurate identification could not be done because of various associations, (i.e. stickiness and clumping Figure 3.2)

The PMC's from the F_1-derived DH plants were sampled at diakinesis, metaphase, telophase I and anaphase I. The PMC's observed in the early stages had a chromosome distribution of 3.5-10 univalents. More than 11 bivalents and occasional trivalents for the F_1-derived DH plants were observed (Table 3.1). At anaphase I the chromosome distribution at the poles was on average 22.8-27.6. This distribution indicated a relatively large number of univalents suggesting loose chromosome associations or partial pairing. The large number of chromosomes during anaphase indicated production of unbalanced gametes. This could be responsible for the low female fertility observed in F_1-derived DH plants. Also secondary chromosome associations as were observed making it impossible to determine in many cases, whether the associations resulted from chromosome stickiness or true homologous pairing.

3.3.1.2. The BC_1F_1-derived DH plants

At anaphase most of the PMC's in BC_1F_1-derived DH plants showed a chromosome distribution of 17-18 (Figures 3.8, 3.10). At diakinesis and prophase the predominant chromosome configuration was 17 bivalents plus 2 univalents (Figures 3.6, 3.12 and 3.13). The presence of trivalents was noted and observed only in a relatively few number of cells studied. On average the BC_1F_1-derived DH plants had a chromosome distribution of more than 14 bivalents, 1-3.9 univalents and a relatively low trivalent chromosome count of 0-0.6 per line (Table 3.2).

Table 3.1. Chromosome distribution in PMC's from F_1-derived DH plants of *B. juncea* x *B. napus*.

Plant	No. of Cells	Diakinesis/Prophase/Metaphase			Anaphase
94-1290	17	II $_{10\text{-}13}$	–	I $_{8\text{-}10}$	23-27
94-1291	19	II $_{14?}$	III $_{0\text{-}1}$	I $_{5\text{-}9}$	21-28
94-1292	7	II $_{14?}$	–	I $_8$	27-27
94-1293	27	II $_{15?}$	–	I $_{0\text{-}7}$	24-29
94-1294	28	II $_{7\text{-}10}$	III $_{0\text{-}1}$	I $_{3\text{-}14}$	22-28
94-1295	25	II $_{10\text{-}17}$	III $_{0\text{-}2}$	I $_{5\text{-}12}$	20-27
	123	II $_{11.7?}$	III $_{0\text{-}0.7}$	I $_{3.5\text{-}10}$	22.8-27.6

91

Figure 3.1. Chromosome configuration of F_1-derived DH plant 94-1294 showing 12 bivalents and 16 univalents (shown with an arrow) at late metaphase 1 (The inset shows the same cell in reduced detail) (Bar 2μm)
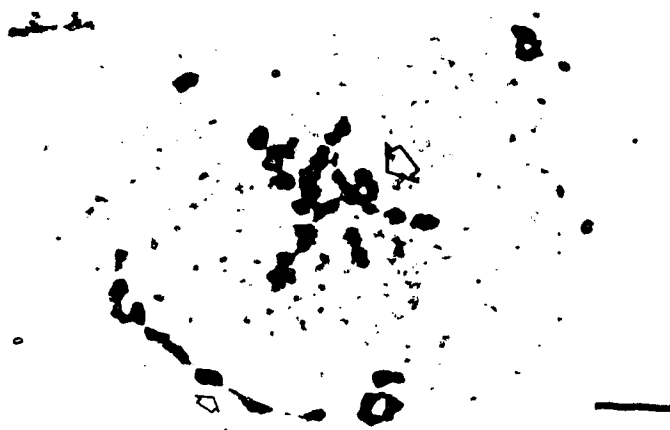
Figure 3.2. Diakinesis stage of F_1-derived DH plant 94-1291 showing chromosome associations, stickiness and clustering. (Bar = 6μm).
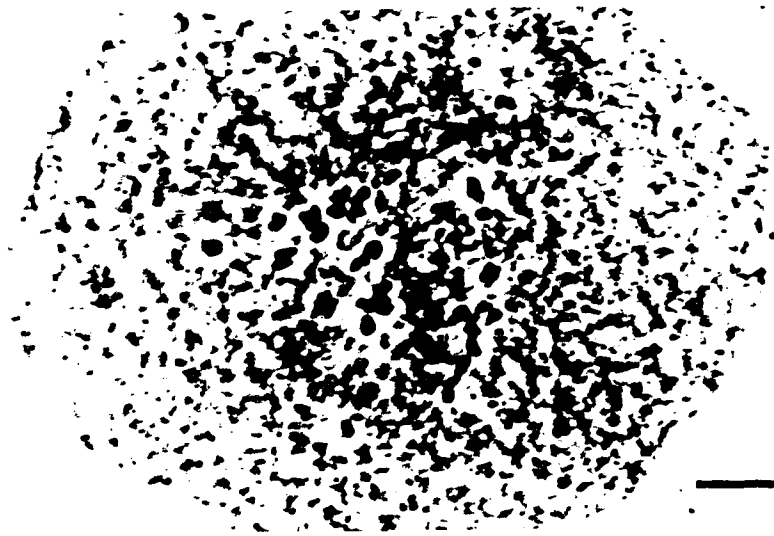
Figure 3.3. Late Prophase chromosomes of F_1-derived DH plant 94-1295 showing 15II + 1III + 6I. The trivalent is shown by an arrow (Bar = 6μm).

Figure 3.4. Prophase chromosomes of F_1-derived DH plant, 94-1295 showing 14II + 13I configuration (Inset shows the same cell in reduced detail) (Bar = 3μm)
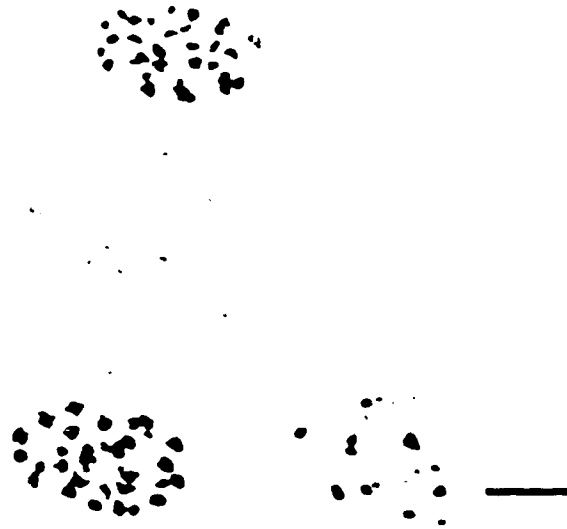
Figure 3.5. Telophase I of F_1-derived DH plant, 94-1290 showing 27 29 configuration at each pole (Bar = 6μm)

Figure 3.6. Diakinesis in BC_1F_1-derived DH plant 94-1280, showing 17II + 2I chromosome configuration (Bar = 7μm).

The univalents may have resulted from dissociation of bivalents after doubling. This may arise because different genomic origins of the chromosomes. At anaphase, the BC_1F_1-derived DH plants had an average chromosome count of 16.6-18.4 at each pole. The large number of bivalents observed indicate a greater degree of pairing within the chromosomes of the different genomes comprising the species involved in the cross. This was also reported in a study between diploid Brassica species by Attia and Robellen (1986). In some cells, a chromosome count of 19 was observed (Figure 3.9 and 3.6) which could have resulted from an extra chromosome from *B. napus*. This, however, could not be substantiated and may result from abnormal chromosome division.

3.3.2. Pollen fertility

There was little variation between the BC_1F_1 plants in terms of pollen fertility (Table 3.3.). The difference between the BC_1F_1 plant population was not significant (p=0.05). The mean pollen fertility of 94-1 and 94-2 was 13.6% and 26.8% respectively. The pollen fertility of the F_1 plants was not determined. There were significant differences between the breeding populations and the parent lines. The parents had a high fertility level ranging from 88.8% in one of the *B. juncea* parents to 96.3% in the other *B. juncea* parent. The fertility of the parents, F_1 and BC_1F_1-derived DH plants is given in Table 3.4.

The fertility of the BC_1F_1 plants increased after undergoing microspore culture and subsequent doubling of chromosomes using colchicine. The 94-1 plants' mean fertility increased from 13.6% to 95.6%. The 94-2 BC_1F_1 plants' mean fertility increased from 26.8% to between 93.2% and 96.5% in BC_1F_1-derived DH plants. The F_1-derived DH plants had a fertility of 88.8% in the DH_2 generation. The difference between the plants was not significantly different although the F_1-derived DH plants had a lower fertility than the other lines.

Table 3.2. Chromosomes distribution in the PMC's of BC_1F_1-derived DH plants of (*B. juncea* x *B. napus*) x *B. juncea*.

Plant	No. of Cells	Chromosome Configuration	
		Diakinesis/Prophase/Metaphase	Anaphase
94-1279	19	II $_{14-15}$ - I $_{0-4}$	16-18
94-1280	24	II $_{15?}$ III $_{0-1}$ I $_{0-2}$	18-18
94-1281	27	II $_{14-17}$ III $_{0-1}$ I $_{1-4}$	17-19
94-1282	14	II $_{16-18}$ III $_{0-1}$ I $_{0-2}$	17-18
94-1283	12	II $_{16-18}$ III $_{0-1}$ I $_{0-2}$	18-19
94-1284	23	II $_{11-13}$ III $_{0-1}$ I $_{4-9}$	16-18
94-1286	29	II $_{16?}$ - I $_{2-3}$	16-18
94-1287	21	II $_{15-18}$ III $_{0-1}$ I $_{0-2}$	14-19
94-1288	27	II $_{14-15}$ - I $_{3-4}$	17-19
94-1289	22	II $_{16-18}$ - I $_{1-7}$	18-18
94-1486	2	?	16-18
	220	II $_{14.7?}$ III $_{0-0.6}$ I $_{1-3.9}$	16.6-18.4

Table 3.3. Pollen fertility levels in BC_1F_1 plants determined by aceto-carmine staining.

BC_1F_1 Plant	No. of Plants	No. of Pollen grains Sampled	Mean Fertility	Maximum Fertility	Std Dev
94-1	13	2143	13.6	40.2	12.6
94-2	5	575	26.8	46.2	17.1
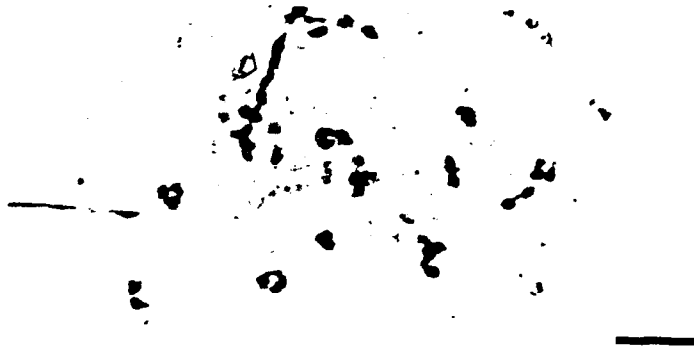
Figure 3.7. PMC of BC_1F_1-derived DH plant 94-1280 at diakinesis showing 17II + 1I Note association of bivalents shown by an arrow (Bar = 10μm)
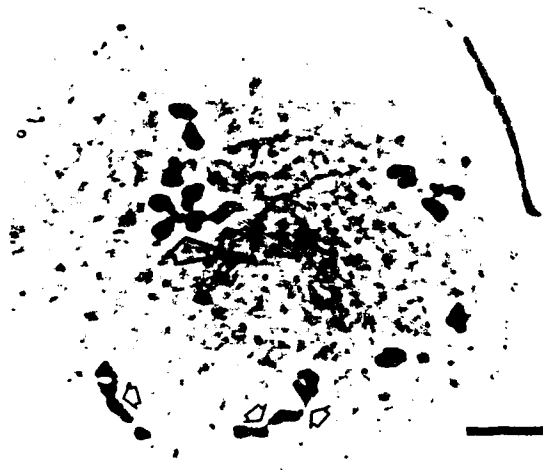
Figure 3.8. Diakinesis of BC_1F_1-derived DH plant 94-1280. The PMC shows 18II. Stickiness is apparent in some of the chromosomes shown by arrows (Bar = 6μm).

Figure 3.9. Metaphase I in BC_1F_1-derived DH plant in 94-1281 The chromosome configuration suggests 18II + 1I. (9 of the bivalents have already divided and diads started to migrate to the opposite poles 10 of the bivalents are still on the metaphase plate) (Bar = 3μm).

Figure 3.10. Early anaphase I of BC_1F_1-derived DH plant 94-1281 showing 17:18 disjunction (Bar = 6μm).
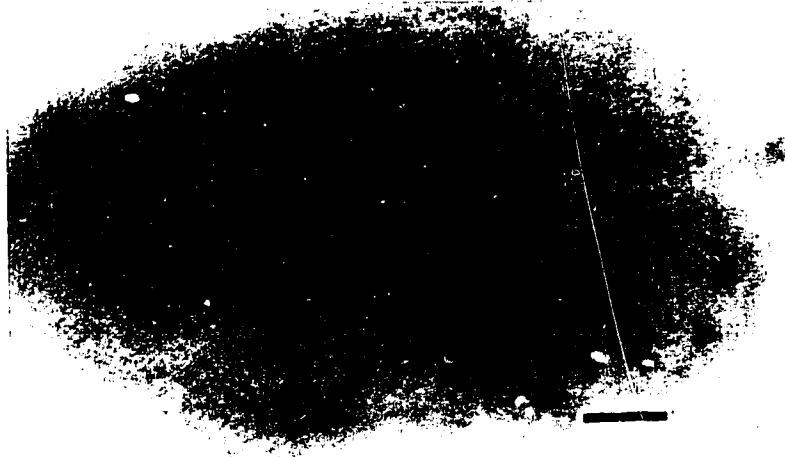
Figure 3.11. Late metaphase I of BC_1F_1-derived DH plant 94-1281 plant showing 18 bivalents. Some of the bivalents have disjoined and diads started to migrate towards the opposite poles (Bar = 4μm).

Figure 3.12. Diakinesis stage of BC_1F_1-derived DH plant 94-1286 PMC showing a 17II + 2I chromosome configuration. The univalents are shown with arrows (Bar = 3μm).

Figure 3.13. Metaphase I of BC₁F₁-derived DH plant 94-1286 with a 17II + 2II configuration Univalents are shown with arrows (Bar 5μm)

Figure 3.14. Anaphase I of plant from BC₁F₁-derived DH plant 94-1288 Separation of the chromosomes at this stage appear normal with a 18 18 chromosome configuration (Bar = 3μm).

3.3.3. Female fertility

Reciprocal crosses were completed for F_1-derived DH lines designated as S4, S6 and S7 (see appendix) and crosses in one direction for F_1-derived DH line S9 using both *B. napus* and *B. juncea*. Fertility index was higher when the parent lines were used as the female in all crosses as compared to the F_1-derived DH lines, but the degree differed from one line to another. When *B. juncea* was used as the female parent the observed fertility was higher than when *B. napus* was used as the female parent. When the *B. juncea* parent was used as the pollen donor, seed set fertility index was higher when compared to *B. napus* (Table 3.5).

Table 3.4. Pollen fertility levels (%) in Parents, F_1-derived DH_2 and BC_1F_1 plants.

Plant	No. of Plants	No. of Pollen grains Sampled	Mean fertility	Maximum Observed Fertility	Std Dev
B. juncea 1	2	269	96.3	96.4	0.1
B. juncea 2	2	150	88.8	91.2	3.4
B. napus	2	334	95.1	95.7	0.8
F_1	10	1272	88.8	95.6	7.1
94-1-5	1	170	95.9	95.9	.
94-2-1	2	344	96.5	98	2.1
94-2-2	5	1017	95.4	100	3.3
94-2-3	12	1557	93.2	100	7.1

Table 3.5. Female fertility of reciprocal crosses between Parents and F_1-derived DH plants of *B. juncea* x *B. napus*.

Cross	No. of Buds Pollinated	No. of Pods at Harvest	No. of Seeds Obtained	Fertility Index (FI)
B. juncea x S4	27	18	66	0.1491
S4 x *B. juncea*	17	17	26	0.0933
B. juncea x S6	32	30	103	0.1963
S6 x *B. juncea*	18	18	4	0.0136
B. juncea x S7	52	38	182	0.2134
S7 x *B. juncea*	43	4	4	0.0057
B. napus x S4	35	11	10	0.0200
S4 x *B. napus*	43	22	5	0.0081
B. napus x S6	39	34	23	0.0412
S6 x *B. napus*	31	27	4	0.0090
B. napus x S7	41	19	5	0.0085
S7 x *B. napus*	20	4	0	0.0000
S9 x *B. juncea*	50	40	55	0.0679
S9 x *B. napus*	37	15	10	0.0189

3.4. Discussion

3.4.1. Cytology

The A-genome chromosomes in the F_1 of a cross between *B. juncea* and *B. napus* are expected to pair, resulting in at least 10 bivalents. If pairing between C and B-genome occurs, more than 10 bivalents may be present. Any occurrence of less than 17 univalents would indicate homoeologous pairing had taken place between the 8 chromosomes of the B-genome and the 9 chromosomes of the C-genome. This would result in possible exchange of genetic material between these genomes. Assuming no allosyndetic pairing of the B and C-genome chromosomes from both parents, only pairing of the A genome chromosomes from *B. juncea* and *B. napus* would occur resulting in 10 bivalents and 17 univalents from B and C-genomes as suggested by Banga (1988). With partial allosyndetic pairing occurring, more than 10

bivalents would be observed and subsequently less than 17 univalents in each PMC. In most of the F_1 and BC_1F_1-derived DH plants in this study, more than 10 bivalents were observed. It has been suggested that homology between the A and B-genome chromosomes is less than between A and C-genome chromosomes (Rajan and Hardas 1963, Attia et al. 1987, Busso et al. 1987 and Namai et al. 1980). In the F_1-derived DH plants in this study, the range of univalents observed was lower than the theoretical expectations. This indicated pairing between the B and C-genomes (Table 3.1). Although Attia et al. (1987) observed a rare complete pairing of A genome in the presence of a single C-genome, 3.5-10 univalents, 11.7 bivalents and 0.7 trivalents were obtained at prophase/metaphase I on average in the 123 PMC's observed in the current study. This suggested a complete A genome chromosome pairing and at least two pairs of chromosomes allosyndetically pairing from the B and C genomes. A low level of chromosome pairing is found between the A and B genomes and between B and C genomes as compared to pairing between A and C genomes (Attia and Robbelen 1986 and Attia et al. 1987). This would suggest pairing occurrs between the A genomes of the parents. This would therefore leave the B and C genomes to pair allosyndetically.

The presence of higher numbers of bivalents than the theoretically expected has been thought to result in more chiasmata formation in the hybrids (Attia et al. 1987) and therefore more chances of genetic material transfer (Ayotte et al. 1988). Such pairing allowed Sacristan and Gerdemann (1986) to transfer disease resistance from *B. nigra* to *B. napus* and Roy (1984) to transfer the blackleg resistance from *B. juncea* to *B. napus* through a series of backcrosses to *B. napus*. This resistance was however lost in subsequent generations because the lines were found to be addition lines. The loss of the extra chromosome in Roy's lines resulted in the loss of blackleg resistance. In the current study at anaphase I the average chromosome count in the F_1-derived DH plants was 22.8-27.6 in each pole. This distribution indicates a possibility of production of unbalanced gametes in the F_1-derived DH plants which may have reduced chromosomal stability. In the BC_1F_1-derived DH plants, a more stable anaphase was obtained with PMC's showing an average of 16.6-18.4

distribution in the poles at anaphase I. Occasionally PMCs with as low as 14 chromosomes at one pole were observed. This was not compensated in the other pole, suggesting complete loss rather than redistribution during metaphase I.

In both *B. juncea* (Prakash and Hinata 1980) and *B. napus* (Attia and Robbelen 1986) homoeologous chromosome pairing from the two diploid ancestors constituting the two amphidiploid species occurs at a very high frequency at meiosis in artificially synthesized *B. juncea* and *B. napus*. This occurs at a reduced frequency in amphihaploid DH plants derived from established amphidiploid cultivars. This is as a result of amphihaploid plants inheriting or evolving mechanisms that prevent homoeologous recombination (Lydiate et al. 1993). In the presence of one single C-genome, complete pairing of two A-genomes was found to be very rare in digenomic triploids (Attia et al. 1987). Pairing was not restricted to only the A homologues, but extended to include the C genome. On the other hand, no tendency of allosyndetic pairing was found between the B and C genomes. This was contrary to findings of Mizushima (1968) who reported four bivalents following hybridization between *B. nigra* (BB) and *B. oleracea* (CC). Banga (1987) observed as many as three bivalents in a spontaneous haploid of *B. carinata* (BC). Banga found that in the presence of one B genome two A genomes exhibited complete homologous pairing resulting in a $I_9 + II_{10}$ configuration. In a cross between *B. napus* and *B. nigra*, in generation of addition lines the trigenomic hybrid when backcrossed to *B. napus* were found to have a $I_{9.41} + II_{16.24} + III_{0.21} + IV_{0.87}$ against a theoretical $I_8 + II_{19}$ chromosome distribution if no promoters or inhibitors were involved (Jahier et al. 1989). The single F_1 plant obtained from the cross in this study had $I_{8.13} + II_{7.26} + III_{0.27} + IV_{0.81} + V_{0.06}$ at meiosis. The F_1 had 18% quadrivalent or pentavalent pairing. Because of the deviation from theoretical expectations, it was reasoned that the B genomes must be influencing the chromosome arrangements at meiosis. According to Banga (1988) there are a few homoeologous regions in some chromosomes belonging to the B and C genomes where pairing is possible. This may influence and disrupt the genome organization resulting in exchange of genetic material. The evidence from these studies show that possibilities exists of C genome chromosomes from *B. napus*

pairing strongly enough not to dissociate (separate) with B genome chromosomes on backcrossing to *B. juncea*. This would result in transfer of traits to *B. juncea* with most of the *B. juncea* characteristics remaining intact. The 2 univalents observed in Figures 3.12 and 3.13 could be a chromosome from *B. juncea* pairing loosely with a chromosome from *B. napus*. On backcrossing, low homology between B and C genomes caused the dissociation of the chromosomes. This resulted in two separate chromosomes with the other pairs coming from the AB genome of *B. juncea*.

In a cross between *B. napus* and *B. juncea* (AB) and between *B. napus* (AC) and *B. carinata* (BC) followed by backcrossing to *B. napus*, Rashid et al. (1994) were able to recover yellow seeds from subsequent generations. This showed that interactions between the A and C genome chromosomes occur. According to Busso et al. (1987) pairing between trigenomic hybrids is assumed to occur mostly between the A and C-genomes, however since less than eight univalents were found in their study involving ACB trigenomic hybrids, some chromosomes from the B-genome must have been involved in the pairing between A and C-genome chromosomes. This was again demonstrated by Zaman (1989) who was able to show introgression of A genome chromosome(s) or gene(s) from *B. rapa/B. juncea* not only into the A genome of *B. napus* but also into the C-genome.

Tai and Ikonen (1988) reported an allosyndetic pairing between the A and C genomes at the rate of II $_{7.73}$ in dihaploids from *B. napus*. Prakash and Hinata (1973) and Mizushima (1950) showed that B genome chromosomes paired infrequently with those of A and C genomes. In the studies with resynthesized *B. juncea*, Rajan and Hardas (1963) found that B genome chromosomes manifested homology only with A genome chromosomes alien to *B. juncea* and originating from another source but not from digenomic species used in its resynthesis. This would then imply that the A genome from *B. napus* would be able to pair with B genome chromosomes from *B. juncea*. On backcrossing the F$_1$, the chromosome pairing would favour the *B. juncea* parents resulting in chromosome configuration of the *B. juncea* type. Backcrossing *B. naporapa* with *B. napus*, McNaughton (1973) showed that stabilization of the cross eventually results from selective elimination of the foreign A-genome leaving the

recurrent parent genomes. There is a possibility of the *B. napus* chromosomes being eliminated as observed by James (1978) in wheat, or suppression of pairing occurring between the genomes as documented in several studies (Rajan and Hardas 1963, Prakash 1974, Lydiate 1993, Song et al. 1993). This leaves the *B. juncea* chromosomes to pair among themselves giving rise to PMC's having 18 chromosomes. Sunberg and Glimelius (1991) found chromosome elimination to occur when species with different ploidy levels are combined. This was thought to occur as a result of dissimilarities within the genomes and manifested in the different cell cycle times in the nucleus.

