University of Alberta

Genetic analysis of the B-genome chromosomes in the *Brassica* species

by

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Dedicated to my beloved parents
Hossein and Pari

and

to my ever supportive brothers
Kamran and Kourosh

Abstract

The family Brassicaceae includes a number of important species used as vegetables, oilseeds and medicine. The Brassica B-genome is significantly diverged from the A- and C-genomes, and species containing the B-genome possess many valuable agronomic and disease resistance traits. In this thesis, two populations of interspecific crosses between *Brassica napus* (AACC) and *Brassica carinata* (BBCC) were studied, and microsatellite (SSR) markers and genomic *in situ* hybridization (GISH) techniques were applied to characterize the B-genome chromosome introgressions in the advanced backcross populations and their effect on disease resistance, morphological and seed quality traits.

A BC_2S_3 derived doubled haploid (DH) population was genotyped with 184 SSR markers and DH lines carrying stable B-genome chromosomal segments were identified. The GISH assay demonstrated that three of the 60 DHs were substitution lines in which the B-genome chromosome J13 was common to all. The lines with B-genome chromosomes, "B+", were significantly different ($P < 0.01$) from the lines without B-genome chromosomes, "B-" for some morphological and seed quality traits, such as days to flowering, days to maturity and erucic acid content. Further study revealed that resistance to Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*) has been inherited from the B or C genomes of *B. carinata* in some of the DH lines.

Several BC_3S_1 families of another cross, derived from well-characterized BC_3 plants containing introgressed B-genome chromosomes, were analyzed using SSR markers and the GISH assay to study the inheritance of the B-genome

chromosome(s) and their association with morphological traits. This study revealed that the B-genome chromosomes tend to transfer through generations, either as whole chromosome(s) or as chromosomes without small terminal segments, as evidenced by low recombination frequencies (~ 0%) between the B and A/C-genome chromosomes. These results were supported by the GISH assay and chromosomes counts, and revealed that many of the BC_3S_1 lines were addition lines carrying extra B-genome chromosomes.

Acknowledgments

Science is the discipline of lighting one more small point of light, adding slowly to a firmament of stars which eventually become bright enough that all of us can see truth clearly. However, the process of sparking that new light, as every candidate for a doctorate can attest to, is a long, arduous road. Thankfully, we are usually not alone in the effort. My journey, in particular, has been aided so many times by tremendous people who have gone far beyond the call of duty. Albert Schweitzer once said: "Sometimes our light goes out but is blown into flame by another human being. Each of us owes deepest thanks to those who have rekindled this light." These are the people who rekindled my light when it wavered. What I have accomplished is directly attributable to their help.

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… whether we like it or not, we can never sever our links with the past, complete with all its errors. It survives in accepted concepts, in the presentation of problems, in the syllabus of formal education, in everyday life, as well as in language and institutions. Concepts are not spontaneously created but are determined by their 'ancestors.' That which has occurred in the past is a greater cause of insecurity -- rather, it only becomes a cause of insecurity -- when our ties with it remain unconscious and unknown.

Ludwik Fleck (1981)

From the book 'Genesis and Development of a Scientific Fact'

University of Chicago Press,

1. INTRODUCTION AND OBJECTIVES:

1.1 General introduction

More than 30 species of the tribe Cruciferae (*Brassiceae*) are important cultivated crops, grown globally for oil, condiments, and vegetables (Barret et al. 1998). They are also now being considered for other products like bio-diesel and medicinal products (Cardone et al. 2002; FAO 2006). Of greatest importance in the world are the species *Brassica napus*, *Brassica rapa*, and *Brassica juncea* as sources of edible oil, *Brassica oleracea* as a source of vegetable crops; and the species *Raphanus sativa* and *Sinapis alba*, which along with *Brassica nigra* are sources of condiment mustard. Wild and allied species of this tribe can potentially be used in the breeding programs of cultivated species, as donors of economically important nuclear genes or as sources of cytoplasmic male-sterility (Becker et al. 1999; Gomez-Campo 1980, Rahman 2001). There are a number of reports of agronomically important traits which have been successfully introgressed into cultivated *Brassica* species through interspecific or intergeneric crosses (Table 1.1).