In the BC_1F_1-derived DH plants of the present study, a relatively higher number of bivalents was obtained (Table 3.1) ranging from 14-18 as compared to F_1-derived DH plants with 7-14 bivalents. The reduction in the univalents observed and the increase in the bivalents found in the BC_1F_1-derived DH plants indicate greater stability. This was also reflected in the quantity of seed set. Each chromosome from synthetic *B. napus* is thought to have a range of partial or complete homoeologues in the A and C-genomes composing them (Lydiate 1993), enabling partial or complete binding with other chromosomes. Although it has proven difficult to associate specific changes to acquisition of specific chromosomes (Chevre et al. 1991 and McGrath and Quiros 1990), the fact that more bivalents than expected were formed in the F_1-derived DH plants in this material could have resulted in exchange of genetic material during chiasma formation. The presence of loosely associated chromosomes in the BC_1F_1-derived DH lines would attest to this. The presence of extra chromosome(s) has been associated with qualitative changes expressed in the plant. Chen et al. (1992) observed a dramatic increase in erucic acid content of the monosomic addition line attributed to the removal of the eight C-genome chromosomes belonging to the C-genome in *B. rapa-alboglabra* addition lines. The presence of many univalents in the F_1-derived DH lines may have been responsible for the low female fertility observed in the lines studied. Because of an increase in the bivalent frequency in the BC_1F_1-derived DH plants, a high female and male fertility was observed (Table 3.2). The individual BC_1F_1-derived DH plants with a high univalent frequency also showed a reduced seed set in the field. Although no drastic phenotypic alterations were observed in the BC_1F_1-

derived DH lines as compared to the *B. juncea* parent, changes at the physiological level are possible (Banga 1988, Chen et al. 1992, Cheng et al. 1994).

3.4.2. Male and Female Fertility

The fertility of the breeding populations differed considerably before and after microspore culture. The lower fertility shown by F_1 plants could be as a result of chromosomal aberrations common in interspecific crosses. Several studies reported similar results (Roy 1984, Olsson 1986, Sabharwal and Dolezel 1993). Subsequent microspore culture followed by colchicine treatment of the F_1 and BC_1F_1 plants 94-1 and 94-2 resulted in increased pollen fertility. The fertility of the F_1-derived DH_2 plants however was still lower than that shown by BC_1F_1-derived DH plants obtained by backcrossing the F_1 plants to *B. juncea*. The high fertility shown by these plants suggests a more stable chromosome division resulting in fertile pollen. This is confirmed by the higher seed set shown by the BC_1F_1-derived DH plants. Although the level of pollen fertility shown by F_1-derived DH_2 plants was high, it was not reflected in greater seed set. This was also reported by Lee and Namai (1992). The seed set in F_1-derived DH lines was generally poor as compared to DH lines derived from BC_1F_1 plants. This would suggest that F_1 plants had fertile pollen grains that could not effectively self-fertilize. The F_1-derived DH plants could have had problems associated with reduced maternal female fertility as suggested by Meng and Zhou (1988), Meng and Yi (1988) and Meng and Lu (1993). Most of the pods formed after self pollination were empty, despite normal pod development, which was consistent with observations made by Sabharwal and Bechyne (1991). Some of the pods had shrunken seeds which did not germinate. A single backcross seemed to have resulted in more stability of the reproductive process resulting in improved seed setting in selfed BC_1F_1-derived DH plants. This could be as a result of improved pairing of chromosomes from the same genome resulting in more even chromosome distribution during meiosis. The resulting pollen would be more likely to effect self-fertilization without the attendant problems associated with interspecific hybrids noted by Nishiyama et al. (1991).

Reciprocal crossing of the F_1-derived DH lines with their parents confirmed that *B. juncea* is more successful as a maternal parent as compared to *B. napus*. This was also reported by Sabharwal and Bechyne (1991) and Sabharwal and Dolezel (1993). In most crosses, the fertility index was 3-7 times higher in *B. juncea* than in *B. napus*. The fact that pollen from F_1-derived DH lines resulted in a higher seed set when used as pollinators, confirms the earlier observation of high pollen fertility. The pollination of the F_1-derived DH plants with pollen from the parent lines resulted in poor seed set, strongly suggesting reduced female fertility of the F_1 lines. The fact that the pollen had a higher fertility and an ensuing high fertilization rate to the parent lines would indicate chromosomal abnormalities in the female gametes, resulting in pairing problems. If pollen had chromosomal problems, presence of empty grains (non staining with acetocarmine) would have been observed in the F_1-derived DH lines. This was not the case in the present material. Incompatibility reactions between pollen and pistil resulting in disorientation of growth of the pollen tubes in the ovary and arrested development of embryos have also been offered as explanations for reduced female fertility (Dolstra 1982, Meng and Zhou 1988). Heavy deposition of callus after pollination has been found to affect pollen germination in an interspecific cross between *B. napus* and *B. juncea* (Meng 1987 and Meng and Yi 1988). Since pollen fertility of both *B. juncea* and *B. napus* in the present study was both 96% and 95% (Table 3.2), it can be concluded that the F_1-derived DH plants had reduced female fertility, which stabilized on backcrossing to *B. juncea*. The male fertility, however, was not affected both in F_1 and BC_1F_1-derived DH lines.

References

Attia T. and G. Robbelen 1986. Cytogenetic relationship within cultivated *Brassica* analyzed in amphihaploids from the three diploid ancestors. Can. J. Genet Cytol. 323-329.

Attia T., C. Busso and G. Robbelen 1987. Digenomic triploids for an assessment of chromosome relationships in the cultivated diploid *Brassica* species. Genome 29: 326-330.

Ayotte R., P.M. Harney and V.S. Machado 1988. The transfer of triazine resistance
from *Brassica napus* L. to *B. oleracea* L. II. Morphology, fertility and cytology
of the F₁ hybrid. Euphytica 37: 189-197.

Banga S.S. 1987. Spontaneous haploidy in *Brassica carinata*. SABRAO J. 19(1): 69-
70.

Banga S.S. 1988. C-genome chromosome substitution lines in *Brassica juncea* (L.)
Coss. Genetica 77: 81-84.

Busso C., T. Attia and G. Robbeъen 1979. Trigenomic combinations for the analysis of
meiotic control in the cultivated *Brassica* species. Genome 29: 331-333.

Chen B.Y., V. Simonsen, C. Lanner-Herrera and W.K. Heneen 1992. A *Brassica*
campestris-alboglabra addition line and its use for gene mapping, intergenomic
gene transfer and generation of trisomics. Theor Appl Genet 84: 592-599.

Cheng B.F., W.K. Heneen and B.Y. Chen 1994. Meiotic studies on a *Brassica*
campestris-alboglabra monosomic addition line and derived *B. campestris*
primary trisomics. Genome 37: 584-589.

Chevre A.M., F. Eber, E. Margale, M.C. Kerlan, C. Primard, F. Vedel, M. Delseny and
G. Pelletier 1993. Comparison of somatic and sexual *Brassica napus-Sinapis alba*
hybrids and their progeny by cytogenetic studies and molecular
characterization. Genome 37: 367-374.

Chevre A.M., P. This, F. Eber, M. Deschamps, M. Renard, M. Delseny and C.F.
Quiros 1991. Chaıacterization of disomic addition lines *Brassica napus - Brassica*
nigra by isozyme, fatty acid and RFLP markers. Theor Appl Genet 81: 43-49.

Dolstra O. 1982. Synthesis and fertility of *Brassicoraphanus* and ways of transferring
Raphanus characters to *Brassica*. Agric. Res. Rep. 917. Pudoc, Wageningen,
pp1-90.

Gomez-Campo C. 1980. Morphology and morpho-taxonomy of the tribe *Brassiceae*.
In S. Tsunoda, K. Hinata and C. Gomez-Campo (Eds.), pp 3-31, *Brassica*
crops and wild allies: Biology and Breeding. Japan Sci. Soc. Press, Tokyo.

Harberd D.J. and E.D. McArthur 1980. Meiotic analysis of some species and genus
hybrids in the Brassiceae. *In Brassica* crops and wild allies, biology and

breeding. Edited by Tsunoda S., K. Hinata and C. Gomez-Campo. Japan Scientific Societies Press, Tokyo pp. 65-87.

Heslop-Harrison J., Y. Heslop-Harrison and R.K. Shivana 1984. The evaluation of pollen quality and a further appraisal of the fluorochromatic (FCR) test procedure. Theor Appl Genet 67: 367-375.

Iwanami Y., T. Sasakuma and Y. Yamada 1988. *In* Pollen: Illustrations and scanning electromicrographs. Springer- Verlag New York.

Jahier J., M. Chevre, A.M. Tanguy and F. Eber 1989. Extraction of disomoic addition lines of *Brassica napus* - *B. nigra*. Genome 32: 408-413.

James J. 1978. New maize x Tripsum hybrids for maize improvements, Euphytica 28: 239-247.

Kianian S.F. and C.F. Quiros 1992. Trait inheritance fertility and genomic relationships of some n=9 *Brassica* species. Genetic Resources and crop Evolution 39: 165-175.

Lee K.H. and H. Namai 1992. Pollen Fertility and seed set Percentage after backcrossing of sesquidiploids (AAC genomes) derived from interspecific hybrid between *Brassica campestris* L. (AA) and *B. oleracea* L. (CC) and frequency distribution of aneuplcids in the progeny. Japan. J. Breed. 42: 43-53.

Lydiate D., A. Sharpe, U. Lagercrantz and I. Parkin 1993. Mapping the *Brassica* genome. Outlook on Agriculture 22: (2) 85-92.

McGrath J.M. and C.F. Quiros 1990. Generation of alien chromosome addition lines from synthetic *Brassica napus* : morphology, cytology, fertility and chromosome transmission. Genome 33: 374-383.

McNaughton I.H. 1973. *Brassica napocampestris* L. (2n=58). 1. Synthesis, Cytology, Fertility and general considerations. Euphytica 22: 301-307.

Meng J. 1987. Study advances on distant hybridization incompatibility in *Brassica*. Oil crops of China 4: 71-77.

Meng J. and G. Yi 1988. Studies on the embryology of reciprocal crosses between *Brassica napus* and *B. juncea*. Sci Agric Sin 21: 46-50.

Meng J. and J. Zhou 1988. The interspecific compatibility of *Brassica napus* x *B. oleracea*. II. The influence of maternal genotypes on the pollen-pistil interaction. J. Huazhong Agric Univ 7: 111-117.

Meng J. and M. Lu 1993. Genotype effects of *Brassica napus* on its reproductive behaviour after pollination with *B. juncea*. Theor Appl Genet 87: 238-242.

Mizushima U. 1950. Karyogenetic studies of species and genus hybrids in the tribe *Brassiceae* of cruciferae. Tohoku J. Agric. Res. 1: 1-14.

Mizushima U. 1968. Phylogenetic studies on some wild *Brassica* species. Tohoku J. Agric. Res. 19: 83-99.

Newcomer E.H. 1953. A new cytological and histological fixing fluid. Science 118: 161.

Nishiyama I., M. Sarashima and Y. Matsuzawa 1991. Critical discussion on abortive interspecific crosses in *Brassica*. Plant Breeding 107: (4) 288-302.

Olin-Fatih M. and W.K. Heneen 1992. C-banded karyotypes of *Brassica campestris*, *B. oleracea* and *B. napus*. Genome 35: 583-589.

Olsson G. 1986. Alloploids in *Brassica*. *In* Olsson G. (ed) Svalof 1886-1986: Research results in plant breeding Lts forlag, Stockholm, pp114-119.

Prakash S. and K. Hinata 1980. Taxonomy, cytogenetics and origin of crop *Brassicas*, a review. Opera Bot. 55: 1-57.

Prakash S. 1973. Non homologous meiotic pairing in the A and B genomes of *Brassica*: its breeding significance in the production of variable amphidiploids. Genet Res 21: 133-137.

Prakash S. and V.L. Chopra 1990. Reconstruction of allopolyploid *Brassicas* through non-homologous recombination: Introgression of resistance to pod shatter in *Brassica napus*. Genet Res. Camb. 56: 1-2.

Rajan S.S. and M.W. Hardas 1963. Autosyndetic and allosyndetic pairing in *Brassiceae*. Indian Journal of Genetics and Plant breeding 24: (1) 15-21.

Rashid A., G. Rakow and R.K. Downey 1994. Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breeding 112: 127-134.

Roy N.N. 1984. Interspecific transfer of *Brassica juncea* type high blackleg resistance to *Brassica napus*. Euphytica 33: 295-303.

Sabharwal P.S. and J. Dolezel 1993. Interspecific hybridization in *Brassica*: Application of flow cytometry for analysis of ploidy and genome composition in hybrid plants. Biologia Plantarum 35(2) 169-177.

Sabharwal P.S. and M. Bechyne 1991. Morphology of F1- Interspecific hybrids of cross *Brassica napus* L. X *Brassica juncea* L. Genet. a Sletcht. 27(2-3) 87-95.

Sacristan M.D. and M. Gerdemann 1986. Different behaviour of *Brassica juncea* and *B. carinata* as sources of *Phoma lingam* resistance in experiments of interspecific transfer to *Brassica napus*. Plant Breeding 97: 304-314.

Shivanna K.R. and N.S. Rangaswamy 1992. Pollen Biology: In A laboratory Manual. Springer, Berlin Heidelberg New York.

Sjodin C. and K. Glimelius 1989. *Brassica naponigra*, a somatic hybrid resistant to *Phoma lingam*. Theor Appl Genet 77: 651-656.

Song K., K. Tang and T.C. Osborn 1993. Development of synthetic *Brassica* amphidiploids by reciprocal hybridization and comparison to natural amphidilpoids. Theor Appl Genet 86: 811-821.

Stringam G.R. 1970. A simple mordant technique for plants with small chromosomes. Can. J. Bot. 48: 1134-1135.

Struss D., U. Bellin and G. Robbelen 1991. Development of B-Genome chromosome addition lines of *B. napus* using different interspecific Brassica hybrids. Plant Breeding 106: 209-214.

Tai W. and H. Ikonen 1988. Incomplete bivalent pairing in dihaploids of *Brassica napus* L. Genome 30: 450-457.

Wang X.H., P. Luo and J.J. Shu 1989. Giemsa N-banding patterns in cabbage and Chinese kale. Euphytica 41: 17-21.

Zaman M.W. 1989. Introgression in *Brassica napus* for adaptation to the growing conditions in Bangladesh. Theor Appl Genet 77: 721-728.

Chapter 4

Agronomic Performance

4.1. Introduction

In Brassica crops, considerable breeding efforts have been directed to improving production potential as well as stability (Prakash 1980). High seed yield in *B. napus* has most commonly been associated with the production of large numbers of pods per plant or per unit area. This has been reported in diverse environments such as the United Kingdom (Allen and Morgan 1972) and western Australia (Thurling 1974b).

Other associated traits such as dry matter accumulation, growth rate and development area indices of different plant parts in *B. napus* have been investigated by Inanaga and Kumura (1974), Thurling (1974a), Allen and Morgan (1975) Tayo and Morgan (1975), Clarke and Simpson (1978), Kasa and Kondra (1986), Augustinussen (1987), Mendham et al. (1990), Morrison et al. (1990) and Kjellstrom (1993). The relationships between seed yield and growth characteristics have been studied by Degenhardt and Kondra (1984), Thurling (1974b) Chay and Thurling (1989) and Varshney et al. (1990). In *B. juncea*, the relationships between growth rate factors, growth and yield, have been analyzed by Roy et al. (1981), Chauhan and Bhargava (1984) and Bhargava and Tomar (1988).

4.1.1. Phenotypic characteristics

A high crop growth rate during vegetative development is an important pre-requisite for high seed yield (Mendham and Scott 1975). The yield advantage of *B. rapa* over *B. napus* has been attributed to a greater assimilate source accumulated before anthesis and available for mobilization to the developing seeds after anthesis (Richards and Thurling 1978c). Studies with winter cultivars of *B. napus* (Mendham and Scott 1975) indicated that the plant must attain some critical size at the time of floral initiation for maximum yield in a given environment. The rate of growth in the early stages could be used as an indicator of vigour at a given time as it reflects the amount of dry matter accumulated by the plant (Clarke and Simpson 1978).

114

The initiation of flowering is a major developmental event and the time between sowing and flowering is another important determinant of yield in short-season environments. Studies have generally shown flowering time to be highly heritable and is often determined by relatively few major genes (Thurling 1993). Flowering time thus appears to have potential as a selection criteria for yield in early generations. Early flowering is essential to the avoidance of stress later in the season in spring cultivars. The days to flowering are important because more than 75% of the pods retained at maturity are formed from flowers opening within 14 days of anthesis (Tayo and Morgan 1975). These flowers are mainly on the terminal raceme and on the basal and middle regions of the axillary inflorescence.

Pods are important as photosynthetic organs (Clarke and Simpson 1978, Chapman et al. 1984, Major et al. 1978). Pod growth and ripening is characterized by a period of rapid growth of older pods on the terminal inflorescence, axillary inflorescences, and abscission of some of the younger and more apically-positioned pods (Allen and Morgan 1975). The contribution of assimilates from pods to rapidly developing seeds are nearly equal to the assimilates from leaves (Brar and Thies 1977), however, the leaves exerts an early effect on seed yield by influencing development of pods (Clarke and Simpson 1978).

Harvest index is a trait that has been extensively studied, especially as an alternative criteria of selection for high yield. Harvest index is defined as the ratio of grain yield to total biomass yield (Donald 1962, Huhn et al. 1991). The harvest index is a relatively simple character to measure although it is a manifestation of complex photosynthetic, translocatory and storage processes associated with seed development (Thurling 1993). Harvest index (HI) has increased with yield improvements in European wheat cultivars even without a conscious effort to select for it. This has however had little effect on biomass production (Austin et al. 1980). Harvest index is highly stable under different environmental conditions (Austin 1980).

4.1.2. Water stress resistance

Plant water relations in Brassica have been studied in an effort to evaluate genotypes suitable to drier areas which are traditionally not suitable to conventional growing of Brassica crops. Woods et al. (1991) compared *B. juncea, B. napus* and *B. rapa* in Western North America. In the Woods et al. (1991) study, *B. juncea* in more than one location of these trials survived drought and shattering, whereas, *B. napus* and *B. rapa* did not. In the Brassica, it has been shown that there is variation in seed yield response to drought both between and within *B. rapa* and *B. napus* (Richards and Thurling 1978a, 1978b). *B. juncea* has a greater ability to extract water from the soil, especially when limited amounts are available. A small loss of water in *B. juncea* results in a higher water potential than *B. napus* (Kumar and Elston 1992). Several methods have been used to estimate various components of water stress. Some of the methods used include water retention in leaves (Oleinikova and Kozhushko 1970, Sandhu and Laude 1958, Salim et al. 1969), leaf water status (Todd and Webster 1965), and electrolyte leakage (Blum and Ebercon 1981, Vasquez et al. 1990).

4.2. Objectives

The objective of this study was to assess the agronomic characteristics of the BC_1F_1 and F_1-derived DH lines developed and described earlier and to determine the degree of introgression of traits from *B. napus* to the BC_1F_1-derived DH plants.

4.3. Material and Methods

4.3.1. Field establishment

The experimental plots were established at the University's Edmonton Research Station Farm at Michener ($53^0 24'N$ latitude $113^0 31'W$ longitude) on black chernozemic soils. Eighteen lines, including two *B. juncea* and one *B. napus* parents were seeded at a rate of 6 kg ha^{-1} using Swift current Fabro four row plot seeder on May 9, 1995. The fertilizer (11-51-00 of NPK) was applied at the rate of 50 lbs/acre to the soil before ploughing in. The lines were grown in four-row plots six metres long replicated three times. Those lines with insufficient seed were planted in two-row plots.

116

Two guard rows were sown around the experiment. Weeds were kept to a minimum by rogueing. Assessment of agronomic and morphological traits based on descriptors by IPBGR (1987, 1990) began 1 month following sowing. The presence or absence of anthocyanin in the leaf was determined one month after planting by observing purple colouration of petioles and stems. A sample was observed at four random spots in all the plots, and presence or absence of the colouration noted. Plant heights were recorded 30 days after sowing to estimate the relative growth rates of the BC_1F_1 and F_1-derived DH lines compared to the parent plants. Four plants were sampled for height at random from the plot. In the two-row plots, two samples were taken. Days to flowering were determined from the day of planting until 50% of the plants had flowered. This was done by observing the plots every day and estimating the time in which 50% of the plants flowered. Primary branching character was observed by counting the number of primary branches from the main raceme. Sample size consisted of four plants selected at random from three replicates. Pods per plant were determined by selecting four plants at random in plots and counting individual pods present per plant. Within the plant, six pods were sampled from the main raceme starting at the base. Pod length and seeds per pod were determined from the six pods. The number of pods on the main raceme were determined from four standing plants in the plots. Seed yield per plant was determined by harvesting four plants at random from each plot, allowing them to dry in the field for three days, and then transferring them into a drying shed to air dry for five days. Individual plants were then weighed to obtain total dry weight per plant, threshed and the seed cleaned and weighed. Harvest index was calculated from seed yield per plant and total dry matter yield. From seed obtained on a single plant yield, a random sample was taken and 200 seeds counted and weighed. Seeds obtained from the single plant harvest was retained for fatty acids and meal quality analysis discussed in chapter 5. Statistical analysis of variance and regression was done using glm procedure of the SAS statisical program (1989).

4.3.2. Water stress resistance

Ten leaf discs 8mm in diameter from leaf numbers three and six were harvested from green house plants grown as previously described in Chapter 2. The discs were then incubated in 10 ml of 10%, 20% 30% and 40% (w/v) of Polyethylene Glycol 8000 (formally 6000) obtained from Sigma dissolved in de-ionized doubled distilled water (Bansal et al. 1991). A control consisting of the same number of discs in 10ml de-ionized water was included. The vials were kept at 10 ^0C for 24 hours (Blum and Ebercon 1981). The discs were then rapidly washed three times in distilled water and incubated again for 24 hours in distilled water. The vials were then well shaken, and conductivity from the solution determined using conductivity meter CDM 83 (Copenhagen). The vials were heated in an oven for three hours at 100 ^0C to kill the tissues and left overnight to equilibrate at room temperature. The conductivity of the solution was determined after shaking. Leak..ge was determined by taking the ratio of first conductivity reading (T1) to the second conductivity reading (T2). Reading obtained from the control (C1) and (C2) were used to calculate injury level percentages using the method of Blum and Ebercon (1981):

$$\% \text{ Injury} = 1 - \{1 - (\frac{T1}{T2}) / 1 - (\frac{C1}{C2})\} \times 100$$

Where

T_1 = First conductivity measurement
T_2 = Second conductivity measurement
C_1 = First conductivity measurement of control
C_2 = Second conductivity measurement of control

4.4. Results

4.4.1. Morphological traits

4.4.1.1. Flower and leaf

The morphology of the BC_1F_1-derived DH lines closely resembled the *B. juncea* parents in most of the leaf and flower characteristics studied. All plants with the exemption of F_1-derived DH plant 94-1291 did not express any anthocyanin in the cotyledons and stems (Table 4.1). The leaves were smooth in seven of the eleven BC_1F_1-derived DH plants with 4 of the lines showing hairy leaf trait. In the F_1-derived

DH plants, only 1 line, 94-1291, had smooth leaves, while the other lines expressed the hairy leaf trait. The lobing of the leaf margin in F_1-derived DH genotypes was rounded (IPBGR 1987, 1990). The BC_1F_1-derived DH lines showed a mixture of both acute and rounded lobes in seven-and four lines respectively. The leaf lamina shape observed in most of the genotypes resembled the *B. juncea* parent with wide elliptic (WE) leaves.

Three BC_1F_1-derived DH lines had orbicular (ORB) leaf shapes (Table 4.1). One line had a narrow elliptic (NE) leaf type found in the *B. napus* parent (IPBGR 1987, 1990). Only 1 F_1-derived DH line had orbicular leaf shape while the rest had wide elliptic leaf lamina shape. The margin dentation in F_1-derived DH lines was undulate with only one line showing rounded dentation. Amongst the BC_1F_1-derived DH lines were 2 lines with sharp and 9 lines with rounded teeth margin dentation. All BC_1F_1-derived DH lines had elongated bract shapes with small dentations as in the *B. juncea* parent (Table 4.2).

4.4.1.2. Pod length

There were significant differences (p=0.05) between the parents and progeny for pod length. *B. napus* had the longest pods (mean length 6.13 cm). The BC_1F_1-derived DH plant 94-1284 had the shortest pods at 2.55 cm. Most lines had *B. juncea*-type pods. There was a significant difference in pod length between the parent *B. napus* and *B. juncea*, BC_1F_1 and F_1-derived DH lines.

4.4.2. Agronomic characteristics

4.4.2.1. Plant height

Plant height was measured to estimate the relative vigour of different genotypes. *B. juncea* parents 1 and 2 had the most vigour with *B. juncea* parent 2 showing superior vigour to all other parents. The *B. napus* parent had the poorest growth vigour at 30 days with a height of 4.17 cm. This was significantly different (p=0.05) from both *B. juncea* parents. The BC_1F_1 and F_1-derived DH plants were not significantly different amongst themselves, but were significantly different from both parents. The height ranged from 10.67 cm in *B. juncea* to 2.0 cm in the F_1-derived DH plant 94-1290.

119

Table 4.1. Some Leaf morphological characteristics of parents, F_1 and BC_1F_1-derived DH genotypes of *B. juncea* x *B. napus* interspecific crosses.

Genotype		Anthocyanin	Hairs	Lobing	Lamina Shape	Margin Denition	Type of Leaf
Parent	*B. juncea* 1	2	2	Rounded	WE	Sharp teeth	1
	B. juncea 2	2	2	Rounded	WE	Sharp teeth	1
	B. napus	2	1	Acute	NE	Undulate	2
BC₁F₁ Derived	94-1279	2	2	Rounded	ORB	Sharp teeth	1
	94-1280	2	1	Acute	WE	Rounded	1
	94-1281	2	1	Acute	WE	Rounded	2
	94-1282	2	1	Rounded	ORB	Rounded	1
	94-1283	2	1	Acute	WE	Rounded	1
	94-1284	2	2	Acute	WE	Rounded	1
	94-1286	2	1	Acute	WE	Rounded	1
	94-1287	2	1	Acute	WE	Sharp teeth	1
	94-1288	2	1	Acute	NE	Rounded	1
	94-1289	2	2	Rounded	WE	Rounded	2
	94-1486	2	2	Rounded	ORB	Rounded	1
F₁ Derived	94-1290	2	2	Rounded	WE	Undulate	1
	94-1291	1	1	Rounded	WE	Undulate	2
	94-1292	2	2	Rounded	WE	Undulate	1
	94-1293	2	2	Rounded	WE	Undulate	1
	94-1295	2	2	Rounded	ORB	Rounded	2

Anthocyanin=1-Present, 2-Absent, Hairiness=1-Smooth, 2-Hairy; Leaf Type=1-Petiolate, 2-Lyrate Lamina Shape (Width /Length)=WE-Wide Elliptic (WE=0.67-0.79), ORB-Orbicular (>0.80), NE-Narrow Elliptic (<0.66).
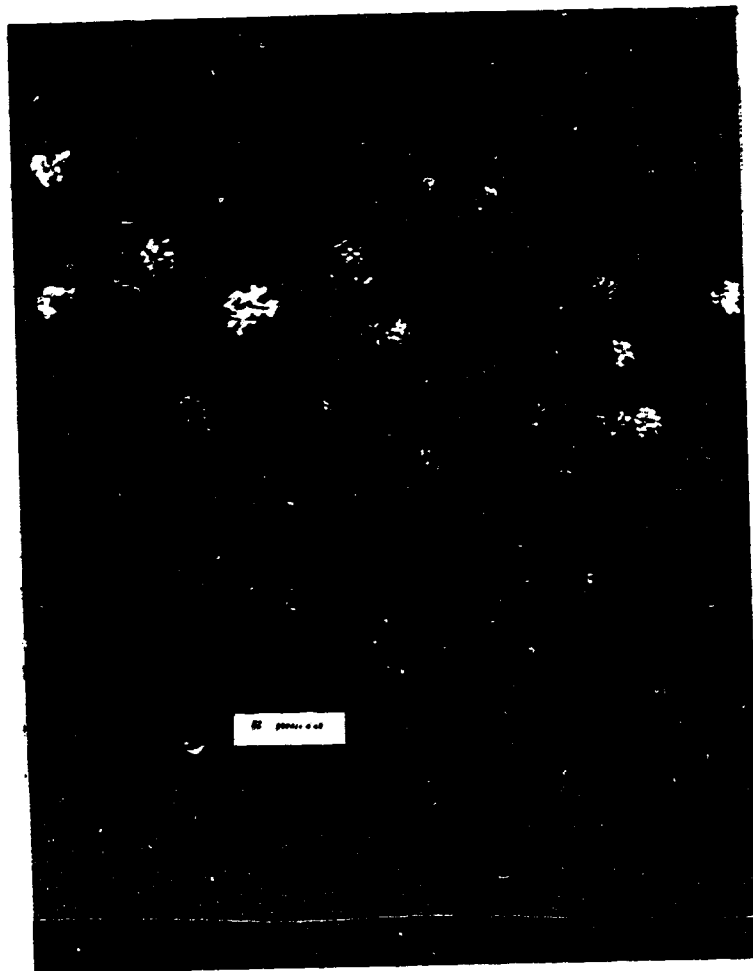
Figure 4.1. Parent plant *B. juncea* 1 near the end of the flowering period. Note the relatively large number of pods.

Figure 4.2. Parent plant line *B. napus*, showing the relatively fewer number of pods, branching and mid flowering as compared to *B. juncea*. The parent line was sown concurrently with *B. juncea* parent shown in Figure 4.1

Table 4.2. Inflorescence characteristics of parents, F_1 and BC_1F_1-derived DH genotypes in *B. juncea* x *B. napus* interspecific crosses.

Genotype		Nature of Flower	Bract	
			Shape	Dentition
Parent	*B. juncea* 1	1	Elongate	Small teeth
	B. juncea 2	1	Elongate	Small teeth
	B. napus	2	Broad Auricles	Coarse Small teeth
F_1-Derived	94-1291	2	Elongate	Coarse Small teeth
	94-1295	3	Broad	Coarse Small teeth
	94-1290	3	Elongate	Smooth
	94-1292	2	Broad Auricles	Sparse Coarse teeth
	94-1293	3	Broad	Sparse Coarse teeth
BC_1F_1-Derived	94-1279	1	Elongate	Small teeth
	94-1280	1	Elongate	Small teeth
	94-1281	1	Elongate	Small teeth
	94-1282	1	Elongate	Small teeth
	94-1283	1	Elongate	Small teeth
	94-1284	1	Elongate	Small teeth
	94-1286	1	Elongate	Small teeth
	94-1287	1	Elongate	Small teeth
	94-1288	1	Elongate	Small teeth
	94-1289	1	Elongate	Small teeth
	94-1486	1	Elongate	Small teeth

Flower type=1-*B. juncea*, 2-*B. napus*, 3-Other
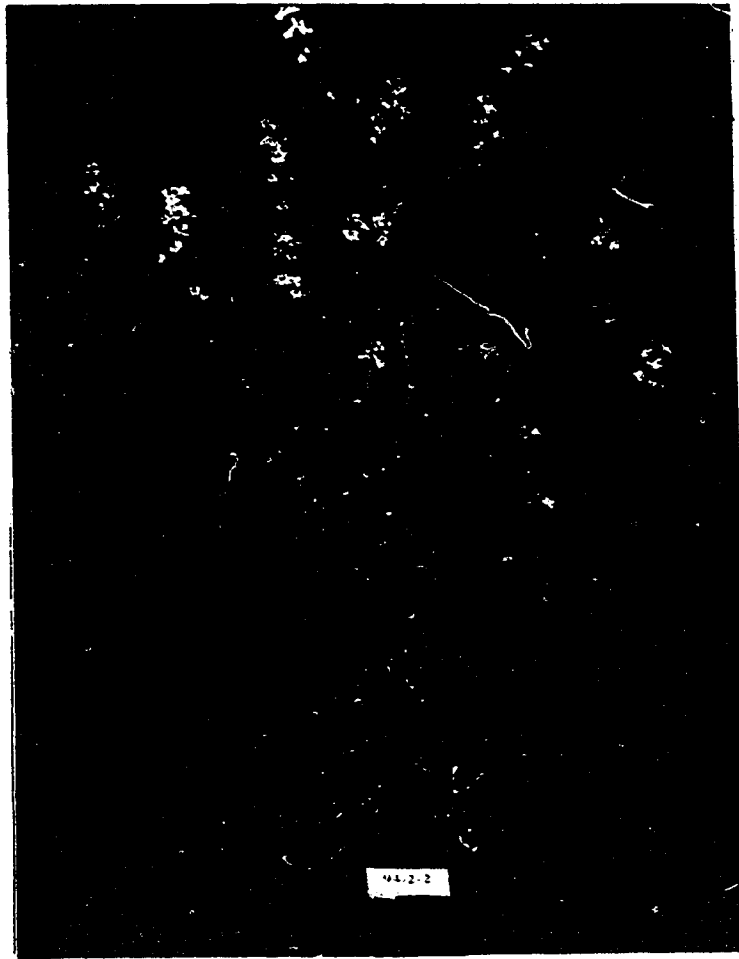
Figure 4.3. BC_1F_1-derived DH plant. The earliness relative to the F_1-derived DH plant (Figure 4.4) is evident. The number of pods present on this plant was greater than those observed in the F_1-derived DH plant.

124
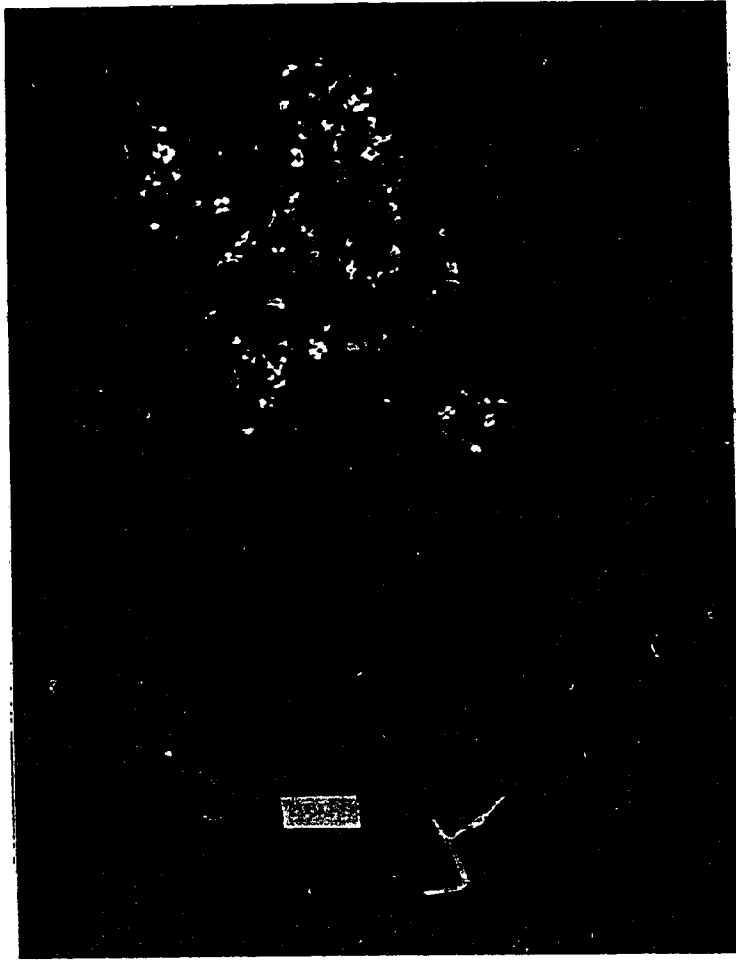
Figure 4.4. F₁-derived DH plant derived from the cross *B. juncea* x *B. napus*. The relative morphological similarity to *B. napus* (Figure 4.2) is evident. Note the developing pods in this plant are fewer than those in BC₁F₁-derived DH plant (Figure 4.3).
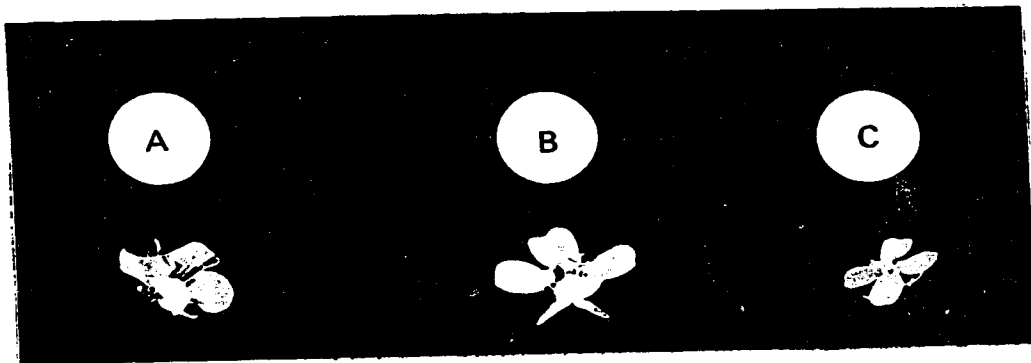
Figure 4.5. Comparison of flower morphology of parent lines, *B. juncea, B. napus,* and F_1-derived DH plants. The flowers of the F_1-derived DH plants are larger, less intense in colour with petals more separated than that in the *B. juncea* parent. (A= *B. napus,* B= F_1-derived DH plants and C= *B. juncea*)
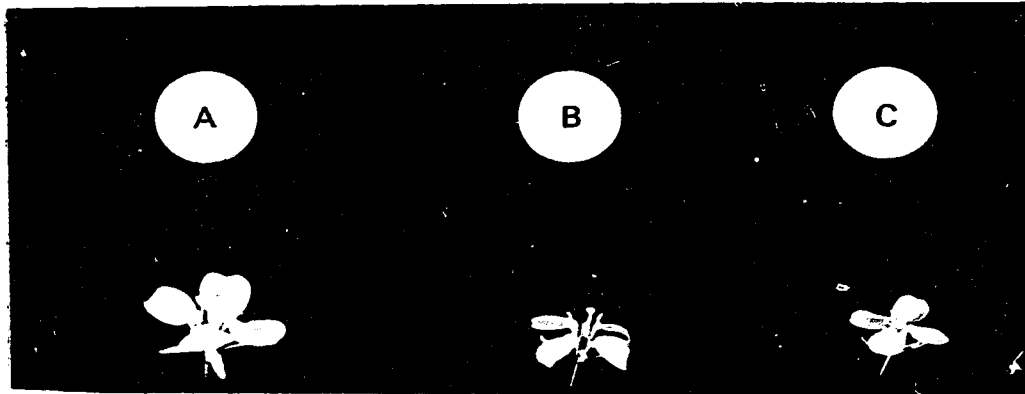
Figure 4.6.   Comparison of flower morphology of BC_1F_1, F_1-derived DH
plants and *B. juncea*. The F_1-derived DH plant flower is larger
than the backcross flower. A= F_1-derived, B=BC_1F_1-derived and
C= *B. juncea*. The petal separation more evident in the *B. napus*
flower is still detectable in the BC_1F_1-derived DH plant flower.

The genotypes were all significantly different at p=0.05 (Table 4.4) when analyzed individually. The F_1-derived DH plants had an intermediate growth rate between the two parents. The *B. juncea* 1 (Bj1) growth rate was significantly different from the other groups. The *B. juncea* parent lines were faster growing than *B. napus* or BC_1F_1-derived DH plants.

4.4.2.2. Primary branching

The number of primary branches ranged from 13.75 in the F_1-derived DH plant 94-1291 to 4.29 in the BC_1F_1-derived DH plant 94-1280. When F_1 and BC_1F_1-derived DH plants were pooled and analyzed as groups, there was no significant differences (p=0.05) detected within the groups. There was no observed pattern in this trait in the F_1 and BC_1F_1-derived DH plants. The F_1 plants had the highest number of branches in the grouping. *B. juncea* 2 (Bj2) had the lowest number of branches per plant. *B. napus* and Bj1 were not significantly different. The BC_1F_1-derived DH plants had a mean branch number of 7.9 which was significantly different from the others at p=0.05.

4.4.2.3. Days to flowering

Days to flowering ranged from 58 days in F_1-derived DH plant 94-1291 and 94-1293 to 43.7 days in the *B. juncea* parents. The F_1-derived DH plants flowered later than the parent lines and the BC_1F_1-derived DH lines. The average of the BC_1F_1-derived DH lines, Bj1 and Bj2 and *B. napus* were 48.3, 44.0, 46.7 and 47 days respectively.

4.4.2.4. Pod characteristics

4.4.2.4.1. Pod number

The total number of pods were significantly different in the parent genotypes as generally compared to both the F_1 and BC_1F_1-derived DH plants, with a few exceptions in the BC_1F_1-derived DH plants (Table 4.3). Pods per main raceme ranged from 60 pods in BC_1F_1-derived DH plant 94-1284 to 29.5 pods in F_1-derived DH line 94-1293.

The F_1-derived DH plants had the highest number of pods per main raceme at 37.0 pods. They also exhibited a higher pod count compared with both parents. This was different from observations in the green house where F_1-derived DH plants had fewer pods than other lines (Figure 4.1, 4.3, and 4.4). This may have occured as a result of reduced stand densities in F_1-derived DH line plots in the field. The BC_1F_1-derived DH plants had an intermediate pod count.

4.4.2.4.2. Seed number

The number of seeds per pod was highest in the *B. napus* parent with an average of 22.07 seeds per pod. The F_1-derived DH plant 94-1295 had the least number of pods with 2.04 seeds per pod. The F_1-derived DH plants had the least number of seeds per pod, however seeds per pod in the BC_1F_1-derived DH plants were not significantly different from the *B. juncea* parent line. The F_1-derived DH lines with very few seeds were probably the result of reduced fertility of the female plant as earlier observed.

4.4.3. Yield characteristics

4.4.3.1. Dry Matter yield

The total single plant dry matter yield varied greatly from 235.58g in BC_1F_1-derived DH plant 94-1286 to 40.33g in 94-1486. Individual BC_1F_1-derived DH lines were significantly differenct with respect to dry matter yield. The F_1-derived DH plants had a relatively higher but non significant average dry matter yield than the parents and BC_1F_1-derived DH lines. *B. juncea* parent 1 had the least average dry matter yield of 40.5g.

Table 4.3. Pod, seed and raceme traits in individual parents, F_1 and BC_1F_1-derived DH genotypes in *B. juncea* x *B. napus* interspecific crosses.

Genotype		Pods Number		Pod Characteristics		Primary Branches
		Total Pods	Main raceme	Length	Seed No	
Parent	*B. juncea* 1	290.7 c	42.1 cdef	3.12 defgh	17.26 cd	5.5 hi
	B. juncea 2	285.3 c	50.9 abc	4.00 c	18.22 bc	7.12 defghi
	B. napus	209.5 c	37.0 efg	6.13 a	22.07 ab	6.00 ghi
F_1 Derived	94-1290	-	-	-	-	8.00 bcdefgh
	94-1291	706.0 b	58.5 a	2.70 gh	3.08 ji	13.75 a
	94-1292	330.5 bc	30.0 g	3.85 cde	10.50 efgh	9.25 bcde
	94-1293	345.0 bc	29.5 g	3.03 efgh	2.75 ji	8.67 bcdef
	94-1295	360.5 bc	46.8 bcde	3.62 cdef	2.04 j	8.25 bcdefg
BC_1F_1-derived	94-1279	431.9 bc	44.0 bcde	3.02 efgh	14.07 cdef	7.58 cdefgh
	94-1280	237.6 c	42.3 cdef	3.84 cde	17.89 bc	4.92 i
	94-1281	324.9 bc	37.4 defg	2.65 h	11.82 efg	8.6 bcdef
	94-1282	474.1 bc	54.3 ab	3.29 cdefgh	13.58 cdef	6.83 efghi
	94-1283	490.8 bc	47.75 bcde	3.30 cdefgh	7.82 gh	6.17 fghi
	94-1284	224.5 c	60.0 a	2.55 h	9.79 efgh	9.50 bcd
	94-1286	1387.4 a	48.0 bcd	3.52 cdefg	6.79 hi	13.75 a
	94-1287	501.0 bc	43.2 cde	2.93 fgh	9.63 fgh	10.00 bc
	94-1288	493.3 bc	39.9 defg	3.91 cd	12.74 def	8.83 bcde
	94-1289	385.2 bc	32.1 fg	3.12 defgh	9.54 fgh	9.42 bcd
	94-1486	284.8 c	37.6 defg	3.71 cdef	14.45 cde	6.83 efghi
	S.E.M	74.6	1.80	0.09	0.54	0.41

Figures followed by the same letters are not significantly different at p=0.05 using Duncan's multiple range test.

4.4.3.2. Seed yield per plant

Seed yield per plant was significantly different within the lines (Table 4.5). Yield was highest in the BC_1F_1-derived DH plant 94-1288 with 26.31g. The lowest yield was obtained from the F_1-derived DH plants with plant 94-1292 yielding only 0.14g. The *B. juncea* 2 parent had 24.69g whereas the *B. napus* parent had 15.99g.

4.4.3.3. Harvest index

The highest harvest index (HI) was obtained from BC_1F_1-derived DH plant (94-1486), with 39.15%. The lowest harvest index values were obtained from the F_1-derived DH line (94-1292) with HI=0.1 %. The parent lines had a relatively high harvest index as compared to the other genotypes.

4.4.4. Correlations: Seed yield, harvest index and other yield components

Analysis of variance detected significant differences in most traits measured. No significant correlations were detected between harvest index and other characteristics (Table 4.6). Pods per main raceme, pod length, seeds per pod, seed yield per plant and 1000 seed weight were all positively correlated to harvest index. Total pods per plant and total dry weight were negatively correlated to harvest index. Components such as total pods per plant, and total dry weight had negative correlations with harvest index. They, however, had positive correlations with seed yield per plant, showing that total pods and total dry weight per plant could be an important predictor for both seed yield per plant and higher harvest index.

Table 4.4. Agronomic characteristics of parents, F$_1$ and BC$_1$F$_1$-derived DH genotypes of *B. juncea* x *B. napus* interspecific crosses.

Genotypes		Height (cm)	Primary Branches/Plant	Days to Flowering
Parents	*B. juncea* 1	6.18 bcdef	5.5 hi	43.7 h
	B. juncea 2	10.67 a	7.17 defghi	43.3 h
	B. napus	4.17 defg	6.00 ghi	52.7 bc
F$_1$ Derived	94-1290	2.00 g	8.00 bcdefgh	45.0 gh
	94-1291	5.00 cdefg	13.75 a	58.0 a
	94-1292	6.5 bcdef	9.25 cbdc	50.0 cdc
	94-1293	9.5 ab	8.68 cbdcf	58.0 a
	94-1295	4.25 defg	8.25 cbdcfg	52.0 bcd
BC$_1$F$_1$ Derived	94-1279	7.92 abc	7.58 cdefgh	47.0 cfgh
	94-1280	6.25 bcdef	4.29 i	45.7 fgh
	94-1281	4.00 defg	8.60 cbdcf	54.3 b
	94-1282	6.83 bcdc	6.83 efghi	46.3 cfgh
	94-1283	7.33 bcd	6.17 fghi	44.0 h
	94-1284	3.5 efg	9.5 bcd	49.0 dcf
	94-1286	4.92 cdefg	10.25 b	48.3 dcfg
	94-1287	3.08 fg	10.00 bc	53.0 bc
	94-1288	5.5 cdefg	8.83 cbdc	48.0 cfg
	94-1289	3.58 cfg	9.42 cbd	48.7 dcfg
	94-1486	8.42 abc	6.83 cfghi	45.3 fgh
	S.E.M	0.76	0.41	0.91

132

Table 4.5. Yield Characteristics of parents, individual F_1 and BC_1F_1-derived DH plants of *B. juncea* x *B. napus* interspecific crosses.

Genotype		Total Dry Weight (g)	Seed Yield/ Plant (g)	Harvest Index (%)
Parent	*B. juncea* 1	40.51 d	13.78 ab	33.70 abc
	B. juncea 2	89.65 bcd	24.69 a	29.02 bc
	B. napus	51.73 cd	15.99 ab	30.67 abc
F_1-Derived	94-1290	48.00 d	-	-
	94-1291	110.00 bcd	0.44 b	0.30 h
	94-1292	95.70 bcd	0.14 b	0.10 h
	94-1293	179.70 ab	2.22 b	1.25 h
	94-1295	69.95 cd	1.92 b	2.45 gh
BC_1F_1-Derived	94-1279	63.04 cd	17.99 ab	27.28 cd
	94-1280	50.83 cd	15.56 ab	31.84 abc
	94-1281	87.69 bcd	17.75 ab	12.17 ef
	94-1282	46.72 cd	12.09 ab	26.46 cd
	94-1283	96.48 bcd	8.68 ab	7.8 efg
	94-1284	127.48 bcd	13.54 ab	10.63 efg
	94-1286	235.58 a	14.60 ab	6.42 fgh
	94-1287	149.23 abc	17.09 ab	11.99 ef
	94-1288	144.28 abcd	26.31 a	19.42 de
	94-1289	94.83 bcd	13.00 ab	12.37 ef
	94-1486	40.33 d	15.49 ab	39.15 a
	SEM	17.4	3.18	0.02

Figures followed by the same letters are not significantly different at p=0.05 using Duncan's multiple range test.