As novel sources of germplasm for crop improvement programs, wild relatives of crop plants can be used to increase genetic diversity for nuclear and cytoplasmic genes (Ky et al. 2000). Therefore the study of the diversity of allied species is important for crop improvement, since the wild relatives found at the centers of origin often have significant genetic diversity.

The *Brassica* B-genome carries many valuable traits which are not found in the *Brassica* A- or C-genomes, such as resistance to the fungal disease blackleg, caused by *Leptosphaeria maculans*, (Rimmer and Vandenberg 1992), heat and drought tolerance (Kumar et al. 1984), aluminum tolerance (Huang et al. 2002) and tolerance to saline conditions (Malik 1990). The *Brassica* species carrying the B-genome (*B. nigra*, *B. carinata* and *B. juncea*) may therefore be important for the improvement of *B. napus* (Table 1.1).

Table 1.1. Introgression efforts for transferring traits from related species into Brassica crop species.

Trait of Interest	Donor species	Recipient species	Reference
Resistance to Blackleg	*B. juncea*	*B. napus*	Chevre et al. (1997); Dixelius and Wahlberg (1999); Roussel et al. (1999)
	B. nigra	*B. napus*	Chevre et al. (1996)
	B. carinata	*B. napus*	Dixelius and Whalberg (1999)
	B. rapa	*B. napus*	Li and Cowling (2003)
Silique shatter resistance	*B. juncea*	*B.napus*	Prakash and Chopra (1988)
Yellow seed	*B. rapa*	*B. napus*	Rahman (2001)
Clubroot resistance	*Raphanus sativus*	*B. oleracea*	Hagimori *et al.* (1992)
Alternaria leaf spot resistance	*Sinapis alba and*	*B. olereacea*	Hansen and Earle (1997)
	Camelina sativa	*B. olereacea*	Sigareva and Earle (1999)
	B. juncea	*B. carinata* *B. rapa*	Sharma and Sing (1992)
Resistance to nematodes	*Sinapis alba*	*B. napus*	Lelivelt (1993)
Intermediate C3-C4 photosynthetic activity	*Moricandia arvensis*	*B. napus*	Zhang et al. (2004)
Resistance to herbicides	*Raphanus sativus*	*B. napus*	Chevre et al. (1997)
Rfo restorer gene for cytoplasmic male-sterility	*Raphanus sativus*	*B. napus*	Delourme et al. (1998), Primard-Brisset et al. (2005)

The amphidiploid *Brassica* species *B. carinata*, carrying the B- and C-genomes, has been used as a source of the B-genome in interspecific crosses of *B. napus* and *B. carinata* to generate the plant material used in this study. I used several approaches including microsatellite molecular markers and fluorescent *in situ* hybridization techniques to study the nature and behavior of the B-genome chromosomes in the interspecific crosses and identify traits associated with them.

1.2. Origin, domestication and evolution of *Brassica* species

Knowledge of the evolutionary relations among the species of the Cruciferae (Brassiceae), benefit from our knowledge of model plant Arabidopsis as it is closely related to the Brassica and the knowledge of the Arabidopsis genome sequence has contributed significantly to the understanding of the genome structure in *Brassica*. In particular, studies of the *Brassica* A- and C-

genomes benefits from our knowledge of Arabidopsis (Lydiate et al. 1993; Panjabi et al. 2008; Parkin et al. 2005). Cytological and molecular techniques have answered some of the questions regarding the origin, evolution, domestication and relationship between the genome of members of the *Brassicacea* family and cultivated *Brassica* species (Table 1.2). However, much remains unknown.

Table 1.2. Taxonomic relationship among species in the Cruciferae family. From Prakash, (1999).