Table 4.6. Coefficients of correlation between seed yield/plant and seven of its components in parents, F_1 and BC$_1$F$_1$-derived DH plants (7 df).

		X_1	X_2	X_3	X_4	X_5	X_6	X_7	Y
Total Pods/Plant	(X_1)	1.00	0.43	0.14	-0.20	0.71	-0.23	0.30	0.08
Pods/Main raceme	(X_2)		1.00	0.40	0.26	0.18	0.22	0.83	0.31
Pod length (cm)	(X_3)			1.00	0.67	-0.02	0.47	0.57	0.40
Seed/Pod	(X_4)				1.00	-0.40	0.88	0.54	0.72
Total Dry wt/Plant	(X_5)					1.00	-0.52	0.11	0.04
Harvest Index (%)	(X_6)						1.00	0.41	0.69
1000 Seed wt. (g)	(X_7)							1.00	0.44
Seed Yield/Plant (g)	(Y)								1.00

4.4.5. Water stress resistance

The electrolyte leakages obtained from the different groups for leaf number three ranged from 7.75% for the discs in distilled water (Control) to 42.28% (40% PEG). There were no significant differences between the genotype lines, although significant differences were observed among the various treatments. Leakage levels increased with increase in PEG concentration levelling off at concentrations between 20 and 30%. At higher concentrations the leakage was almost equal. *B. napus* had consistently low leakage levels at all treatments, the differences becoming smaller as concentrations of PEG increased.

The F_1 and BC_1F_1-derived DH plants had intermediate values between the two parents. At higher concentrations, the BC_1F_1-derived DH plants had leakage levels higher than both parents. Leakage levels for the sixth leaf had a different pattern as compared to the third leaf (Figure 4.8). As in leaf three, *B. napus* had low leakage levels when compared to *B. juncea* at low concentrations. The differences became smaller at higher concentrations. In the highest concentration tested, *B. juncea* had lower injury levels when compared to *B. napus*. As in leaf three, the BC_1F_1-derived DH lines had leakage levels midway between the two parents. Leakage levels for the F_1-derived DH plants were lower than either parent. In all the treatments, leakage was higher in BC_1F_1 than the F_1-derived DH lines.

Injury levels (Blum and Ebercon 1981), showed similar trends to leakage levels (Figure 4.9). *B. juncea* had the highest injury levels tapering off at 20% PEG concentration. The highest injury levels obtained for both *B. juncea* and *B. napus* was 38.22% and 38.12% at the highest concentrations tested which was not significantly different at $p=0.05$. At lower concentrations, *B. napus* showed consistently less injury than *B. juncea*. As in the case of leakage, the F_1 and BC_1F_1-derived DH had intermediate injury levels to those of both parents. F_1-derived DH lines had higher injury levels than BC_1F_1-derived DH lines in all the treatments for the third leaf.

135

Figure 4.7. Electrolyte leakage for the third (3rd) leaf profiles of parents, F_1 and BC_1F_1-derived DH genotypes in *B. juncea* x *B. napus* interspecific crosses.
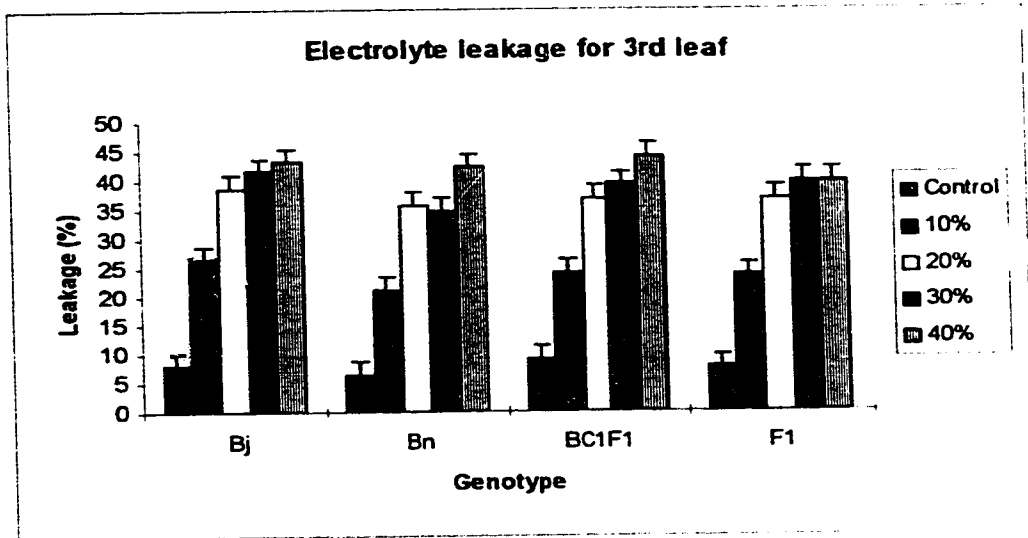
Figure 4.8. Electrolyte leakage for the sixth (6th) leaf profiles of parents, F_1 and BC_1F_1-derived DH genotypes in *B. juncea* x *B. napus* interspecific crosses.
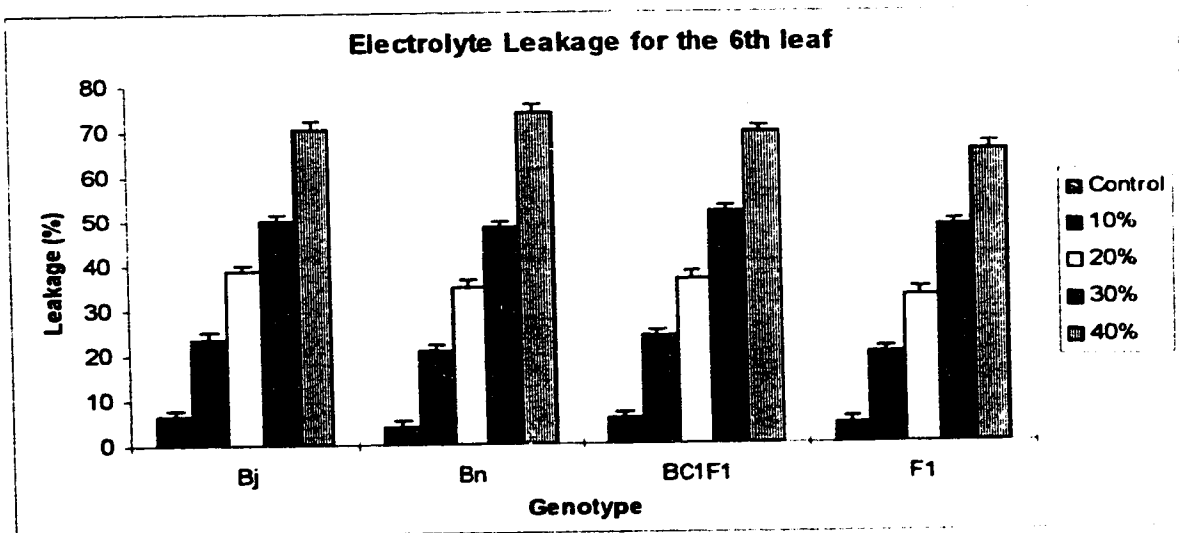
The injury levels obtained for leaf six showed an almost linear relationship between injury levels and PEG concentrations. The injury levels were lowest in the F_1-derived DH group contrasting with leaf three, in which *B. napus* had the lowest injury. At higher concentrations, *B. napus* showed higher injury levels than all other lines. At higher levels of PEG, *B. juncea* injury levels were relatively low as compared to the other lines.

Figure 4.9. Injury (%) caused by different PEG concentrations to the 3rd and 6th of parents, F_1 and BC_1F_1-derived DH genotypes in *B. juncea* x *B. napus* interspecific crosses.

Injury (%) to the 3rd and 6th leaf
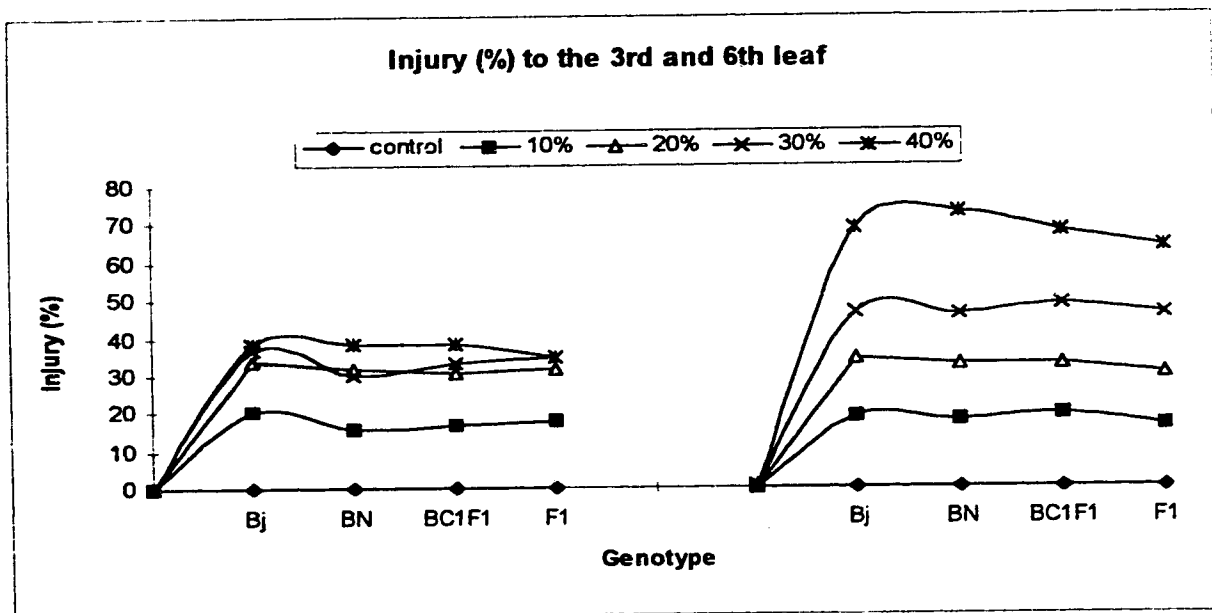
4.5. Discussion

4.5.1. Agronomic and morphological traits

According to Thurling (1993), alternative criteria used for yield selection must be simple to measure, significantly correlated to plot yield, and highly heritable. The most widely used alternative criteria have been primary morphological components of seed yield such as the number of pods per plant, number of seeds per pod and weight per 1000 seed. Adams (1967) found that although these characters are appropriate, they have sometimes proved unreliable because of existence of compensatory mechanisms. This resullts in reductions in one or more yield components in response to selection for another specific component. In other instances, it has proven effective as observed in the case of selection for seed weight in *B. juncea* (Gupta and Labana 1985). Seed yield is linearly related to biomass and days to 50% flowering. Seed yield depends primarily on the number of pods and number of seeds per pod produced (Kumari et al. 1994, Campbell and Kondra 1978). Studies by Taylor and Smith (1992) found no correlation between seeds per pod and yield in any of the *B. napus* varieties studied. Negative correlations between number of pods per plant and number of seeds per pod were reported in both *B. napus* and *B. rapa* by Thurling (1974b).

In the current material, the hairy leaf trait found in the *B. juncea* parent lines was retained in some BC_1F_1-derived DH lines and lost in others. Since absence of the hairy leaf trait found in the *B. napus* parent was expressed in the backcross-derived progeny, the genes responsible for the trait expression must have been introgressed from *B. napus*. In the F_1-derived DH lines, only one line expressed the glabrous trait, while the other lines retained the hairiness gene, showing that it was possible to lose some of the genes from *B. juncea* through the cross.

Preliminary results on the various yield characteristics and yield components studied had different effects on seed yield per plant. Although the F_1-derived DH plants had a high dry matter yield (106.81g plant^{-1}) it was not reflected in seed yield (1.27g plant^{-1}). Among the genotypes studied the F_1-derived DH plants had the highest total pod number (533.3) with the lowest found in the *B. napus* parent (154.5). There was considerable variation in pod numbers per plant, mainly as a result of differences in

plant sizes associated with uneven stands in the plots. Because of the compensatory nature in Brassicas growth (Adams 1967, Chay and Thurling 1989), the plants displayed ...siderable variability to compensate for lower stand density in some plots. Although Taylor and Smith (1992) contend that yields are relatively unaffected by differences in plant density, the single plants in this study seem to have adjusted to compensate for low plant density.

There was a general observation that pods on the main raceme were directly related to yield, except for *B. napus* and F_1-derived DH plants which had low pod numbers (37) on the main raceme but had a higher seed yield (15.99g plant^{-1}) (Table 4.3). Apparently the genotypes with long pods also seemed to respond by producing fewer pods to compensate for increased requirement for photosynthates (Adams 1967). Pods from the *B. napus* parent were significantly longer than all other genotypes. The *B. juncea* parent (Bj2) was significantly different from *B. napus* but was similar to BC_1F_1 and F_1-derived DH plants. The shortest pods were obtained from one of the *B. juncea* parents (Bj1). The length of pods did not result in significantly higher yields in the preliminary data obtained. Pod lengths in the BC_1F_1-derived DH plants, however, were similar to *B. juncea* types. Since no increase in pod length was observed in the F_1-derived DH plants greater than the *B. juncea* parent, the genes controlling the pod length were either not transferred, or expressed in the F_1-derived DH genotypes (recessive genes). The short pods observed in both F_1 and BC_1F_1-derived DH lines could have originated from *B. juncea*. Since this trait is highly heritable (Chay and Thurling 1989), the phenotype would have been expressed if the genes inherited from *B. napus* were dominant. This cannot be said with certainity because the expression of the genes may occur also if in the homozygous recessive condition.

The genotype with the highest harvest index was *B. juncea* parent Bj1 (Table 4.6) The genotype with the lowest harvest index were the F_1-derived DH plants. Individual plants (94-1486) had a harvest index higher than the best parent an increase of 27.6% (Table 4.6). This shows that it was possible to improve the efficiency of the parents as reflected by the improved harvest index. From this study, it has been possible to identify genotypes agronomically similar if not better than the higher value parent.

139

Presence of genotypes with potential for superior yield to both *B. juncea* and *B. napus* parents (like 94-1288 line) suggests that recombination of genes from A, B and C-genomes of *B. juncea* and *B. napus* occurred, resulting in genotypes with desirable genetic constitution. For example, preliminary data from both BC_1F_1-derived DH line 94-1280 and 94-1486 with a yield of 15.56g plant^{-1} (HI= 31.84) 15.49g plant^{-1} (HI=39.15) respectively suggests that the lines were more efficient in allocation of assimilates to seeds than both *B. napus* and *B. juncea*. Although line 94-1286 had a high yield, its high dry matter yield could not be converted to seed yield. These three lines should be investigated further for yield stability and other qualitative traits.

4.5.2. Water stress resistance

When electrolyte leakage was determined for leaf number 3, there were no significant differences among the parents, F_1, and BC_1F_1-derived DH lines. The leakage levels for leaf 3 were far lower than the leakage levels for leaf 6, especially at increased PEG concentration, showing that they suffered less cell membrane damage. Leakage levels for leaf 6 were significantly different (P=0.05) within the groups. This suggests that older leaves (leaf 3) were more dehydration tolerant than intermediate and younger leaves (leaf 6). This finding was constant across the groups at higher concentrations (higher stress levels) for both older and younger leaves (Figures 4.7, 4.8). Other than being more dehydration tolerant than leaf 6, the leaves had similar injury patterns (Figure 4.9). Studies by Levitt (1985) found that detached younger leaves were more dehydration tolerant than older leaves when stressed. The findings were based on relative water content in detached cabbage leaves. Since *B. napus* had a lower electrolyte leakage on average apart from highest level of stress, it can be argued that it has more dehydration tolerance than *B. juncea* when rapidly dehydrated. As the stress level increases, the cell membrane stability seems to break down as evidenced by the increased leakage as compared to *B. juncea*. According to Levitt (1985), in slowly dehydrated leaves, increased stress results in increased dehydration tolerance by permitting drought induced acclimation. Premachandra et al. (1991) found that electrolyte leakage was affected by leaf thickness and surface wax content in maize.

Leakages taken for both F_1 and BC_1F_1-derived DH plants for leaf 3 were intermediate between the parents. This would be expected if genetic material is transferred from the parents to the F_1 and BC_1F_1-derived DH lines. At higher levels of stress, F_1-derived DH plants had reduced leakage but the BC_1F_1-derived DH lines had a higher leakage level than the parents. Conformational alterations of the cell membranes suggested by Shcherbakova and Kacperska-Palacz (1980) and increased solutes has been found to result from acclimation. Other reports by Good and Maclagan (1993), Shcherbakova and Kacperska-Palacz (1980), Shcherbakova and Kacperska (1983) found no beneficial effects on drought tolerance arising from these conformational changes. *B. juncea* is slower in adjusting to rapid dehydration stress resulting in more injury reflected in higher leakages. Ashraf and Mehmood (1990) reported highest rate of deposition of epicuticular wax in nature in *B. napus* at the onset of drought, but as drought progressed, the epicuticular wax deposition was highest in *B. juncea*. This, and the fact that *B. napus'* relative water content decreases slowly but continuously as compared to *B. juncea* which loses water more rapidly (Good and Maclagan 1993), probably explains the higher leakage values observed in *B. juncea* as compared to *B. napus*. The F_1-derived DH lines leakage is less at higher stress levels than the parents and BC_1F_1-derived DH lines. This suggests that the F_1-derived DH lines had the genetic potential to resist drought, but took longer to adjust (acclimatize) to the stress, similar to the *B. juncea* parent. The BC_1F_1-derived DH lines were more sensitive to rapid dehydration and would probably require more time to acclimatize to the stress because leakage levels increased with increased stress levels. The backcrossing to *B. juncea* appears to have resulted in loss of genetic material obtained from *B. napus* allowing for rapid acclimation to sudden stress levels.

References

Adams M.W. 1967. Basis of yield component compensation in crop plants with special reference to the field bean, *Phaseolus vulgaris* L. Crop Sci. 7: 505-510.

Allen E.J. and D.G. Morgan 1972. A quantitative analysis of the effects of nitrogen on the growth, development and yield of oilseed rape. J. Agric. Sci Camb 78 315-324.

Allen E.J. and D.G. Morgan 1975. A quantitative comparison of the growth, development and yield of different varieties of oilseed rape. J. Agric. Sci Camb 85: 159-174.

Ashraf M. and S. Mehmood 1990. Response of four Brassica species to drought stress Environmental and experimental botany 30:93-100.

Augustinussen E. 1987. The influence of nitrogen fertilizing on growth and development and yield of different varieties of oilseed rape. Tidskr. Planeavl. 91: 33-44.

Austin R.B. 1980. Physiological limitations to cereal yields and ways of reducing them by breeding. In: R.G Hurd et al. (eds.), Opportunity for increasing crop yields p3-18. Pitman Publishers Ltd. London.

Austin R.B., J. Bingham, R.D. Blackwell, L.T. Evans, M.A. Ford, C.L. Morgan and M Taylor 1980. Genetic improvement in wheat yields since 1900 and associated physiological changes. J. Agric Sci Camb 94: 675-689.

Bansal K.C., S. Nagarajan and N.P. Sukumaran 1991. A rapid screening technique for drought resistance in potato (*Solanum tuberosum* L.) Potato Research 34, 241-248.

Bhargava S.C. and D.P.S. Tomar 1988. Physiological contribution for increased yields in rapeseed-mustard. Proc. int. Cong. Plant Physiology. Feb 15-20. New Delhi, India. p352-360.

Blum A. and A. Ebercon 1981. Cell membrane stability as a measure of drought and heat tolerance in wheat, Crop Sc. 21: 43-47.

Brar G. and W. Thies 1977. Contribution of leaves, stem, siliquae and seeds to dry matter accumulation in ripening seeds of rapeseed (*Brassica napus* L.). Z. Pflanzenphysiol 82: 1-13.

Campbell D.C. and Z.P. Kondra 1978. Relationships among growth patterns, yield components and yield of rapeseed. Can. J. Plant Sci. 58: 87-93.

Chapman J.F., R.W. Daniels and D.H. Scarisbrick 1984. Field studies on ^{14}C assimilate fixation and movement in oilseed rape (*Brassica napus*). J. Agric. Sci Camb 102: 23-31.

Chauhan Y.S. and S.C. Bhargava 1984. Physiological analysis of growth and yield variation of rapeseed and mustard. J. Agric. Sci. Camb 103: 249-252.

Chay P. and N. Thurling 1989. Variation in pod length in spring rape (*Brassica napus*) and its effect on seed yield and yield components. J. Agric. Sci. Camb 113: 139-149.

Clarke J.M. and G.M. Simpson 1978. Growth analysis of *Brassica napus* cv. Tower. Can. J. Plant Sci. 58: 587-595.

Degenhardt D.F. and Z.P. Kondra 1984. Relationships between seed yield and growth characters, yield components, seed quality of summer-type oilseed rape (*Brassica napus* L.). Euphytica 33: 885-889.

Donald C.M. 1962. In search of yield. J. Aust. Inst. Agric. Sci 28: 171-178.

Good A.G. and J.L. Maclagan 1993. Effects of drought stress on the water relations in *Brassica* species. Can. J. Plant Sci. 73: 525-529.

Gupta M.L. and K.S. Labana 1985. Effects of selection for seed size and its correlated response in Indian mustard. Crop Improv 12: 193-194.

Huhn M., F. Grosse and J. Leone 1991. On harvest indices of winter oilseed rape (*Brassica napus* L.). J. Agronomy & Crop Science 167: 299-309.

IBPGR 1987. Descriptors for *Brassica campestris* L. International Board for Plant Genetic Resources, Rome.

IBPGR 1990. Descriptors for *Brassica* and *Raphanus*. International Board for Plant Genetic Resources, Rome.

Inanaga S. and A. Kumura 1974. Studies on matter production of rape plant (*Brassica napus* L.) I. Changes with growth in rates of photosynthesis and respiration of rape plant population. Proc. Crop Sci. Soc. Japan 43(2): 261-266.

Kasa G.R. and Z.P. Kondra 1986. Growth analysis of spring-type oilseed rape. Field Crops Res. 14: 361-370.

Kjellstrom C. 1993. Comparative growth analysis of *Brassica juncea* under Swedish conditions. Can. J. Plant Sci. 73: 795-801.

Kumar A. and J. Elston 1992. Genotypic differences in leaf water relations between *Brassica juncea* and *B. napus*. Annals of Botany 70: 3-9.

Kumari N., S.C. Bhargava and S.N. Bhardwaj 1994. Physiological basis of yield and its components in rape seed mustard with reference to photoperiod. Indian J. Plant Physiol. 37(3): 142-146.

Levitt J. 1985. Relationship of dehydration rate to drought avoidance, dehydration tolerance and dehydration avoidance of cabbage leaves, and to their acclimation during drought -induced water stress. Plant, Cell and Environment 8:287-296.

Major D.J., J.B. Bole and W.A. Charnetski 1978. $^{14}CO_2$ assimilation by stems, leaves and pods of rape plants. Can. J. Plant Sci. 58: 783-787.

Mendham N.J. and R.K. Scott 1975. The effect of plant size at inflorescence initiation on subsequent growth and yield of oilseed rape (*Brassica napus*). J. Agric. Sci. Camb 84: 487-502.

Mendham N.J., J. Russell and N.K. Russell 1990. Response to sowing time of three contrasting Australian cultivars of oilseed rape (*Brassica napus*). J. Agric. Sci. Camb 114: 275-283.

Morrison M.J., P.B.E. McVetty and R. Scarth 1990. Effect of altering plant density on growth characteristics of summer rape. Can. J. Plant Sci. 70: 139-149.

Oleinikova T.V. and N.N. Kozhushko 1970. Laboratory methods of drought resistance evaluation of some cereal crops Tr. Prikl. Bot. Genet. Sel. 43(1): 100-111.

Prakash S. 1980. Cruciferous oilseeds in India. In: Brassica crops and wild Allies, Biology and Breeding(ed. S. Tsunoda, K. Hinata and C. Gomez Campo), p151-163. Tokyo: Japan Scientific Soc. Press.

Premachandra G.S., H. Saneoka, M. Kanaya and S. Ogata 1991. Cell membrane stability and leaf wax content as affected by increasing water deficits in maize. Journal of experimental botany 42(235):161-171.

Richards R.A. and N. Thurling 1978a. Genetic analysis of drought stress response in Rapeseed (*Brassica campestris* and *B. napus*). III. Physiological Characters. Euphytica 38: 755-759.

Richards R.A. and N. Thurling 1978b. Variation between and within species of rapeseed (*Brassica campestris* and *B. napus*) in response to drought stress. I Sensitivity at different stages of development. Aust. J. Agric. Res 29:469-477.

Richards R.A. and N. Thurling 1978c. Variation between and within species of rapeseed *Brassica campestris* and *Brassica napus* in response to drought stress. II. Growth and development under natural drought stress. Aust. J. Agric. Res. 29: 479-490.

Roy V., S. Maiti and B.N. Chatterjee 1981. Growth analysis and fertilizer response of 'Varuna' Indian mustard. Indian J. Agric. Sci. 51: 173-180.

Salim M.H., G.W. Todd and C.A. Stuutte 1969. Evaluation of techniques for measuring drought avoidance in cereal seedlings. Agronomy J. 61: 182-185.

Sandhu A.S. and H.H. Laude 1958. Test of drought and heat hardiness of wheat Agronomy J. 61:182-185.

SAS 1989. SAS institute Inc. Cary NC USA 27513-2414.

Shcherbakova A. and A. Kacperska 1983. Water stress injuries and tolerance as related to potassium efflux from injured cells from winter rape hypocotyls. Physiol. Plantarum 57: 296-300.

Shcherbakova A. and A. Kecperska-Palacz 1980. Modification of stress tolerance by dehydration pretreatment in winter rape hypocotyls. Physiol. Plantarum 48: 560-563.

Taylor A.J. and C.J. Smith 1992. Effects of sowing date and seeding rate on yield and yield components of irrigated canola (*Brassica napus* L.) grown on a red-brown earth in south-eastern Australia. Aust. J. Agric. Res. 43: 1629-1641.

Tayo T.O. and D.G. Morgan 1975. Quantitative analysis of the growth, development and distribution of flowers and pods in oilseed rape (*Brassica napus* L.). J. Agric. Sci. Camb 85: 103-110.

Thurling N. 1974a. Morphological determinants of yield in rapeseed (*Brassica campestris* and *Brassica napus*). I. Growth and morphological characters. Aust J. Agric Res 25: 697-710.

Thurling N. 1974b. Morphological determinants of yield in rapeseed (*Brassica campestris* and *Brassica napus*). II. Yield components. Aust J. Agric Res 25: 711-721.

Thurling N. 1993. Physiological constraints and their genetic manipulation. Monor Theor App Genet. New York Springer-Verlag. 1993 Vol. 19: 45-66.

Todd G.W. and D.L. Webster 1965. Effects of repeated drought period on photosynthesis and survival of cereal seedlings. Agron. J. 57:399-404.

Varshney S.K., B. Rai and B. Singh 1990. Analysis of harvest index in three cultivated species of *Brassica*. Indian J. Genet. 50(3): 289-295.

Vasquez T.A., Y. Zuily-Fodil, A.T. P. Thi and J.B. Vieira da Silva 1990. Electrolyte and Pi leakage and soluble sugar content as physiological test for screening resistance to water stress in *Phaseolus* and *Vigna* species. Journal of Experimental Botany vol. 41 No. 228: 827-837.

Woods D.L., J.J. Capcara and R.K. Downey 1991. The potential of mustard (*Brassica juncea* (L.) Coss) as an edible oil crop on the Canadian Prairies. Can. J. Plant Sci. 71: 195-198.

Chapter 5
Quantitative Study

5.1. Introduction

Brassica species like *B. napus*, *B. rapa* and *B. juncea* are important source of vegetable oils. Significant oil quality improvements from the first two species have been done by shifting cultivation from traditional cultivars to cultivars producing more nutritionally desirable oils and meal. Besides being high in caloric value, oils and fats are important in human and animal nutrition as they provide a source of essential fatty acids like linolenic and arachidonic acids. Fats and oils transport fat soluble vitamins like A, D and E. Oils and fats also provide an efficient heat transfer medium (frying) for rapid uniform cooking and contribute to the flavour and palatability of foods.

Canola meal has relatively low digestible and metabolizable energy attributed to its high level of fibre content associated with black and brown seed coat colour (Bell 1993). Improvements in the quality of canola meal can be achieved by breeding for further genetic reduction in glucosinolate levels, for yellow seed coat colour, and for increased meal protein content. Resistance to blackleg and blackspot diseases is important because of serious losses from this disease. The best control has been suggested to be development of resistant cultivars (Newman and Bailey 1987).

5.1.1. Oil

The unique polyunsaturated fatty acid composition of canola oil differentiates it from other oils. The low level of palmitic acid (C16:0) and other saturated fatty acids (< 5%) are considered nutritionally desirable. An increase of short chain fatty acids such as palmitic acid, however, could improve the suitability of canola oil for manufacture of margarines and shortenings (Davik and Heneen 1993, Tanhuanpaa et al. 1995). Canola oil has a higher oleic acid (C18:1) (about 55%) and a lower linoleic acid (C18:2) (about 26%) than is found in most vegetable oils. The highly unsaturated fatty acid content especially C18:3 in canola influences oil stability and quality, and results in reduced shelf life.

A reduction in linolenate levels in canola oils can effectively retard oil autoxidation and flavour deterioration. Genetic alteration of fatty acids such as linolenic acid (C18:3) has resulted in better quality oils with improved flavour and storage ability (Scarth et al. 1988, Eskin et al. 1989). However in the long run, this acid is necessary because it is one of the essential fatty acids for humans. Linolenic acid has been shown to be effective in lowering serum triglyceride levels as well as in reducing platelet aggregation thereby increasing blood clotting time. This 'anti-thrombic' and 'anti-blood clotting' effects appear to play a role in reducing coronary heart disease (Canola Council of Canada 1995a).

The control of biosynthesis of fatty acids has been reported to be under several genetic mechanisms and is in different genomes in the Brassicas. Erucic acid in *B. juncea* was found to be under embryogenic control (Downey and Harvey 1962) with genes from both A and B genomes (Kirk and Hurlstone 1983). Erucic acid is also thought to be controlled by at least two genes acting in an additive manner in *B. napus* (Jack et al. 1994, Harvey and Downey 1964, Downey and Dorrell 1971, Chen et al. 1988a) with genes located in the A and C genomes (Anand and Downey 1981). In a study of a *B. carinata* x *B. napus* cross Fernandez-Escobar et al. (1988) confirmed the presence of erucic acid-controlling genes in each of the three *Brassica* genomes A, B and C. Stringam and McGregor (1980) reported the control of inheritance of linoleic and linolenic acid to be under the developing embryo rather than the maternal tissue in *B. rapa*. Other mechanisms for controlling C18 fatty acids have been proposed. Cytoplasmic effects (Diepenbrock and Wilson 1987, Thomas and Kondra 1973), and the influence of the environment were reported to have a considerable effect on the final concentration of linolenic and linoleic fatty acids (Tremolieres et al. 1982, Pleines and Friedt 1988, 1989).

The presence of fatty acid controlling genes in all genomes, (Fernandez-Escobar et al. 1988, Chen et al. 1988a) and the ability for complete and partial pairing between A, B and C genomes of different Brassica species (Fernandez-Escobar 1988, U 1935) makes it possible to recombine specific genes and select for desired fatty acid composition (Roy and Tarr 1986) through interspecific crosses.

The strong association between chlorophyll and linolenic acid seems to make selection for reduced levels of this acid difficult (Thies 1970, cited by Roy and Tarr 1986), but other research findings suggests otherwise (Stringam and McGregor 1980). The expression of low linolenic acid in Brassica is therefore feasible without the problems associated with chlorophyll (Scarth et al 1988, 1995).

5.1.2. Glucosinolates

Glucosinolates are a class of about 100 naturally occurring thioglucosides that are characteristic of the cruciferae and related families in the order Capparales (Bones and Rossiter 1995). Glucosinolates are found in all parts of the plant; sometimes more than 15 different types of glucosinolates occurring in one plant (Olesen 1981, Sang et al. 1984). The major glucosinolates occurring in the six *Brassica* species in the U triangle (U 1935) are gluconapin (3-butenyl glucosinolate), progoitrin (2-hydroxy-3-butenyl glucosinolate) and glucobrassicanapin (4-pentenyl glucosinolate) comprising the alkenyl group and 4-hydroxy-glucobrassicin (4-hydroxy-indolylmethyl glucosinolates) and glucobrassicin comprising the indole group (Kraling et al. 1990). The latter lack any sulphur containing functional group in the 3-5 carbon side chain (Gland et al. 1981). The modification of the side chain produces alkenyl hydroxyalkenyl methylthioalkyl and methylsulphinylalkyl homologues. The total aliphatic glucosinolate content of the seed of *B. napus* is determined by the genotype of the maternal parent rather than the genotype of the zygote (Kondra and Steffanson 1970, Love et al. 1990) and is controlled by nuclear genes. Environment and nutrition (Sones et al. 1984, Josefsson and Appelqvst 1968), and time of sowing (Sang et al. 1986) affect the level of glucosinolates in the seeds of *B. napus*. Most of the glucosinolates in the seed come from redistribution from other parts of the plant (Drozdowka and Rogozinska 1982, McGregor 1988, Merrien 1989). Magrath and Mithen (1993) found the rate of glucosinolate synthesis and degradation in the pod tissue as factors responsible for glucosinolate levels in the seeds. Reduction of the level of glucosinolates in the pods decreases as the seeds develop as a result of their transport to the seed (De March et al.

1989). This suggests that glucosinolates are a storage form for nitrogen, carbon and sulphur. This also explains why five to ten times the levels of glucosinolates are found in the seeds as compared to the vegetative parts (Clossais-Besnard and Larher 1991). Indolyl glucosinolates in Brassica occur in significant quantities, with little differences between low and high alkenyl glucosinolate cultivars (McGregor 1978). The main difference between cultivars with high and low glucosinolate content come from differences in progoitrin (2-Hydroxy 3-butenyl glucosinolates) and gluconapin (3-butenyl glucosinolate) (Bilsborrow and Evans 1989). As a result of presence of seed-specific glucosinolates, it has been suggested that vegetative parts provide mainly glucosinolate precursors. The final steps for glucosinolate synthesis occur in the seed (Clossais-Besnard and Larher 1991). In the double zero winter rapeseed types, decreasing proportion of alkenyl glucosinolates was found to be accompanied by an increase in the proportion of the indole glucosinolates (Musnicki et al. 1995). The restrictive nature of aliphatic glucosinolates in *B. napus* has been found to be of little taxonomic value (Adams et al. 1985). However the glucosinolate profiles could be utilized for identification in crosses between low and high glucosinolate species

The evidence that rapeseed meal contained goitrogens was first noted in rats (Kennedy and Purves 1941), then swine and poultry (Daun and McGregor 1991, Bell and Belzile 1965) and in growing bulls (Iwarsson et al. 1973). The problem of meal utilization in animal feed results from hydroxyalkenyls that affect iodine metabolism. This depresses growth, causes haemorrhaging liver as a result of epithionitriles and causes skeletal abnormalities in poultry. Upon hydrolysis of glucosinolates by myrosinase (thioglucoside glucohydrolase E.C. 3.2.3.1) at neutral pH, the glucose and sulphate are split off to free oxazolidinethione isothiocyanate which are goitrogenic (Bell 1984). Feeding experiments with indolyl glucosinolates and their transformation products demonstrated non detrimental effects on animals fed with diets containing these glucosinolates if kept below 3μm g^{-1} of dry matter of the diet. The diet is usually below this level even when 30% of the diet comes from double low rapeseed meal (Bell 1994) containing normal indolyl glucosinolate.

Glucosinolate amounts do not appear to be directly involved in resistance to *L. maculans* but the concentration on a fresh weight basis does (Van den Berg and Rimmer 1991). Glucosinolates are thought to be rapidly metabolized to sustain plant growth and catabolized during the reproductive phase especially during flowering (Clossais-Besnard and Larher 1991). The reduction of the level of glucosinolate contents in the seed and subsequently in the meal has been a long term breeding objective in canola. Low glucosinolate lines are desirable to produce glucosinolate-free meal for the animal feed industry and possibly for human consumption.

5.1.3. Protein

Canola meal is a source of high quality protein supplement but its use is limited because it has low available energy and low protein digestibility (81%) (Bell et al. 1981, Bell 1993). The protein is of high biological value (Ballester et al. 1977, Sauer et al. 1982), a feature unusual for proteins of vegetable origin. Canola meal also contains an exceptionally high content of phosphorus (1.1-1.2%) (Guenter et al. ι 95). Rapeseed meal contains about 43% crude protein (dry basis) (Bell 1984), with canola meal showing a 41.9% crude protein (Bell and Keith 1991). Canola meal with improved protein but with high digestibility and amino acids availability in animals digestive systems would allow for its extensive use in animal feeds if competitively priced (Bell 1993). The new initiative of developing canola quality *B. juncea* is based on superior qualities shown by this species over *B. napus*, among which its pure yellow coat colour associated with higher protein and less fibre in its meal (Slominski et al. 1995).

5.1.4. Disease resistance

5.1.4.1. Blackleg resistance (*Leptosphaeria maculans*)

Blackleg is a serious disease of rapeseed and canola, and causes severe losses in many parts of the world. The disease is caused by *Phoma lingam* (Tode ex Fr.) Desm.

with the perfect stage of the fungus being *Leptosphaeria maculans* (Desm) Ces & de Not. The disease causes premature ripening of the plant resulting in serious yield losses. The disease has been effectively controlled in Europe since the introduction of resistant cultivars (Newman and Bailey 1987, Rimmer and Van den Berg 1992). Partially resistant seedlings get leaf-lesions caused by ascospores (primary infection) and later pycnidiophores (secondary infection) discharged from the infected leaves. In susceptible plants, the infection spreads from the leaf to the base of the stem, forming cankers. Cultivars resistant to the fungus remain 60-80% canker free in their adult stage (Roy 1984). Only partial or adult resistance has been found in *B. napus* (Roy 1984), although recently the University of Alberta has released a blackleg resistant variety 'Quantum' adapted to western Canada (Stringam et al. 1995a, 1995b). *B. juncea* shows high resistance in both seedling and adult plant stage (Roy 1984). Because of the systemic nature of the disease, the use of resistant cultivars still remains the chief method of controlling blackleg, and new cultivars should be highly resistant (Newman and Bailey 1987). One method of achieving such resistance would be through interspecific crosses between *B. juncea* and *B. napus*. The interspecific crosses involve interaction and exchange of genetic material between the various genomes of the species involved in the cross.

5.1.4.2. Blackspot resistance (*Alternaria brassicae*)

Blackspot disease of rapeseed is an economically important disease in western Canada caused by *Alternaria brassicae* (Conn et al. 1990, Tewari and Conn 1988). The fungus attacks all parts of the plant, especially leaves and pods causing lesions that have necrotic centres surrounded by chlorotic areas (Ayer and Pena-Rodriguez 1987). The pathogen synthesizes abscisic acid which accelerates leaf senescence (Dahiya and Rimmer 1988). Consequently, substantial reductions occur in canola and rapeseed seed yield and oil quality (Degenhardt et al. 1974, Ayer and Pena-Rodriguez 1987), because of the production of small and wrinkled seeds resulting from premature ripening, and pod shattering (Grontoft and O'Connor 1990). Generally, *B. napus* cultivars are less susceptible than those of *B. rapa* (Skoropad and Tewari 1977, Tewari 1991, Rai and

Kumar 1995) although all commercial cultivars of Brassica species are susceptible (Tewari 1991). The factors important for disease spread include epicuticular wax on leaves (Conn and Tewari 1989), temperature (Degenhardt et al. 1982, Kennedy and Graham 1995), leaf age, and duration of wet periods (Mridha and Wheeler 1993, Hong and Fitt 1995). Sources of resistance to the fungus have been identified in wild and allied Brassica species such as *Camelina sativa* and *Capsella bursa* (Tewari 1991). Rai and Kumar (1995) reported F_1 plants obtained from *B. juncea* x *B. napus* as being moderately resistant to the disease, although the F_1 from the cross *B. napus* x *B. rapa* were susceptible. In the study, resistant F_1 plants from *B. carinata* x *B. juncea* and *B. napus* x *B. carinata* crosses were obtained.

5.2. Materials and Methods

The materials studied included 3 field grown parent lines, 4 F_1 and 11 BC_1F_1-derived DH lines as discussed in Chapter 4. Two of the F_1-derived DH lines did not produce enough seed for the various analytical processes. Protein analysis was done on meal obtained after oil extraction. Part of the meal was also used for glucosinolate determination. Different sample sizes were used for the various seed components discussed as a result of seed limitations.

5.2.1. Oil content

Four samples of 1.25g each per line per replicate were obtained from field grown seeds. Oil content was determined by nuclear magnetic resonance (NMR) analysis on a whole seed basis using an Oxford 4000 NMR Analyzer (Oxford Analytical Instruments Ltd). The oil content was expressed as percentage oil content in the air dried seed (8-12% moisture content).

5.2.2. Fatty acids

Two samples from each line per replicate were analysed in duplicates and the analysis repeated three times. The average of the three repeats was used for statistical analysis in the BC_1F_1-derived DH lines. Fatty acids were determined by the ISO (1990)

procedure for animal and vegetable oils using gas chromatography, briefly described below. Approximately three to four grams of seed were placed in a Raney oilseed crusher (Canadian Centre for Advanced Instrumentation) and seven ml of petroleum ether added. The loaded crusher was then closed and placed on a shaker and run for thirty minutes. The Raney crusher was then opened and the solvent evaporated overnight. One ml of pentane was added to the oil to dilute it. 2 ml of sodium methanoate were added to esterify the oil, and the mixture allowed to stand for thirty minutes. The methylated oil was placed in autosampler vials for gas liquid chromatography analysis. using a GLC (HP model 5890 series I) equipped with an autosampler and an integrator. The autosampler was set to inject 1-3 μl of the sample for analysis. The injector and detector were programmed at 280 $^{\circ}$C and 300 $^{\circ}$C while the column temperature was adjusted to 200-240 $^{\circ}$C rising at a rate of 10 $^{\circ}$C min^{-1}. The pressure of the helium carrier gas was adjusted to 40lbs in^{-2} (2.9kg cm^{-2}). The detector range was set up at 1. The autosampler run time was 5 minutes with a flush time of 13-15 seconds. The integrator settings included a stop time of 5 minutes, with a minimum area of 50. The slope was set for 0.1mV/minute. The chart was plotted to 5mm/minute on a 1mV full scale. The fatty acids were expressed as percentage of total fatty acids present in the sample. The free fatty acids were not determined.

5.2.3. Glucosinolates

Samples used for glucosinolate analysis were equal to those used for fatty acid analysis as the meal obtained was used for glucosinolate extraction. The air dried oil free meal was prepared for glucosinolate extraction by treating with aqueous methanol to inactivate endogenous myrosinase. The glucosinolates were then desulphated on an ion exchange column. The desulphated glucosinolates were derivatized to form volatile trimethylsilyl (TMS) ethers that were separated and quantified by gas liquid chromatography (Heaney and Fenwick 1980).

5.2.4. Protein

Two oil free meal samples from the BC_1F_1-derived DH lines were obtained from the meal remaining after glucosinolate analysis. Since seed was limited, only one F_1-derived DH line was analysed for meal protein. The meal was weighed carefully and loaded using an attached autosampler into a Leco FP-2000 Analyzer fitted with a nitrogen probe. The meal was combusted, the resulting gases analyzed, and the contents expressed as percentage protein by multiplying N value by 6.25.

5.2.5. Disease resistance

5.2.5.1. Blackleg resistance (*Leptosphaeria maculans*)

Twelve lines (11 BC_1F_1 and 1 F_1-derived DH lines) and three parent lines (2 *B. juncea* and 1 *B. napus*) were screened. The method used to screen the material was that described by Bansal et al. (1994). The seeds from the 14 lines were each planted in sixteen 6 x 6 cm pots by placing several seeds in the pots which were later thinned on germination to two plants. The plants were maintained at approximately 21 °C and 16 hr photoperiod supplemented with 400W high pressure sodium lamps. Isolates of *L. maculans* cultured on V8 agar supplemented with rose bengal (0.4µm/ml) and incubated at room temperature under 12 hr photoperiod were used as the inoculum. Pycnidiospore suspensions were prepared separately in sterile distilled water from 12-14 day old growing cultures and adjusted to 1×10^6 spores per millilitre. The seedlings per line were divided into four groups of eight plants each and then randomized into trays. Inoculations were made by placing 10µl of the suspension dispensed from an Eppendorf micro pippette on a wound made on each cotyledon by a No. 1 entomological needle. The inoculated seedlings were incubated in a mist chamber (100% RH) for two days. The reactions on cotyledonary leaves were recorded 10 days after inoculation on a 0-4 scale, and the disease severity calculated using the formula below:

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 diffuse margin
4	Most of the tissue collapsed with pycnidia formation

155

Disease severity (DS) % = $\dfrac{\Sigma(\text{No. of plants in categories} \times \text{Category value}) \times 100}{\text{Total no. of plants} \times 4}$

and the data transformed to the logarithmic values and analyzed using the glm procedure of SAS (1989) statistical software.

5.2.5.2. Blackspot resistance (*Alternaria brassicae*)

The disease score was taken on field grown parents, F_1 and BC_1F_1-derived DH lines. The disease severity score was taken on pods prior to harvesting. This was done by visually estimating the percent area covered with lesions using the assessment key developed by Conn et al. (1990). The data were then transformed to the logarithm values and analyzed using the GLM procedure of SAS (1989) statistics software.

5.3. Results

5.3.1. Oil Content and fatty acids

The oil contents in the parents *B. napus*, *B. juncea* 1 and *B. juncea* 2, F_1 and backcross F_1-derived DH plants were significantly different (p=0.05) (Table 5.1). The oil content was highest in *B. napus* with 46.5% oil. The lowest percentage of oil was found in the F_1-derived DH plants with a mean oil content of 30.3%. The BC_1F_1-derived DH lines had an oil content of 40.1%. This was lower lower than that found in both parents. The fatty acids were dominated by oleic, linoleic and linolenic fatty acids (Table 5.1), but the proportions were significantly different within the different genotypes (p=0.05). *B. napus* had the highest level of oleic acid while *B. juncea* had the lowest. The F_1-derived DH plants as group had a high oleic acid (49.5%) second only to *B. napus*. In the BC_1F_1-derived DH plants, the level dropped to 40.2% which was slightly but not significantly lower than the backcross parent at p=0.05. Means for linoleic acid were highest in the BC_1F_1-derived DH lines (33.2%) while the lowest level was found in the parent *B. napus*. The F_1-derived DH plants had a mean of 26.7%, intermediate between the two parents. Linolenic acid was highest in *B. juncea* 2 and BC_1F_1-derived DH plants (16.3%). *B. napus* and F_1-derived DH plants had lower levels (11.7 and 14.1% respectively). The levels of palmitic acid were generally low (3.1-

156

4.7%). The highest levels were found in the F_1-derived DH lines (4.7%) (Table 5.1). BC_1F_1-derived DH lines had higher levels (4.1%) than *B. juncea* 2 parent. Stearic acid was also expressed at low levels (1.4-2.6%) with *B. napus* and *B. juncea* expressing lower levels when compared to both F_1 and BC_1F_1-derived DH lines. When individual lines were analyzed, the oil content was significantly different within the genotypes analyzed. The F_1-derived DH plant 94-1291 had the lowest oil content. The BC_1F_1-derived DH line with highest percentage of oil content was 94-1279. The F_1-derived DH plant with the highest percentage of oil content was 91-1295. All fatty acid contents (percentages oil) were significantly different (p=0.05) in all the genotypes studied except for the lignoceric fatty acid. Oleic acid was highest in the F_1-derived DH plant 94-1291. The F_1-derived DH plants had relatively higher levels of this particular fatty acid than *B. juncea* and BC_1F_1-derived DH lines. Linoleic acid was lowest in the F_1-derived DH line 94-1291 and highest in the BC_1F_1-derived DH plant 94-1284. Linolenic acid levels were approximately one half those of linoleic acid in most of the genotypes.

The correlation between oil content and oleic acid was low but positive (r=0.3140) (Table 5.2). Linoleic acid had a negative correlation coefficient (r=-0.2636) with oil content while linolenic acid had a low negative correlation coefficient (r=-0.1997). The relationship between oleic, linoleic and linolenic acids was high (oleic and linoleic r=-0.9812, oleic and linolenic r=-0.8262 linoleic and linolenic r=0.7534). The coefficients between oil content and arachidic acid (r=-0.6050) and behenic (r=-0.2962) were significant (p=0.05).