Family	Cruciferae
Tribe	Brassiceae
Genus	Brassica (27 species) Moricandia (1 species) Raphanus (2 species) Sinapis (8 species) Diplotaxis (4 species)
Species	*Brassica oleracea* *Brassica nigra* *Brassica rapa* *Brassica carinata* *Brassica juncea* *Brassica napus*

1.2.1 Geographical distribution and domestication

Species of the family Cruciferae are believed to have originated in the Himalayan region (Hedge 1976). The tribe *Brassicaceae* is dispersed throughout the Mediterranean, the Irano-Turanian and the Saharo-Sindian phytogeographic regions (Hege, 1976). These three regions are different in climate, in altitude, and ecological diversity (Fig. 1.1; Hege, 1976). Most of the variability in the *Brassica* species occurs in the western part of this range i.e., the southwest Mediterranean area, comprising Morocco, Spain, Algeria, and some of the Atlantic islands (Gomez-Campo 1980). It is believed that the southwest Mediterranean region is where the family *Brassiceae* originated first (Hege, 1976). Currently though, it is accepted that significant evolutionary change has taken place in the

Mediterranean area, suggesting a secondary centre of origin in this part of the world (Gomez-Campo 1980).

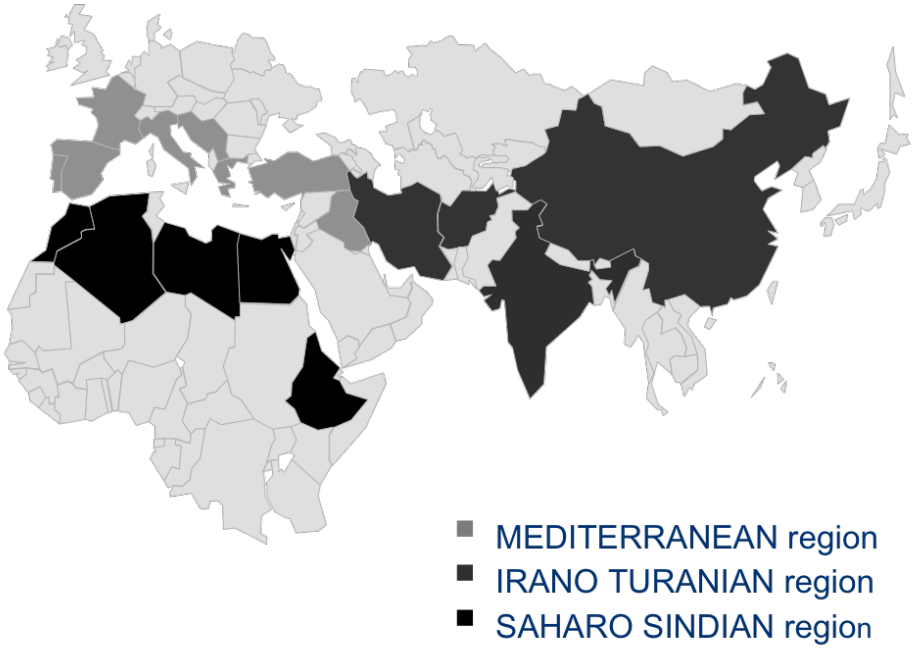

Fig.1.1. Geographical distribution of the *Brassiceae*.

1.2.1.1 Centers of origin and domestication of diploid *Brassica* species

Brassica rapa (Syn *B. campestris*) (n=10, A-genome), which includes oil seed type, Chinese cabbage, turnip and mustard, is one of the most primitive *Brassica* species and is believed to be the first to be domesticated (Gomez-Campo and Prakash 1999). It is primarily found in the plateau regions of the Irano-Turanian region on the Mediterranean coast (Tsunoda 1980). It has been suggested that the first domestication occurred in Europe as a biennial plant, from which annual forms were selected. South India, central Asia and China are considered to be secondary centers of origin, where three distinct ecotypes of *B. rapa*, brown sarson, yellow sarson and toria, have evolved (Burkil 1930; Gomez-Campo and Prakash 1999; Quijada et al. 2007).

Brassica oleracea (n=9, C-genome), which includes the vegetables kale, cabbage, broccoli and cauliflower, grows perennially on the coastal rocky cliffs of the Atlantic coasts of northern Spain, eastern France and southern Britain (Tsunoda 1980). It is believed to have been domesticated from diverse sources of germplasm (Gomez-Campo and Prakash 1999). Selection in different climates, natural hybridization and gene introgression has expanded the level of genetic diversity in this species (Gomez-Campo and Prakash, 1999). *Braccisa oleracea* generally grows slowly and has a large storage capacity for nutrients, which accounts for its adaptation to diverse natural habitats. It has a recent history of cultivation (Gomez-Campo C. 1999).