5.3.2. Glucosinolates

Total glucosinolate content was significantly different between the parents, F_1 and BC_1F_1-derived DH plants. The *B. juncea* 2 parent had a high total glucosinolate content in 100mg oil free meal (Table 5.3). The *B. napus* parent had the lowest total glucosinolate content among the three parents used. The F_1-derived DH plants had higher glucosinolate levels than both parents. Total alkenyls were highest in *B. juncea* 1

parent while the *B. napus* parent had the lowest. *B. juncea* 2 the maternal parent used as the female parent in all the BC_1F_1-derived DH lines apart from line 94-1289 had a total alkenyl glucosinolate content of 45.2 μmg^{-1}. The BC_1F_1-derived DH plants had an average of 59.6 μmg^{-1} of alkenyl glucosinolates. The total indoles were significantly different within the parents and F_1 and BC_1F_1-derived DH groups. The lowest level of total indoles was found in the F_1-derived DH plants with 5.4 μmg^{-1} and the highest in *B. napus* parent. The presence of 4-OH-indolylmethyl glucosinolate was highest in the *B. napus* and lowest in the F_1-derived DH plants (Table 5.3). 2-hydroxy-3-butenyl glucosinolates were high in F_1-derived DH plants. The lowest levels of this glucosinolate was found in *B. juncea*. BC_1F_1-derived DH plants and *B. juncea* were not significantly different (p=0.05) in this indole glucosinolate, but *B. napus* was. Allyl glucosinolate were mainly found in the BC_1F_1, F_1-derived DH lines and *B. juncea* plants. Among individual genotypes, the lowest level of allyl glucosinolate was found in the F_1-derived DH plant (94-1292).

Total glucosinolates ranged from 123.5 μmg^{-1} in F_1-derived DH line 94-1295 to 11.5 μmg^{-1} in F_1-derived DH plant 94-1292. The lines with the lowest total glucosinolate content were all BC_1F_1-derived DH lines 94-1281, 94-1288 and 94-1279 having 31.4, 37.3 and 39.3 μmg^{-1} respectively. These lines were significantly different (p=0.05) from both parents, all having lower levels than *B. juncea*, but higher levels than *B. napus*.

Total alkenyl glucosinolates, currently used to set the canola standard was significantly different among the genotypes analyzed. The plant with the highest level of total alkenyl glucosinolate was F_1-derived DH plant 94-1295 (Table 5.3). The lowest levels of alkenyl glucosinolates were obtained from 94-1281 with 24.3 μmg^{-1} and 94-1288 with 27.4 μmg^{-1}. Although these genotypes had higher glucosinolate contents than the *B. napus* parent, they were below the current 30.0 μmg^{-1} limit set for lines to be considered as canola quality. This will however change by 1997 to include both aliphatic and indole glucosinolates (Canola Council of Canada 1995b). Specific

glucosinolates like 3-butenyls, 4-pentenyl and 2-OH-3-butenyls, were all significantly different (p=0.05) between parents, F_1 and BC_1F_1-derived lines. The 3-butenyl and 4-pentenyl glucosinolates were found mainly in the $B. juncea$ and BC_1F_1-derived DH lines. They also occured in lower concentrations in $B. napus$ and F_1-derived DH lines. The BC_1F_1-derived DH line 94-1289 was derived from $B. juncea$ 1, which probably explains the high levels obtained for these glucosinolates. The BC_1F_1-derived DH lines with the lowest 3-butenyl glucosinolates included 94-1281, 94-1288, and 94-1279 (Table 5.3).

5.3.3. Proteins

The meal protein was highest in the F_1-derived DH line (Table 5.5). The parent lines had a narrow range of protein (43.7-44.5%). The BC_1F_1-derived DH lines had a wider variation (39.5-43.3%), with none of the lines showing higher protein content than the parents.

Table 5.1. Oil content (% in seed) and fatty acid profiles (% of oil content) of parents, F_1 and BC_1F_1-derived DH genotypes of *B. juncea* x *B. napus* interspecific crosses.

Genotype		Fatty acids								Oil Content (%)
Type	Plant	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Arachidic	Eicosenoic	Lignoceric	
Parent	*B. napus*	4.35bcde	1.40h	54.44b	24.52e	11.68h	0.58fgh	1.66a	0.21abc	46.5a
	B. juncea 1	3.35hi	2.64bc	44.76d	32.31bcd	13.75g	0.61efg	1.18fghi	0.20abc	43.3abc
	B. juncea 2	3.75efgh	2.17cdef	41.12fg	32.29bcd	17.70a	0.57gh	1.17ghi	0.18abc	41.5bc
F_1	94-1291	3.94defgh	1.84fgh	57.93a	21.86f	12.00h	0.68bcde	1.496b	0.00e	25.4h
	94-1292	4.61bc	2.38cde	52.62b	25.89e	13.31g	0.00j	1.20fgh	0.00e	nd
	94-1293	6.27a	3.30a	38.98gh	33.06bcd	15.62cdef	1.01a	0.93k	0.18abc	29.0g
	94-1295	3.94defgh	1.96efg	50.01c	25.74e	14.97f	0.64defg	1.35cd	0.14bcd	36.6de
BC_1F_1	94-1279	3.64fghi	2.11def	44.49de	31.48d	15.36ef	0.52hi	1.05j	0.18abc	46.3ab
	94-1280	3.84efgh	2.45cde	42.05ef	32.10cd	16.11bcdef	0.64defg	1.33cde	0.15bcd	43.8abc
	94-1281	4.12cdefg	2.43cde	40.53fg	30.80d	15.35ef	0.71bcd	1.23fg	0.07de	36.8de
	94-1282	3.90defgh	2.09def	39.14gh	34.15abc	17.31ab	0.58gh	1.35cd	0.12cd	42.0abc
	94-1283	4.24bcdef	2.51cd	39.22gh	34.08abc	16.48abcde	0.64defg	1.11hij	0.22abc	35.1c
	94-1284	4.67bc	2.51cd	37.21hi	35.77a	16.48abcde	0.67cdef	1.32cde	0.12cd	32.1ef
	94-1286	4.50bcd	3.02ab	35.92i	35.49a	16.91abcd	0.76b	1.26efg	0.23ab	36.4de
	94-1287	4.47bcd	2.25cdef	38.40ghi	34.62ab	16.55abcde	0.63defg	1.27def	0.24ab	35.7e
	94-1288	3.59ghi	2.24cdef	40.52fg	33.16bcd	16.99abc	0.59fgh	1.38c	0.15bcd	42.2abc
	94-1289	4.84b	3.06ab	40.39fg	32.01cd	15.96cdef	0.73bc	1.21fg	0.27a	40.6cd
	94-1486	3.71fgh	2.26cdef	41.99cf	32.81bcd	15.87cdef	0.60cfg	1.19fgh	0.15bcd	44.4abc
	SEM	0.140	0.118	0.633	0.548	0.299	0.020	0.022	0.023	0.892

Figures followed by the same letter (s) are not significantly different at p=0.05 using Duncan's Multiple range test. nd = not determined.

160

Table 5.2. Correlation coefficients of various fatty acids to total oil content profiles of parents, F_1 and BC_1F_1-derived DH genotypes of *B. juncea* x *B. napus* interspecific crosses (10 d.f)

	Lauric	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Aracidic	Eicosenoic	Behenic	Lignoceric	Oil Content
Lauric	1.000										
Palmitic	0.0257	1.000									
Stearic	0.2650	0.5341	1.000								
Oleic	-0.1740	-0.4284	-0.6870	1.000							
Linoleic	0.1751	0.3509	0.6537	-0.9812	1.000						
Linolenic	0.0800	0.1390	0.3903	-0.8262	0.7534	1.000					
Aracidic	0.0889	0.8094	0.7861	-0.4718	0.4084	0.1651	1.000				
Eicosenoic	-0.0679	-0.0005	-0.3743	0.2647	-0.2546	-0.3479	-0.0704	1.000			
Behenic	-0.0107	0.7168	0.3531	-0.2627	0.2166	0.0226	0.6849	0.0873	1.000		
Lignoceric	0.2158	0.2864	0.2598	-0.2868	0.2788	0.0573	0.1736	-0.0279	0.1884	1.000	
Oil Content	0.1079	-0.5355	-0.4004	0.3140	-0.2636	-0.1997	-0.6050*	0.0916	-0.2962*	-0.0560	1.000

* Significant at p=0.05

Table 5.3. Glucosinolate (μm g⁻¹) profiles of parents, F₁ and BC₁F₁-derived DH genotypes in *B. juncea* x *B. napus* interspecific crosses.

Type	Genotype Plant	Glucosinolate Allyl	Butenyl	Pentenyl	OHBUT	OHINDOMETH	Total Alkenyls	Total Indoles	Total Glucosinolates
Parent	*B. napus*	0.129e	1.824f	0.455f	7.217e	14.820a	9.92h	16.008a	25.93gh
	B. juncea 1	1.908c	81.568a	7.786b	1.830g	93.21abc	9.447bc	102.66ab	
	B. juncea 2	23.257bcd	18.844de	1.895def	1.109g	8.037cde	45.24efg	8.252bcde	53.49defg
F₁	94-1291	10.835de	11.325ef	0.737f	23.104c	5.336fg	46.20efg	5.500cde	51.70defg
	94-1292	0.107e	1.171f	0.344f	5.349ef	2.816h	7.30h	4.200e	11.50h
	94-1293	20.054bcd	14.954ef	4.823c	35.571b	5.245fg	69.00bcde	4.300e	73.30cde
	94-1295	39.183a	13.906ef	3.678bcde	57.674a	6.90cfg	116.50a	7.028bcde	123.53a
BC₁F₁	94-1279	17.853cd	12.096ef	0.885f	0.718g	9.350bcd	31.58gh	7.769bcde	39.34fgh
	94-1280	30.98abc	14.875ef	0.392f	2.733fg	6.958efg	49.16defg	8.138bcde	57.30def
	94-1281	14.348de	8.700ef	0.684f	0.452g	6.773efg	24.29gh	7.143bcde	31.43fgh
	94-1282	41.976a	36.544c	4.314cd	1.243g	7.671cde	84.20bc	7.935bcde	92.13bc
	94-1283	42.051a	11.734ef	1.826def	16.172d	4.942g	72.45bcde	5.18cde	77.63bcd
	94-1284	34.161ab	30.362cd	2.013def	0.574g	5.303fg	67.17cdef	5.521cde	72.69cde
	94-1286	42.909a	29.910cd	1.385ef	0.973g	4.717gh	75.31bcd	4.936de	80.25bcd
	94-1287	41.201a	40.733c	3.469cde	1.073g	6.140efg	86.59bc	6.249bcde	92.84bc
	94-1288	15.968cde	10.093ef	0.686f	0.527g	9.713bc	27.40gh	9.871b	37.27fgh
	94-1289	0.488c	64.478b	30.144a	1.487g	7.346def	96.80ab	6.043bcde	102.84ab
	94-1486	19.037bcd	16.932c	2.118def	1.139g	10.820b	39.33fg	9.051bcd	48.38efg
	SEM	3.731	3.128	0.562	0.750	0.520	6.666	0.948	6.582

OHBUT=2-Hydroxy-3-Butenyl; OHINDOMETH=4-Hydroxy-3-indolylmethyl

162

Table 5.4. Correlation of individual glucosinolates to total glucosinolates of parents, F_1 and BC_1F_1-derived DH genotypes in *B. juncea* x *B. napus* interspecific crosses (9d.f).

	Allyl	Butenyl	Pentenyl	OHBUT	OHPENT	INDOMETH	OHINDOMETH	TOTALK	TOTINDOLES	TOTGLUCS
Allyl	1.000									
Butenyl	0.0538	1.000								
Pentenyl	-0.3030	0.5904	1.000							
OHBUT	0.0571	-0.1948	0.0414	1.000						
OHPENT	-0.1630	-0.1576	0.2189	0.6381	1.000					
INDOMETH	-0.0801	-0.2675	-0.0943	0.2442	0.2046	1.000				
OHINDOMETH	-0.5804	-0.2250	0.0087	-0.0345	0.3505	0.2637	1.000			
TOTALK	0.5175	0.7749	0.4699	0.2062	0.0362	-0.1586	-0.4632	1.000		
TOTINDOLES	-0.4969	-0.2422	-0.0531	-0.0126	0.3507	0.3794	0.8538	-0.4340	1.000	
TOTGLUCS	0.4820*	0.7816*	0.4852*	0.2142*	0.0797	-0.1207	-0.3827*	0.9942*	-0.3348*	1.000

* Significant at p=0.05

OHBUT= 2-Hydroxy-3-Butenyl, OHPENT=2-Hydroxy-4-Pentenyl; INDOMETH=3-Indolylmethyl; OHINDOMETH=4-Hydroxy-3-indolylmethyl

163

Table 5.5. Protein content of oil free meal (%) of parents and BC₁F₁-derived DH genotypes of *B. juncea* x *B. napus* interspecific crosses.

Genotype	Plant	Protein (%)
Parents	*B. napus*	44.2b
	B. juncea 1	44.5b
	B. juncea 2	43.7ab
F₁-derived DH	94-1295	47.0a
BC₁F₁-derived DH	94-1279	40.4de
	94-1280	43.2bc
	94-1281	41.5cde
	94-1282	41.1cde
	94-1283	39.8e
	94-1284	39.5e
	94-1286	42.0bcde
	94-1287	41.2cde
	94-1288	43.3bc
	94-1289	39.8e
	94-1486	42.8bcd
	SEM	0.676

5.3.4. Disease Resistance

5.3.4.1. Blackleg (*Leptosphaeria maculans*)

Blackleg resistance was high in *B. juncea* (Figure 5.1). *B. napus* had the highest disease severity score. There were significant differences observed between *B. juncea* and *B. napus* parents (p=0.05). The F_1-derived DH line screened for blackleg had an intermediate reaction to the disease with a severity score of log10 1.29 (19.5%) between the two parents (other F_1-derived DH lines did not germinate). A wide range of hypersensitive reactions were obtained from the BC_1F_1-derived DH lines ranging from very resistant (94-1486) with a score of log10 DS of 0.5987 (3.1%) to fairly susceptible DH lines (94-1288) with a log10 DS score of 1.43 (27.0%).

5.3.4.2. Blackspot (*Alternaria brassicae*)

The scores were taken from field grown plants. Although different methods were used in scoring the disease reaction of the lines, standardization of the data enabled valid comparisons to be made. Contrary to previous reports, *B. napus* was more susceptible than *B. juncea* to *Alternaria brassicae* (Rai and Kumar 1995, Saharan 1991). The disease score for *B. napus* was Log10 DS of 1.0398 (10.9%) as compared to *B. juncea*'s score of Log10 DS of 0.9143 (8.2%) (Figure. 5.4). The F_1-derived DH lines 94-1291 and 94-1295 had log10 DS of 1.044 (11.8%) and 0.9248 (8.4%). According Rai and Kumar (1995), the F_1 of a similar cross were characterized as moderately resistant. The BC_1F_1-derived DH lines had a wide range of phenotypic expression to blackspot disease. Very susceptible DH lines (94-1289 and 94-1287) with log10 DS scores of 1.2906 (19.5%) and 1.1505 (14.1%) and very resistant lines (94-1280 and 94-1281) with log10 DS of 0.3495 (2.2%) and 0.5714 (3.7%) were obtained which had values well above and below the parents.

Figure 5.1. Blackleg and Black spot disease severity (DS) score on laboratory grown (Blackleg) and Field grown (Blackspot) parents. F_1 and BC_1F_1 derived DH lines.
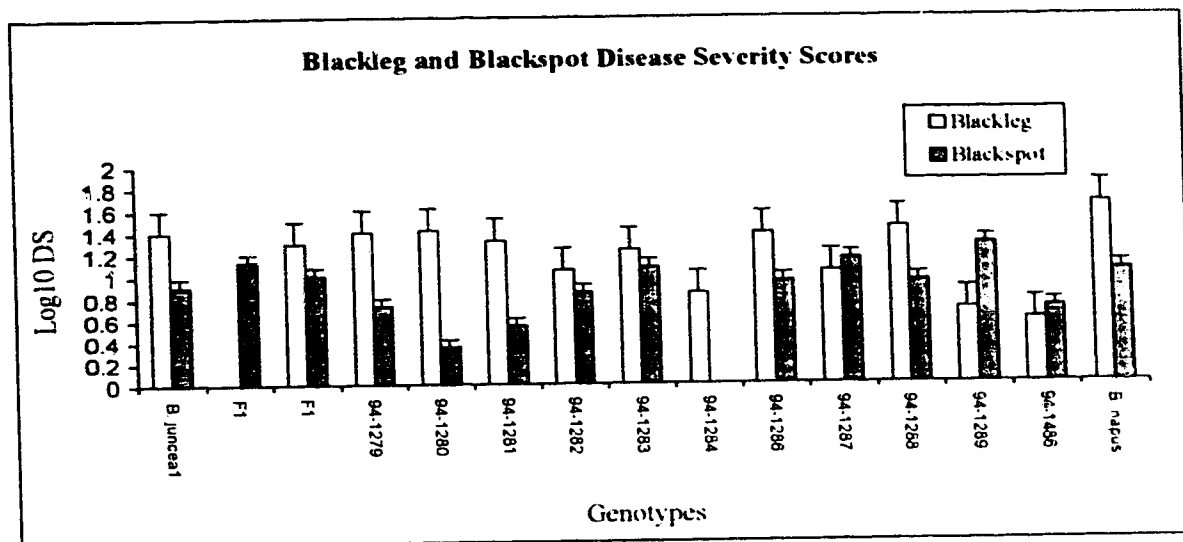
5.3.5. Seed Colour

The F_1 seeds from the *B. juncea* x *B. napus* cross had a black seed coat. On backcrossing, the seed coat colour of the BC_1F_1 plants varied, with 3 BC_1F_1-derived DH lines showing seeds that could not be classified as completely black or completely brown. Eight of the 11 BC_1F_1-derived DH lines had yellow seed coats. Seeds from reciprocal crosses of the F_1-derived DH lines with the parents had seed coats similar to those of the maternal plant, but the shades of brown obtained with the BC_1F_1-derived DH lines created a problem in classification. As a result, distinct classes of yellow, brown, brown/black and black were used in the classification of seed colour (Table 5.6).

Table 5.6.   Seed coat colour of parents, F$_1$, BC$_1$F$_1$-DH lines and reciprocal crosses of F$_1$-derived DH and parent lines.

Genotype		Seed Coat Colour
Type	**Plant**	
Parent	*B. napus*	Black
	B. juncea 1	Yellow
	B. juncea 2	Yellow
F$_1$ derived DH	94-1291	Black
	94-1292	Black
	94-1293	Black
	94-1295	Black
BC$_1$F$_1$ derived DH	94-1279	Yellow
	94-1280	Yellow
	94-1281	Yellow
	94-1282	Yellow
	94-1283	Yellow
	94-1284	Brown/Black
	94-1286	Yellow
	94-1287	Brown/Black
	94-1288	Yellow
	94-1289	Brown/Black
	94-1486	Yellow
F$_1$ derived DH x Parents	94-1292 x *B. juncea* 2	Brown/Black
	94-1293 x *B. juncea* 2	Brown
	94-1293 x *B. napus*	Brown
	94-1295 x *B. juncea* 2	Brown/Black
	94-1295 x *B. napus*	Brown
	94-1290 x *B. juncea* 2	Black
	94-1290 x *B. napus*	Brown/Black
Parents x F$_1$-derived	*B. juncea* 2 x 94-1292	Yellow
	B. juncea 2 x 94-1293	Yellow
	B. juncea 2 x 94-1295	Yellow
	B. napus x 94-1292	Brown/Black
	B. napus x 94-1293	Brown/Black
	B. napus x 94-1295	Brown/Black

5.4. Discussion

5.4.1. Oil Content and Fatty acid Profiles

The range of phenotypic expression in oil content ranged from 46.3% to 25.4%o in both the F_1 and BC_1F_1-derived DH lines. The fact that three DH lines had oil contents similar to the B. *napus* parent (46.5%) than to the B. *juncea* (41.5%o) parent suggests that introgression of genes for this trait from B. *napus* to B. *juncea* had occurred (Figure 4.7). Recombination however, also resulted in genotypes with values lower than the B. *juncea* parent, implying that some recombinations from the cross were undesirable. The F_1-derived DH lines had generally low oil contents, which were easily shifted on backcrossing towards the B. *juncea* background.

5.4.2. Fatty acid Profiles

5.4.2.1. Oleic acid

B. *napus*' with the preferred fatty acid profile registered a mean of 54.4% oleic acid. Most of the F_1-derived DH lines showed a skewed oleic acid level towards B. *napus*. These lines had oleic acid levels >50% apart from the line 94-1293 with a mean of 39.9%. This F_1-derived DH line expressed more B. *juncea* type characteristics than the other F_1-derived DH lines. Among the 6 F_1-derived lines studied cytologically, the line 94-1291 had an average of 24-29 chromosomes during anaphase I of meiosis, a higher count than all the other lines. It is possible that during meiosis, PMCs with unequal chromosome distribution were produced (Table 3.1). This could have eventually resulted in the DH line possessing more B genome gene(s) from B. *juncea* than the other F_1-derived DH lines. In a study by Chen et al. (1992) removal of C-genome chromosomes in B. *rapa-alboglabra* monosomic addition lines resulted in a dramatic increase in erucic acid. This was attributed to a loss of genes responsible for biochemical process leading to fatty acid desaturation step. In the current study, B. *juncea* had lower levels of oleic acid (41.1%). This level was improved from 41.1% in to levels as high as 44.5% in 3 of the 11 BC_1F_1-derived DH lines. The reduction in oleic acid contents in five of the lines suggests the possibility that the gene loci controlling this trait could be on different chromosomes or may be physically located

further apart in the same chromosome allowing exchange during crossing over (Chen et al. 1992). Three BC_1F_1-derived DH lines had oleic acid levels statistically similar to *B. juncea*. Thus it should be possible to obtain a wide range of expressed phenotypes if chromosomes from different genomes occurred in various combinations in the DH lines.

5.4.2.2. Linoleic acid

Linoleic acid was significantly increased in 6 BC_1F_1-derived DH lines. These lines had higher linoleic acid levels than *B. juncea* (Table 5.1). Apparently, there must have been introgression of genes from *B. napus* to *B. juncea* to result in such an increment in levels of this fatty acid. This may be possible if the desaturation step from oleic acid is altered, or further desaturation to linolenic is blocked. These genes were probably not expressed fully in *B. napus* (24.5%), suggesting they could be partially masked or the recombination with *B. juncea* genes enhanced the expression of this trait. The F_1-derived DH lines had a skewed distribution towards the *B. napus* parent except for the F_1-derived DH line 94-1293 which had more *B. juncea* characteristics. The level of this fatty acid which was highest in the *B. juncea* parent, was not retained in most the F_1-derived DH lines, but was likely tempered in its expression by genes from the *B. napus* parent in 6 of the BC_1F_1-derived DH lines (Table 5.1).

5.4.2.3. Linolenic acid

The level of linolenic acid in the *B. juncea* (17.7%) was higher than all of the lines studied. Only a slight reduction in the linolenic acid levels was observed from the *B. juncea*. Although the reduction was significant in some cases, the profiles of all the BC_1F_1-derived DH lines were *B. juncea* types. The F_1-derived DH lines however had profiles closer to *B. napus* profiles than *B. juncea*. It could be argued that the genes responsible for low linolenic acid were in the C genome chromosomes in the *B. napus*, which were introgressed into the F_1-derived lines, but were subsequently lost on backcross. The slight reduction in the linolenic acid levels in the BC_1F_1-derived DH lines was probably as a result of some gene(s) being retained in the BC_1F_1-derived DH

lines, but their influence was not sufficient to reduce levels of the acid. This trait was not as easily altered as the other fatty acid profiles.

5.4.2.4. Other Fatty Acids

One of the problem fatty acids is erucic acid, of which canola must contain less than 2% of the total fatty acid content. In the current material, the genotypes did not show any erucic acid (Retention time of 5.80 min in GLC). The genes regulating synthesis of this fatty acid present in the A and B genome in *B. juncea*, and in A and C in *B. napus* (Kirk and Hurlstone 1983, Fernadez-Escobar 1988, Chen et al. 1988a) seem to have been lost or their expression insignificant in the studied material. It is also possible that minute quantities may have been synthesized, but could not be detected by the GC at that sensitivity level.

Low levels of palmitic acid (C16:0) are necessary to reduce the level of saturation in the oil, however this acid is necessary to improve the suitability of canola oil for manufacture of margarine and shc·t ·nir.gs because it reduces crystallization in the margarine (Davik and Heneen 1993). A a.· ·om F_1-derived DH line 94-1293 the genotypes studied had a narrow profile r.·:.gr ior r.':.; fatty acid. Slight modification in the elongation process may have resulted from the influence of genes from the various genomes consituting the species used in the cross. The percentage of palmitic acid was negatively correlated with oleic acid, suggesting an increase in the biochemical process leading to the carbon chain elongation process from C16 to C18. Stearic acid (C18:0) was positively correlated with levels of palmitic acid. The level of this fatty acid was lower than palmitic acid, ranging from 1.4-3.3%. The eicosenoic acid (C20:1) synthesized from elongation of oleic acid (C18:1) was low, probably because of altered elongation blockage at this step. The reduced amount of this fatty acid may have contributed to the lack of erucic acid as elongation of eicosenoic acid leads to erucic acid. Other minor fatty acids like arachidic (C22:0) and lignoceric acid (C24:0) constituted less than 1.9% of the fatty acids. This is probably as a result of the lack of their fatty acid precursor compounds resulting from blockage and subsequent elongation step from oleic acid through eicosenoic and erucic acids.

5.4.3. Glucosinolates

The variation in glucosinolate content could be attributed to genetic instability. The presence of two univalents in some BC_1F_1-derived DH lines appears to be associated with glucosinolate content in the seeds. Higher levels were obtained in lines showing higher instability (Table 3.2, Figures 3.7, 3.13), with lower glucosinolate levels observed in lines showing a more stable configuration (Figures 3.10, 3.11). Chromosomes from different species but from the same genome (homologous chromosomes) may pair closely because of structural homology, hence greater stability. These chromosomes would have a greater chance of being retained in subsequent backcross than would be the case with homoeologous pairing. It is therefore possible that some chromosome(s) or gene(s) from both the A and C-genome of *B. napus* may have been introgressed into this material complimenting the genes already present in *B. juncea*. These would be important traits expressed by recessive genes.

5.4.3.1. Total Glucosinolates

Total glucosinolates in the present study included both indole and alkenyl glucosinolates. A wide range of phenotypes were observed ranging from extremes of both parents to intermediate types. Two BC_1F_1-derived DH lines (94-1281 and 94-1288) with 31.4 μmg^{-1} and 37.3 μmg^{-1} were obtained, which had lower and significantly different total glucosinolates than *B. juncea*. However the levels of total glucosinolates were greater than those observed in *B. napus*. Although a significant reduction of glucosinolates from 53.5 μmg^{-1} (*B. juncea*) to as low as 31.4 μmg^{-1} (94-1281) was achieved (Table 5.3), these lines do not meet the canola quality standard.

Six BC_1F_1-derived DH lines had total glucosinolate levels (57.3-92.8 μmg^{-1}) higher than *B. juncea* 2 parent. This glucosinolate elevation could occur through recombination of genes controlling this trait from various genomes through either chromosomal substitution (Chen et al. 1988b) or crossing over during meiosis. The result would be chromosomes with modified gene constitution, thus enhancing expression of this trait. The presence of extremes of phenotypes makes it possible to

select for lower total glucosinolates, and because intermediate phenotypes were obtained, there appears to be partial dominance or modification of expression of this trait.

5.4.3.2. Total alkenyls

To conform with current canola standards, total contents of the four major alkenyl glucosinolates must not exceed 30 μmg^{-1} of oil free meal (Aldophe 1980). Only two of the BC_1F_1-derived DH lines had levels below this value. Four of the BC_1F_1-derived DH lines had significantly lower amounts of these glucosinolates (31.6-39.3 μmg^{-1}) than *B. juncea* 2 (45.2 μmg^{-1}).The drastic reduction and increase of alkenyl glucosinolates over the *B. juncea* parent in some lines (Table 5.3), could only arise from a loss or gain of major genes from the C genome of *B. napus* (Kondra and Stefansson 1970, Parkin et al. 199 Magrath et al. 1993). The possibility of inheriting one or both genes responsible for chain elongation and side chain structure (Magrath et al. 1993) may explain the wide variation in alkenyl glucosinolates observed in the BC_1F_1-derived DH lines.

Since the major difference in glucosinolate content in canola quality varieties results from the alkenyl glucosinolates (Bilsborrow 1993), a decrease in alkenyl glucosinolates would be highly desirable. The expression of low alkenyl glucosinolates requires four recessive alleles, and up to 6-9 recessive alleles in backcross populations in *B. juncea* (Stringam and Thiagarajah 1995). Inheritance of some of these alleles may have the effect of reducing alkenyl glucosinolates at varying degrees without displaying profiles from either parent (Kondra and Stefansson 1970) allowing selection for desired genotypes.

5.4.3.3. Total indole Glucosinolates

The definition of canola (Aldophe 1980) did not include the amount of indole glucosinolates in the meal, although they constitute a substantial amount of total glucosinolates. In fact, with the development of low glucosinolate cultivars, indole glucosinolates represented a greater proportion of total glucosinolates since their levels

remain relatively constant (Bilsborrow 1993). The definition of canola has been proposed to include indole glucosinolates (Fenwick 1985). The Canola Council of Canada has proposed that beginning 1997, new glucosinolate level requirements for seed will be 18 μmg^{-1}, whereas the oil free air dried meal will contain <30 μmg^{-1} total glucosinolates (Canola Council of Canada 1995b). In the current study, the profile of *B. juncea* was preferred because of lower levels of indole glucosinolates it exhibited. *B. napus* with the lowest total alkenyl glucosinolates, had the highest level of indole glucosinolates. It was however possible to obtain BC_1F_1-derived DH lines with even lower levels of indole glucosinolates than the lower parent value *B. juncea*. Lines with as low as 4.9 μmg^{-1} of indole glucosinolates were obtained showing possibilities exist of reducing both alkenyl and indole glucosinolates even when the two fatty acids are negatively correlated (Table 5.4). The present data suggest the genes controlling indole glucosinolates may be in different chromosomes, located further apart on the various chromosomes allowing exchange of genetic material during crossing over, or transfer of whole chromosomes between the genomes occured. Although the control of indolyl glucosinolates was thought to be under complex polygenic control (Rucker and Rudloff 1992, Mithen 1992), it appears possible to easily modify the profile of these glucosinolates. If indeed this is the case, then a high probability exists that whole chromosomes may have been introgressed into *B. juncea* in this material. The correlation index between the total indoles and total alkenyls was negative (r = -0.434) and were significant when correlated to total glucosinolates (Table 5.4). This would mean reduction of alkenyl glucosinolates would result in increased indole glucosinolates which the data presented implies. This was also observed by Musnicki et al. (1995). This would make it difficult to completely eliminate both types of glucosinolates unless the apparent link is broken.

5.4.3.4. Allyl Glucosinolates

The allyl glucosinolates are more abundant in the *B. juncea* than in *B. napus* (Sang et al. 1984). In the interspecific material between *B. juncea* and *B. napus* studied, the highest level of this glucosinolate was found in *B. juncea* as expected.

Extreme phenotypes obtained (0.49-42.9 μmg^{-1}), show that selections could be made for lower levels of this glucosinolate. As low as 0.49 μmg^{-1} was obtained in the BC_1F_1-derived DH lines (94-1289) which was derived from *B. juncea* 1 parent (Appendix 1), with the highest in BC_1F_1-derived DH line (94-1286). It is probable that genes introgressed from *B. napus* had the effect of enhancing allyl glucosinolate synthesis resulting in the observed increase in some F_1 and BC_1F_1-derived DH lines, well beyond the high parent range.

5.4.3.5. Proteins

The wide variation obtained in protein content may be a reflection of the differential loss of genetic material inherited from *B. napus* after backcrossing to *B. juncea*. In the process, the data suggests that loss or substitution of genetic material from *B. juncea* may have occurred, making it possible to obtain genotypes of lower meal protein value than either parent. However two of the lines had protein contents similar to the *B. juncea* parent. Only three of the lines did not have meal protein content of >40% (Table 5.6). Two of the brown /black seeded BC_1F_1-derived DH lines had low protein content (Tables 5.5 and 5.6) showing a link between black seeded colour and low meal protein (Slominski et al. 1995).

5.4.4. Disease Resistance

5.4.4.1. Blackleg Disease (*Leptosphaeria maculans*) resistance

Brassica species with the B genome (*B. nigra*, *B. carinata* and *B. juncea*) possess a hypersensitive type of resistance to blackleg that is effective throughout the life of the species (Rouxel et al. 1990, Rimmer and Van den Berg 1992). Introgression of the resistance gene(s) from the B genome may occur more readily if *B. carinata* is used in crosses with *B. napus*, but the resistance breaks down quickly (Rimmer and Van den Berg 1992). It has therefore been found more beneficial to use the long lasting but more difficult path of introgression through interspecific crossing with *B. juncea* (Sacristan and Gerdemann 1986). In the present study, the F_1 and BC_1F_1-derived DH lines screened had blackleg resistance equal to or lower than the *B. juncea* parents.

On backcrossing the F_1 to *B. juncea*, the resulting progeny would be expected to display the *B. juncea* type of resistance, because of increased proportion of the *B. juncea* genome (Table 2.8), but the current material had a wide range of phenotypes, from very high resistance (DS=3.1%) to *B. juncea* parent type (DS=25%). This suggests that resistance genes from *B. napus* were enhancing the already existing resistance genes in *B. juncea*.

Roy (1978, 1984) was successful in transferring *B. juncea* type blackleg resistance into *B. napus* through an interspecific cross. Continued segregation at the F_6-F_7, and cytological observations by Rimmer and Van den Berg (1992) indicated the observed resistance was as a result of aneuploidy. Roy's lines were found to contain 39 or 40 chromosomes. In the current study, the BC_1F_1-derived DH lines had normal chromosome counts apart from 94-1286 with 17II + 2I at metaphase 1. In most other lines at anaphase, chromosome segregation was 18:18 suggesting stability in the lines. Although Roy (1978) described resistance to blackleg in *B. juncea* as absolute and durable, Sjodin and Glimelius (1988) reported susceptibility in *B. juncea* accessions, implying that it was possible to lose the genes conferring the blackleg resistance in some cases. Keri (1991) (cited by Rimmer and Van den Berg 1992) showed resistance in *B. juncea* as resulting from the action of two epistatic genes. The epistatic effects of these genes in the present material may have been altered by the presence of *B. napus* genes producing the observed effects. Instead of a reduction in resistance to blackleg because of the presence of *B. napus* gene(s) (Rouxel et al. 1990), resistance to blackleg was actually enhanced in most of the BC_1F_1-derived DH lines.

5.4.4.2. Blackspot Disease (*Alternaria brassicae*) resistance

Several reports (Shivanna and Sawhney 1993, Tewari 1991, Rai and Kumar 1995) suggested that *B. napus* was more resistant to Alternaria blackspot than *B. juncea*, but in the material studied, *B. juncea* was more resistant. The presence of transgressive segregation for this trait observed in the BC_1F_1-derived DH lines for this trait implies selection was possible. On backcrossing to *B. juncea*, the continuous variation obtained suggests polygenic control. Recombinations with desirable traits

175

apparently did occur between the F_1 and *B. juncea* parent gene(s) resulting in individual lines with better resistance than either parent, however susceptible genotypes were also obtained. Some of the BC_1F_1-derived DH lines had very high susceptibility (94-1289) with log10 DS of 1.2906 (19.5%) while other lines had as low as log10 DS of 0.3495 (2.2%) in 94-1280. It appears that crossing of *B. juncea* with *B. napus*, followed by backcrossing, enabled recombination of resistance genes from the various genomes especially from A genome of *B. napus* and *B. juncea* to occur. This overcame the effects of genes from B genome which have been suggested as possessing a susceptibility conditioning effect on *B. nigra* and *B. juncea* (Rai and Kumar 1995)

5.4.5. Seed colour

Genes for the black colour present in *B. napus* were obviously introgressed into some of the BC_1F_1-derived DH lines. Vera and Woods (1982) identified genes for yellow colour in the A and C genomes in *B. juncea* and *B. carinata*. The seed colour has been thought to be under one, two, three, or five genes in different Brassica species (Hawk 1982, Chauhan et al. 1995, Getinet et al. 1987, Vera et al. 1979, Vera and Woods 1982, Stringam 1980, Mohammad et al. 1942, Shirzadegan 1986, Schwetka 1982). It has been suggested that seed colour is maternally determined (Meng 1995, Shirzadegan 1986). In reciprocal crosses done in the current study with the F_1-derived DH lines and the parents, the seeds obtained had shades of colour similar to the maternal parent. Schwetka (1982) reported that seed colour was predominantly but not completely determined by the maternal parent, however the maternal influence in the backcross seems reduced in the present material since three out of eleven lines had brown seeds. The three BC_1F_1-derived DH lines therefore must have had heterozygous alleles due to the expression of the brown colour. Seeds obtained by reciprocal crosses (Table 5.6) with the parents tended to express the phenotypic colour trait of the maternal plant, showing the strong cytoplasmic influence of this trait.

Yellow seed colour is expressed only when the loci are homozygous and in the recessive condition with any other combination resulting in brown or black seeds (Schwetka 1982), however, a strong cytoplasmic influence from the maternal parent

seems to be enough to allow for phenotypic expression of the yellow colour trait even when genes for black colour are present as shown by the reciprocal seeds (Table 5 o) Intergenomic complementation has been suggested as a result of existing homology between the Brassica genomes (Chen et al. 1988b), however individual genomes can function independently of each other to determine seed colour. The possibility of genes for yellow colour from A and C genomes (Chen et al. 1988b, Rashid et al. 1994) recombining therefore exists, increasing the probability of achieving seeds with a yellow coat. The fact that brown seeded DH lines were obtained after backcrossing the F_1 to B. juncea shows that it was also possible to introgress undesirable dark coloured gene(s) to the progeny.

References

Adams H., J.G. Vaughan and G.R. Fenwick 1985. The limited chemotaxonomic value of glucosinolates for cultivar identification in Brassica napus L. var. oleifera (Metzg.); Z. Pflanzenzuchtg 95: 97-102.

Adolphe D. 1980. Canola, Canada's Rapeseed Crop. Canola Council of Canada, Winnipeg.

Anand I.J. and R.K. Downey 1981. A study of erucic acid alleles in digenomic rapeseed (Brassica napus L.); Can. J. Plant Sci. 61: 199-203.

Ayer W.A. and L.M. Pena-Rodriguez 1987. Metabolites produced by Alternaria brassicae, the blackspot pathogen of canola. Part I, The phytotoxic components. J. Nat. Prod. 50(3): 400-407.

Ballester D., J. King, P. Vera, O. Brunser, E. Yanez and F. Monckerberg 1977. Toxicity of water extracted rapeseed meal in the rat. J. Sci. Food and Agric. 28 855-862.

Bansal V.K., P.D. Kharbanda, G.R. Stringam, M.R. Thiagarajah and J.P. Tewari 1994 A comparison of greenhouse and field screening methods for blackleg resistance in doubled haploid lines of Brassica napus. Plant Disease 78(3): 276-281.

Bell J.M. 1984. Nutrients and Toxicants in Rapeseed Meal: A review; Journal of
Animal Science, 58(4): 996-1010.

Bell J.M. 1993. Factors affecting the nutritional value of canola meal: A review. Can. J.
Anim. Sci. 73: 679-697.

Bell J.M. 1994. Recent Research on canola meal in Canada: Bulletin GCIRC 10: 104-
108.

Bell J.M. and M.O. Keith 1991. A survey of variation in the chemical composition of
commercial canola meal produced in Western Canadian crushing plants. Can. J.
Anim. Sci. 71:469-480.

Bell J.M. and R. Belzile 1965. Goitrogenic properties: Rapeseed meal for livestock and
poultry - A review, Canada Department of Agriculture Publication No. 1257
Ottawa Ontario.p45-60.

Bell J.M., D.M. Anderston and A. Shires 1981. Evaluation of Candle rapeseed meal as
a protein supplement for swine. Can. J. Anim. Sci. 61:453.

Bilsborrow P.E. and E.J. Evans 1989. Changes in glucosinolate concentrations of
single and double low varieties of autumn sown oilseed rape during seed
development. Aspects of applied Biology 23: 91-99.

Bilsborrow P.E., E.J. Evans, F. Murray and F.J. Zhao 1993. Glucosinolate changes in
developing pods of single and double low varieties of autumn sown oilseed rape
(*B. napus*). Ann. Appl. Biol 122: 135-143.

Bones A. and J. Rossiter 1995. Glucosinolates in cruciferous crops. In: New Horizons
for oilseed rape. eds. D.H. Scarisbrick and A.J. Ferguson. Published by
Semundo Ltd.

Canola Council of Canada 1995a. Canada's canola. Winnipeg Manitoba. pp 16.

Canola Council of Canada 1995b. Canola Council's policy positions. Canola digest.
Vol. 29(6): 2

Chauhan Y.S., K. Kumar, R. Bhajan and K.N. Maurya 1995. Inheritance of seed coat
colour and its use in determining natural cross pollination in yellow sarson.
Cruciferae Newsletter No. 17 pp60-61.

Chen B.Y., V. Simonsen, C. Lanner-Herrera and W.K. Heneen 1992. A *Brassica campestris-alboglabra* addition line and its use for gene mapping, intergenomic gene transfer and generation of trisomics. Theor Appl Genet 84: 592-599

Chen B.Y., W.K. Heneen and R. Johnsson 1988a. Independent inheritance of erucic acid content and flower colour in the C-Genome of *Brassica napus* L., Plant Breeding 100: 147-149.

Chen B.Y., W.K. Heneen and R. Johnsson 1988b. Resynthesis of *Brassica napus* L. through interspecific hybridization between *B. alboglabra* Bailey and *B. campestris* L. with special emphasis on seed colour. Plant Breeding 101(1): 52-59.

Clossais-Besnard N. and F. Larher 1991. Glucosinolates accumulation pattern during the development of single low and double low oilseed rape (*Brassica napus* L.) In: Changes in levels of glucosinolates in leaves of oilseed rape (*Brassica napus*) during growth, Rapeseed in a changing world: Proceedings Vol 6 1714-1717.

Conn K.L. and J.P. Tewari 1989. Interactions of *Alternaria brassicae* conidia with leaf epicuticular wax of canola. Mycological Rasearch 93(2): 240-242.

Conn K.L., J.P. Tewari and R.P. Awasthi 1990. A disease assessment key for Alternaria blackspot in rapeseed and mustard. Canadian Plant Disease Survey 70(1): 19-22.

Dahiya J.S. and S.R. Rimmer 1988. Phytoalexin accumulation in tissues of *Brassica napus* inoculated with *Leptosphaeria maculans*. Phytochemistry 27: 3105-3107.

Daun J.K. and D.I. McGregor 1991. Glucosinolates in seeds and residues In: Analysis of oilseeds, fats and fatty foods Ed. J.B Rossell and J.L.R Pritchard, Elsevier Science Publishers Ltd. p185-211.

Davik J. and W.K. Heneen 1993. Identification of oilseed Turnip (*Brassica rapa* L. var *oleifera*) Cultivar groups by their fatty acid and glucosinolate profiles J Sci Food Agric. 63: 385-390.

De March G., D.I. McGregor and G. Seguin-Shwartz 1989. Glucosinolate content of maturing pods and seeds of high and low glucosinolate summer rape; Can. J. Plant Sci. 69: 929-932.

Degenhardt K.J., G.A. Petrie and R.A.A. Morrall 1982. Effects of temperature on germination and infection of rapeseed by *Alternaria brassicae*, *A. brassicola* and *A. raphani*. Can. J. Plant Pathol. 4: 115-118.

Degenhardt K.J., W.P. Scoropad and Z.P. Kondra 1974. Effects on alternaria blackspot on yield, oil content and protein content of rapeseed. Can. J. Plant Sci. 54: 795-799.

Diepenbrock W. and R.F. Wilson 1987. Genetic regulation of linolenic acid concentration in rapeseed. Crop Sci 27: 75-77.

Downey R.K. and B.L. Harvey 1962. Methods of breeding for oil quality in rape; Can. J. Plant Sci 43: 271-275.

Downey R.K. and D.G. Dorrell 1971. Genetic control of fatty acid composition in oilseed crops; Proc. Flax Inst. Nov. 1971, ed. Fargo N.D.

Drozdowska L. and J. Rogozinska 1982. The occurrence of glucosinolates during the flowering and maturation of oilseed rape. Acta Agrobotanica 35: 25-29.

Eskin N.A.M., M. Vaisey-Genser, S. Durance-Todd and R. Przybylski 1989. Stability of low linolenic acid canola oil to frying temperatures. J Amer. Oil Chem Soc. 66: 1081-1084.

Fenwick G.R. 1985. The analysis of glucosinolates in rapeseed. The current situation. GCIRC Bulletin No. 2: 110-112.

Fernandez-Escobar J., J. Dominguez, A. Martin and J.M. Fernandez-Martinez 1988. Genetics of the erucic acid content in interspecific hybrids of Ethiopian Mustard (*Brassica carinata* Braun) and Rapeseed (*B. napus* L.); Plant Breeding 100: 310-315.

Getinet A., G. Rakow and R.K. Downey 1987. Seed colour inheritance in *Brassica carinata* A. Braun, Cultivar S-67. Plant breeding 99: 80-82.

Gland A., G. Robbelen and W. Thies 1981. Variation of Alkenyl glucosinolates in seed of *Brassica* species; Z. Pflanzenzuchtg 87: 96-110.

Grontoft M. and D. O'Connor 1990. Greenhouse method for testing of resistance of young Brassica plants to *Alternaria brassicae*. Plant Breeding 105: 160-164

Guenter W., B.A. Slominski., J. Simbaya and L.D. Campbell 1995. Potential for improved utilization of canola meal using exogenous enymes. Rapeseed today and tomorrow. Proceedings: Vol. 2: 164-166. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Harvey B.L. and R.K. Downey 1964. The inheritance of erucic acid content in rapeseed (*Brassica napus*); Can. J. Plant Sci. 44: 104-111.

Hawk J.A. 1982. Single gene control of seed colour and hypocotyl colour in turnip rape. Can. J. Plant Sci. 62: 331-334.

Heany R.K. and G.R. Fenwick 1980. The analysis of glucosinolates in Brassica species using Gas Chromatography Direct Determination of thiocyanate ion precursors, Glucobrassicin and Neoglucobrassicin; J. Sci. Food Agric. 31: 593-599.

Hong C.X. and B.D.L. Fitt 1995. Effects of infection conditions on the incubation period of dark leaf spot (*Alternaria brassicae*) on oilseed rape (*Brassica napus*). In: Rapeseed. Today and Tomorrow. Vol. 3. Proceedings: 9th International Rapeseed Congress Cambridge, UK 4-7 July 1995. F10 595-597

ISO 1990. Animal and vegetable fats and oils - Analysis by gas chromatography of methyl esters of fatty acids. ISO 5508:1990(E).

Iwarsson K., L. Ekman, B.R. Everitt, H. Figueiras and P.O. Nilsson 1973. The effect of feeding rapeseed meal on thyroid function and morphology in growing bulls. Acta Vet. Scand 14:1

Jack B., A. P. Brown, J.B. Davis and D. Erickson 1994. Selection for fatty acid composition of *Brassica napus* using microspore culture; Cruciferae Newsletter 16: 102-103.

Josefsson E. and L.A. Appelqvist 1968. Glucosinolates in seed of rape and Turnip rape as affected by variety and environment. J. Sci. Food Agric. 19: 564-570.

Kennedy R. and A.M. Graham 1995. Infection oilseed rape by *Alternaria brassicae* under varying conditions of temperature and wetness. In: Rapeseed Today and

Tomorrow. Vol. 3. Proceedings: 9th International Rapeseed Congress Cambridge, UK 4-7 July 1995. F12: 601-603.

Kennedy T.H. and H.D. Purves 1941. Studies on experimental goitre. I. The effects of *Brassica* seed diets on rats. Brit. J. Exp. Pathol. 22: 241.

Kirk J.T.O. and C.J. Hurlstone 1983. Variation and inheritance of erucic acid content in *Brassica juncea*; Z. Pflanzenzuchtg. 90: 331-338.

Kondra Z.P. and B.R. Stefansson 1970. Inheritance of the major glucosinolates of rapeseed (*Brassica napus* L.) meal; Can. J. Plant Sci. 50: 643-647.

Kraling K., G. Robbelen, W. Thies, M. Hermann and M.R. Ahmadi 1990. Variation of seed glucosinolates in lines of *Brassica napus*; Plant Breeding 105: 33-39.

Love H.K., G. Rakow, J.P. Raney and R.K. Downey 1990. Genetic control of 2-Propenyl and 3-Butenyl glucosinolate synthesis in mustard; Can. J. Plant Sci. 70: 425-429.

Magrath R. and R. Mithen 1993. Maternal effects on the expression of individual aliphatic glucosinolates in seeds and seedlings of *Brassica napus*; Plant Breeding 111: 249-252.

Magrath R., C. Herron, A. Giamoustaris and R. Mithen 1993. The inheritance of aliphatic glucosinolates in *Brassica napus*; Plant Breeding 111: 55-72.

McGregor D.I. 1978. Thiocyanate ion, A hydrolysis product of glucosinolates from rape and mustard seed; Can. J. Plant Sci. 58: 795-800.

McGregor D.I. 1988. Glucosinolate content of developing rapeseed (*Brassica napus* L. 'Midas') seedlings. Can. J. Plant Sci. 68: 367-380.

Meng J. 1995. Interspecific hybridization and seed coat quality. In: Rapeseed. Today and Tomorrow. Vol. 4. Proceedings: 9th International Rapeseed Congress Cambridge, UK 4-7 July 1995. J25: 1128-1133.

Merrien A. 1989. Double low oilseed rape in France: Factors affecting glucosinolate levels. Aspects of Applied Biology 23: 109-116.

Mithen R. 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica 63: 71-83.

Mohammad A., S.M. Sikka and M.A. Aziz 1942. Inheritance of seed colour in some oleiferous *Brassicae*. Ind. J. Genet. Plant Breeding 2: 112-127.

Mridha M.A.U. and B.E.J. Wheeler 1993. *In vitro* effects of temperature and wet periods on infection of oilseed rape by *Alternaria brassicae*. Plant Pathol. 42: 671-675.

Musnicki C.Z., W. Mroczyk and A. Potkanski 1995. The qualitative composition of glucosinolates in the seeds of different winter rapeseed cultivars. In Rapeseed Today and Tomorrow. Vol. 3. Proceedings: 9th International Rapeseed Congress Cambridge, UK 4-7 July 1995. H30: 922-924.

Newman P. and D.J. Bailey 1987. Screening for resistance to canker (*Leptosphaeria maculans*) in winter oilseed rape (Brassica napus ssp. oleifera). Plant Pathol. 36: 346-354.

Olesen L. P. 1981. Glucosinolates. In: The biochemistry of plants. Vol. 7. Secondary Plant Products. Ed. E.E. Conn. pp 502-525. Academic Press New York.

Parkin I., R. Magrath, D. Keith, A. Sharpe, R. Mithen and D. Lydiate 1994. Genetics of aliphatic glucosinolates. II. Hydroxylation of alkenyl glucosinolates in *Brassica napus*. Heredity 72: 594-598.

Pleines S. and W. Friedt 1988. Breeding for improved C18 fatty acid composition in rapeseed (*Brassica napus* L.). Fat Sci Technol 90: 167-171.

Pleines S. and W. Friedt 1989. Genetic control of linolenic acid concentration in seed oil of rapeseed (*Brassica napus* L.). Theor Appl Genet 78: 793-797.

Rai B. and H. Kumar 1995. Reaction of different oilseed Brassica species and some hybrids to infection of Alternaria leaf blight disease. Cruciferae Newsletter No. 17 pp 84-85.

Rashid A., G. Rakow and R.K. Downey 1994. Development of Yellow seeded *Brassica napus* through interspecific crosses. Plant Breeding 112: 127-134.

Rimmer S.R. and C.G.J. Van den Berg 1992. Resistance of oilseed Brassica spp. to blackleg caused by *Leptosphaeria maculans*. Can. J. Plant Pathol. 14: 56-66.

Rouxel T., M. Renard, A. Kollmann, and J.F. Bousquet 1990. Brassilexin accumulation and resistance to *Leptosphaeria maculans* in Brassica spp. and progeny of an interspecific cross *B. juncea* x *B. napus*. Euphytica 46: 175-181.

Roy N. N. 1984. Interspecific transfer of *B. juncea* type high blackleg resistance to *B. napus*. Euphytica 33: 295-303.

Roy N. N. and A. W. Tarr 1986. Development of near zero linolenic acid (18:3) of rapeseed *B. napus*. Z. Pflanzenzuchtg 96: 218-223.

Roy N.N. 1978. A study on disease variation in the populations of an interspecific cross of *Brassica juncea* L. x *Brassica napus* L. Euphytica 27: 145-149.

Rucker B. and E. Rudloff 1992. Investigations on the inheritance of the glucosinolate content in seeds of winter oilseed rape (Brassica napus). In: Proceedings, Eighth International Rapeseed Congress, Saskatoon, Canada pp191-196.

Sacristan M.D. and M. Gerdemann 1986. Different behaviour of *Brassica juncea* and *B. carinata* as sources of *Phoma lingam* resistance in experiments of interspecific transfer to *B. napus*. J. Plant Breed. 97: 304-314.

Saharan G.S. 1991. Assessment of losses epidemiology and management of blackspot disease of rapeseed-mustard. In: Rapeseed in a changing world. Proceedings GCIRC Rapeseed Congress, Saskatoon Canada 1991. Vol 2. 465-469.

Sang J.P., C.A. Bluett, B.R. Elliott and R.J.W. Truscott 1986. Effect of time of sowing on oil content, erucic acid and glucosinolate contents in rapeseed (*Brassica napus* L. cv. Marnoo); Aust. J. Exp. Agric. 26: 607-611.

Sang J.P., I.R. Minchton, P.K. Johnstone and J.W. Truscutt 1984. Glucosinolate profiles in the seed, root and leaf tissue of cabbage, mustard, rape seed radish and swede. Can. J. Plant Sci. 64: 77-93.

SAS 1989. SAS institute Inc. Cary NC USA 27513-2414.

Sauer W.C., R. Cichon and R. Misir 1982. Amino acid availability and protein quality of canola and rapeseed mealfor pigs and rats. J. Anim. Sci. 54: 292-301.

Scarth R., P.B.E. McVetty, S.R. Rimmer and B.R. Stefansson 1988. Stellar low linolenic-High linoleic acid summer rape; Can. J. Plant Sci. 68: 509-511.

Scarth R., S.R. Rimmer and P.B.E. McVetty 1995. Stellar low Apollo low linolenic summer rape; Can. J. Plant Sci. 75: 203-204.

Schwetka A. 1982. Inheritance of seed colour in turnip rape (*Brassica campestris* L.) Theor Appl Genet 62: 161-169.

Scoropad W.P. and J.P. Tewari 1977. Field evaluation of the role of epicuticular wax in rapeseed and mustard in resistance to Alternaria blackspot. Can J. Plant Sci. 56: 781-785.

Shirzadegan M. 1986. Inheritance of seed color in *Brassica napus* L. Z. Pflanzenzuchtg 96: 140-146.

Shivanna K.R. and V.K. Sawhney 1993. Pollen selection for *Alternaria* resistance in oilseed brassicas: responses of pollen grains and leaves to a toxin of A. *brassicae*. Theor Appl Genet 86: 339-344.

Sjodin C. and K. Glimelius 1988. Screening for resistance to blackleg *Phoma lingam* (Tode ex Fr.) Desm. within Brassicaceae. J. Phytopathol. 123: 322-332.

Slominski B.A., J. Simbaya, L.D. Campbell and W. Guenter 1995. Nutritive profile of yellow seeded canola/rapeseed. Rapeseed today and tomorrow. Proceedings Vol. 1: 148-149. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Sones K., R.K. Heaney and G.R. Fenwick 1984. Glucosinolates in *Brassica* vegetables. Analysis of twenty seven cauliflower cultivars (*Brassica oleracea* L. var. *botrytis* subvar. *cauliflora* DC); J. Sci. Food Agric. 35: 762-766.

Stringam G.R. 1980. Inheritance of seed colour in turnip rape. Can. J. Plant Sci. 60: 331-335.

Stringam G.R. and D.I. McGregor 1980. Inheritance and fatty acid composition of a yellow embryo mutant in turnip rape (Brassica campestris). Can. J. Plant Sci. 60: 97-102.

Stringam G.R. and M. R. Thiagarajah 1995. Inheritance of Alkenyl glucosinolates in traditional and microspore-derived doubled haploid populations of *Brassica juncea* L. Czern and Coss. Rapeseed today and tomorrow. Proceedings: Vol. 2: 413-415. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Stringam G.R., D.F. Degenhardt, M. R. Thiagarajah and V.K. Bansal 1995a. Quantum summer rape. Can. J. Plant Sci. 75:903-904.

Stringam G.R., V.K. Bansal, M. R. Thiagarajah, D.F. Degenhardt, and J.P. Tewari 1995b. Development of an agronomically superior blackleg resistant canola cultivar in *Brassica napus* L. using doubled haploidy. Can. J. Plant Sci. 75:437-439.

Tanhuanpaa P.K., J.P. Vilkki and H.J. Vilkki 1995. Identification of a RAPD marker for palmitic acid concentration in the seed oil of turnip rape (*Brassica rapa* ssp. oleifera). Theor Appl Genet 91: 477-480.

Tewari J.P. 1991. Current understanding of resistance to *Alternaria brassicae* in crucifers. In: Rapeseed in a changing world. Proceedings GCIRC Rapeseed Congress, Saskatoon Canada 1991. Vol 2. 471-476.

Tewari J.P. and K.L. Conn 1988. Incidence of the blackspot of canola caused by *Alternaria brassicae* (Berk.) Sacc. during 1987. Can. Plant Dis. Surv. 68(1): 103.

Thomas P.M. and Z.P. Kondra 1973. Maternal effects on the oleic linoleic and linolenic acid content of rapeseed oil. Can. J. Plant Sci. 53: 221-225.

Tremolieres A . J.P. Dubacq and D. Drapier 1982. Unsaturated fatty acid in maturing seeds of sunflower and rape: Regulation by temperature and light. Phytochemistry 21: 41-45.

U N. 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jap J Bot 7: 389-452.

Van den Berg C.G.J. and S.R. Rimmer 1991. Changes in levels of glucosinolates in leaves of oilseed rape (*Brassica napus*) during growth, Rapeseed in a changing world: Proceedings Vol. 6: 1697-1699.

Vera C.L. and D.L. Woods 1982. Isolation of independent gene pairs at two loci for seed coat colour in *Brassica juncea*. Can. J. Plant Sci. 62: 47-50.

Vera C.L., D.L. Woods and R.K. Downey 1979. Inheritance of seed coat colour in *Brassica juncea*. Can. J. Plant Sci. 59: 635-637.

Chapter 6

Summary and Conclusions

6.1. Summary

6.1.1. Breeding Material Development and Genetic characterization

The cross between *B. juncea* and *B. napus* was achieved without major problems, however microspore culture performed on the F_1 and BC_1F_1 proved difficult The F_1 from one of the interspecific crosses (93-700-2) was not responsive and F_1-derived DH lines from this cross were not obtained. This may reflect the recalcitrant nature of *B. juncea* to microspore culture. The backrossing scheme (Figure 2.1) resulted in 21 BC_1F_1 donor lines of which only three resembled *B. napus* (Table 2.1) The microspore culture carried out in all donor lines was successful in only eight of the BC_1F_1 plants, with some more responsive than others (Table 2.2). Because of the problems associated with infertility and colchicine toxicity, only eleven BC_1F_1 and six F_1-derived DH lines were obtained.

The genetic characterization of this material using twenty nine RAPD markers (Table 2.4, 2.5) generated by six primers (Table 2.3) between parent lines, F_1 and BC_1F_1-derived DH lines revealed genetic similarities between them. Because of their common genome, *B. juncea* and *B. napus* had a similarity value (SI) of 0.42. The BC_1F_1-derived DH lines had an increased similarity index (SI) of 0.508 as compared to the *B. napus* parent showing an increased genetic component from *B. napus* (Table 2.9 and 2.10). The presence of genome specific markers in both F_1 and BC_1F_1-derived DH lines confirmed the presence of *B. napus* genes in the developed lines (Table 2.6, Figure 2.2, 2.7). Some of the markers were also observed by Quiros et al. (1991).

The preliminary agronomic performance data on the F_1 and BC_1F_1-derived DH lines in the field revealed good yield potential in the BC_1F_1-derived DH lines. The lines had delayed flowering as compared to the *B. juncea* parent, but were generally earlier flowering than *B. napus*. The F_1-derived DH lines had very low fertility resulting in poor seed set (Table 3.3). All the DH lines had good branching (4.9-13.8 branches) with no distinct patterns differentiating the F_1 and BC_1F_1-derived DH lines. Preliminary yield characteristics of BC_1F_1-derived DH lines (Table 3.5) taken on individual single

plants showed some superior lines yielding better than the parents. This indicates that recombination for some of the components of high yield occurred, resulting in superior genotypes.

6.1.2. Stability

The stability of the DH lines was determined through cytological observations, pollen staining and reciprocal crossing of the F_1 to *B. napus* and *B. juncea*. The meiotic data for the F_1-derived DH showed an average chromosome distribution of 11.7II + 0-0.7III + 3.5-10I. The higher level of univalents suggested reduced fertility stability, a factor observed in yield evaluation as very low seed formation was obtained. Pairing between chromosomes from different genomes must have occurred as reflected in varying numbers of univalents obtained from the various lines (Table 3.1, Figures 3.1, 3.2, 3.4 and 3.5).

The expected reduction in male fertility was not observed (Table 3.4), however, seed set was low, a problem attributed to the female fertility. Reciprocal crossing between the F_1-derived DH lines and the parent lines revealed that the fertility index was very low when the F_1-derived DH lines were used as the female parent (Table 3.5), and that improved seed set was obtained when *B. juncea* was used as the female parent. The BC_1F_1-derived DH lines showed a more stable meiosis than the F_1-derived DH lines, with an average meiotic configuration of 14.7II + 0.06III + 1-3.9I at prophase and metaphase (Table 3.2) which later stabilized in most of the lines to an 18:18 configuration at anaphase I (Figure 3.11). The presence of 17II + 2I could be attributed to a loose association between a B genome and a C genome chromosome which dissociated afterwards to give the observed configurations (Figures 3.7, 3.8, 3.15). This association appeared strong in some BC_1F_1-derived DH lines resulting in 18II (Figures 3.9, 3.14, and 3.16). This apparent instability did not affect their agronomic performance (based on one season data), but since only one generation was observed further observation is needed to establish their long term stability.

6.1.3. Quantitative traits

The quantitative traits in the developed F_1 and BC_1F_1-derived DH lines were determined for oil content, fatty acid profiles glucosinolate content, water stress, disease resistance and seed colour. A wide variation in oil content indicates a wide range of genetic recombination, particularly after backcrossing, since no F_1-derived DH line had high oil content. This shows it was possible to extract desirable genes from both species to improve the oil content of the parent whose desired background was sought (Table 5.1). The levels of oleic acid in three BC_1F_1-derived DH lines was generally intermediate between the high parent value (*B. napus* = 54.4%) and low parent value (*B. juncea* = 41.1%). F_1-derived DH lines had highest levels of this fatty acid of up to 57.9%. The improved profile in three BC_1F_1-derived DH lines over that in *B. juncea* indicate it was possible to introgress oil content genes from *B. napus* to *B. juncea* (Table 5.1). It was difficult to modify the linolenic acid profiles as indicated by the fact that BC_1F_1-derived DH lines had profiles similar to *B. juncea*. The genes controlling low linolenic acid from *B. napus* may have been lost during backcrossing Total glucosinolates determined on an oil free meal basis was reduced in some BC_1F_1-derived DH lines over that in *B. juncea* and increased in others. The reduced levels were however not as low as those in *B. napus*. Such observations were also reported by Rakow and Raney (1995). Elevated concentrations of total glucosinolates in the F_1-derived DH lines may have occurred as a result of genes from *B. juncea* and *B. napus* associating in the F_1, enhancing synthesis of glucosinolates. The fatty acid profile was not as variable as that observed in the glucosinolate profile, suggesting this trait was more stable.

6.1.4. Water stress resistance

Electrolyte leakage for 3rd and 6th leaf showed that *B. juncea* is more susceptible to water stress prior to acclimation, but is more tolerant following acclimation than *B. napus*. This could be achieved through physiological processes like osmotic adjustments and increase in amino acid concentration in the cells enabling them to resist drought stress. The effect of the drought stress is also thought to result in

increase in cell wall elasticity or a decrease in cell size. The electrolyte leakage followed similar trends for leaf 3 and 6 (Figure 4.7 and 5.2). The BC_1F_1-derived DH lines were however more susceptible than F_1-derived DH genotypes. This is probably as a result of higher non acclimated tolerance capability inherited from *B. napus*. Injury levels (Figure 4.9) exhibited a similar trend. The superior field water stress resistance observed in *B. juncea* as compared to *B. napus* (Woods et al. 1991) comes as result of acclimation when stress is gradually applied but is not evident in rapid stress to which *B. napus* appears adapted.

6.1.5. Disease resistance

The blackleg resistance recovered from the BC_1F_1-derived DH lines improved from 25% disease severity (DS) in the highly resistant parent (*B. juncea*) using the cotyledon test (Bansal et al. 1994) to about 3.2% DS in BC_1F_1-derived DH line 94-1486. This can be explained by recombination of introgressed gene(s) from *B. napus* with those from *B. juncea* enhancing the already existing resistance to blackleg. The genes for high resistance shown by *B. juncea* expressed both at seedling and adult stages seem to have interacted with the genes for partial resistance in *B. napus* to produce a more resistant genotype expressing a better phenotype than *B. juncea*. Although screening was done at the cotyledonary stage, it is expected the observed resistance will be stable and long lasting as reported in a previous study (Rimmer and Van den Berg 1992). The lack of non aggressive strains at the University Farm (Bansal, personal communication) did not allow for field screening to establish whether this was the case. The developed lines should be challenged in fields with aggressive forms of *Leptosphaeria maculans*.

Resistance to blackspot segregated transgressively in the BC_1F_1-derived DH lines. Differential sensitivity shown by *B. napus* and *B. juncea* was also expressed by BC_1F_1-derived DH lines. It was possible to obtain both highly resistant and highly susceptible lines through interspecific crossing.

The yellow seed coat colour was recovered from the backcrossed plants in eight of the eleven BC_1F_1-derived DH lines. This showed that it was possible to introgress undesirable traits from *B. napus*. The F_1-derived DH lines all had black seed coat

because of maternal effects and dominant nature of genes controlling seed colour. Since the genes from *B. juncea* for yellow colour were present in BC_1F_1-derived DH lines, the homozygous recessive condition necessary for the yellow colour was present (Schwetka 1982).

6.2. Conclusions

1. Genetic material was introgressed from *B. napus* to *B. juncea*, since RAPD markers from *B. napus* were recovered in the BC_1F_1-derived DH lines. Genetic similarities (Nei and Li 1979) calculated between BC_1F_1 lines and *B. napus* showed genetic similarities greater than can be attributed to the common A genome shared between the two species, suggesting introgression from the C genome had occurred.

2. BC_1F_1-derived DH lines were cytologically more stable than the F_1-derived DH lines with an n=18 configuration at diakinesis and a loose association of one bivalent dissociating to give two univalents. The bivalents are probably made up of chromosomes or chromosome segments from the C and B genomes. The F_1-derived DH lines were cytologically unstable, giving rise to PMCs with higher numbers of univalents. A high level of female sterility was observed in F_1-derived DH lines resulting in poor seed set.

3. From the preliminary data on agronomic performance, some BC_1F_1-derived DH lines were superior to *B. juncea* and *B. napus* in both yield and harvest index.

4. The BC_1F_1-derived DH lines were lower in oil content than the *B. napus* parent but showed improved oil content over that of the *B. juncea* parent. Improved fatty acid profiles for oleic and linoleic acid were obtained in specific lines. It was difficult to modify the linolenic acid profile, but it was possible to introgress genes for low total alkenyl glucosinolates from *B. napus* to *B. juncea*.

5. Resistance to blackleg was improved by introgressing genes from *B. napus* to *B. juncea* resulting in BC_1F_1-derived DH lines with higher resistance scores than parental *B. juncea* line. Resistance to blackspot disease was introgressed

from *B. napus* to *B. juncea* resulting in resistant BC₁F₁-derived DH lines to blackspot disease.

6. The desirable yellow seed coat colour was recovered from *B. juncea* on backcrossing, although genes for the black colour were retained in three out of eleven BC_1F_1-derived DH lines.

The effects of introgressing traits through interspecific crosses between *B. juncea* and *B. napus* has been demonstrated although undesirable genes such as dark seed coat colour and high indole glucosinolates can also be introgressed to the species with the desired background. To recover most of the desirable traits in a *B. juncea* background, backcrossing the F_1 to *B. juncea* is necessary, although some desirable quality traits expressed best in the F_1 such as high oleic acid, apparently are lost.

References

Bansal V.K., P.D. Kharbanda, G.R. Stringam, M.R. Thiagarajah and J.P. Tewari 1994. A comparison of greenhouse and field screening methods for blackleg resistance in doubled haploid lines of *Brassica napus*. Plant Disease 78(3): 276-281.

Nei M. and Li W.H. 1979. Mathematical model for study of genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 74: 5267-5273.

Quiros C.F., J. Hu, P. This, A.M. Chevre and M. Delseny 1991. Development and chromosomal localization of genome-specific markers by polymerase chain reaction in *Brassica*. Theor Appl Genet 82: 627-632.

Rakow G. and J.P. Raney 1995. Field Performance of canola quality *Brassica juncea*. Rapeseed today and tomorrow. Proceedings: Vol. 2: 428-430. 9th International Rapeseed Congress Cambridge, UK 4-7th July 1995.

Rimmer S.R. and C.G.J. Van den Berg 1992. Resistance of oilseed Brassica spp. to blackleg caused by *Leptosphaeria maculans*. Can. J. Plant Pathol. 14: 56-66.

Schwetka A. 1982. Inheritance of seed colour in turnip rape (*Brassica campestris* L.). Theor Appl Genet 62: 161-169.

Appendix 1   Parents, F_1 and BC_1F_1 derived DH genotypes codes of *B. juncea* x *B. napus* interspecific crosses.

Plants' name	Source	Sample
B. juncea 1	Parent (Bj1)	S2
B. juncea 2	Parent (Bj2)	S1
B. napus	Parent (BN)	S3
94-1279	94-2-3	S18
94-1280	94-2-3	S17
94-1281	94-2-3	S16
94-1282	94-2-3	S15
94-1283	94-2-3	S14
94-1284	94-2-2	S19
94-1286	94-2-2	S13
94-1287	94-2-2	S12
94-1288	94-2-3	S11
94-1289	94-1-5	S10
94-1486	94-2-1	S20
94-1290	F_1	S9
94-1291	F_1	S8
94-1292	F_1	S7
94-1293	F_1	S6
94-1294	F_1	S5
94-1295	F_1	S4

Appendix 2a

B5x 10 (frozen stock) (1L)

KNO₃	12.5g
MgSO₄-7H₂O	1.25g
CaCl₂-2H₂O	3.75g
(NH₄)₂SO₄	0.67g
NaH₂PO₄-H₂O	0.75g
Fe 330	0.20g
B5x 10 vitamin stock	50.0mL
B5x 100 micronutrient stock	50.0mL
KI stock (0.83g in 1L)	5.0mL

ddH₂O added to a final volume of 1L
Store in freezer as 100 ml samples

Appendix 2b

B5x 10 (frozen vitamin stock) (1L)

myosinositol	10.0g
nicotinic acid	0.10g
pyridoxine HCl	0.10g
thiamine HCl	1.00g

ddH₂O added to a final volume of 1L

Appendix 2c

B5x 10 (frozen micronutrient stock) (1L)

MnSO₄-H₂O	1.0g
H₃BO₃	0.30g
ZnSO₄-7H₂O	0.20g
Na₂MnO₄-2H₂O	0.025g
CuSO₄-5H₂O	0.0025g
CoCl₂-6H₂O	0.0025g

ddH2O added to a final volume of 1L

Appendix 2d

B5 Wash Medium (1L)

	100mL
B5x 10 stock	200mL
Sucrose	20g

ddH₂O added to a final volume of 1 L.
pH adjusted to 6.0
Sterilize in autoclave

Appendix 2e

B5 Solid Culture Media (1L)

B5x 10 stock	200mL
Sucrose	130g
GA3 (0.15mg/L)	1mL
ddH2O added to a final volume of 1 L.	
pH adjusted to 5.7	
Agar	8g
Sterilize in autoclave	

Appendix 3

NLN Culture Medium (1L)

ddH$_2$O	500mL
KNO$_3$	0.125g
MgSO$_4$-7H$_2$O	0.125g
Ca(NO$_3$)$_2$-4H$_2$O	0.50g
KH$_2$SO$_4$	0.125g
Fe 330	0.04g
B5x 100 vitamin stock	10.0mL
B5x 100 micronutrient stock	10.0mL
Glutathione	0.03g
L-glutamine	0.80g
L-serine	0.10g
Sucrose	130g
ddH$_2$O added to a final volume of 1L	
pH adjusted to 6.0	
filter sterilize and store in fridge (2-5 °C)	