Brassica nigra (n=8, B-genome) or black mustard, is found in the Mediterranean region, extending into central Asia and the Middle East, where winters are relatively mild, and in Morocco and Egypt (Tsunoda 1980). In the climatic conditions of the Mediterranean regions, *B. nigra* grows relatively quickly (Tsunoda 1980).

1.2.1.2 Centers of origin and domestication of amphidiploid *Brassica* species

Brassica juncea (n=18, AABB) or Indian mustard is an amphidiploid derived from *B. rapa* and *B. nigra*. Compared to *B. rapa*, it has a higher leaf area and more rapid growth rate and is morphologically similar to *B. nigra* (Mizushima and Tsunoda 1967). *Brassica juncea* has a long history of cultivation in temperate and humid parts of southern China, (Gomez-Campo and Prakash 1999). The Middle East regions are more likely to be the primary center of origin of *Brassica juncea*, where the oil seed form evolved (Gomez-Campo and Prakash 1999; Mizushima and Tsunoda 1967). Consequently, central and western China, the Near East, the arid plateaus of Asia Minor and southern Iran were considered as secondary centers of origin, where its wild relatives are found (Vavilov 1949). Recent cytological, biochemical and molecular evidence suggest a polyphyletic origin for *B. juncea*, in areas where the parental species have a sympatric distribution (Vaughan 1977).

Brassica carinata (n=17, BBCC) or Ethiopian mustard, is an amphidiploid derived from *B. oleracea* and *B. nigra*. Its cultivation had been limited locally to the Ethiopian plateaus and parts of Kenya for centuries (Gomez-Campo and Prakash 1999). Tsunoda (1980) observed *B. oleracea* and *B. nigra* growing near each other as crop plants or weeds on the Ethiopian plateau, where the temperature is very stable throughout the year, favoring the lengthy cultivation time of these species (Astley 1982; Riley and Belayneh 1982; Tsunoda 1980). However, no wild forms of *B. carinata* have been found on the Ethiopian plateau (Mizushima and Tsunoda 1967).

Brassica napus (n=19, AACC), commonly known as canola or oilseed rape, is the amphidiploid of *B. rapa* and *B. oleracea* (Tsunoda 1980). The term "canola" was introduced in 1978 by the Canola Council of Canada, and often refers to the *B. napus*, *B. juncea* and *B. rapa* species whose seed oil contains less than 2% erucic acid and seed meal contain less than 30 micromoles of the aliphatic glucosinolates per gram of meal on an oil-free basis.

Brassica napus has a recent history of domestication. Wild forms have been reported to occur in northern parts of Europe, including Sweden, the Netherlands and Britain (Rakow 2004). Naturalized forms of *B. napus*, which are quite different from cultivated forms are found on coastal cliffs in Southwest Europe, where *B. oleracea* and *B. rapa* grow wild (Tsunoda 1980). It is believed that *B. napus* originated in the Mediterranean regions or western and northern regions of Europe (Tsunoda 1980). However, according to Gomez-Campo and Prakash (1999), the overlap of the two parents may not exist in these areas and it may have originated in an agricultural environment outside the Mediterranean region. They speculated that hybridization between *B. oleracea* and *B. rapa* occurred several times. With only 400 years of domestication/cultivation, it is now one of the most economically important edible crops in the world (Gomez-Campo and Prakash 1999).

1.3 Evolution of cultivated *Brassica* species

Evolution and the phylogenic relations among the species of the family Brassicaceae have been studied extensively for over 90 years. Some of these species are interesting models for studies of the origin and genetic relationships of the genus *Brassica*. Not surprisingly, over time, the best available techniques for phylogenic studies in the *Brassica* have included morphological traits (mostly in early 20th century, e.g., Schulz 1936), cytogenetic studies (pioneered in Japan in 1910 by Takamine and followed by Morinaga during 1928-1934), chloroplast cytoplasmic studies (mostly during the 1980's to mid-1990's), biochemical studies (during the 1980's and 1990's) and more recently molecular studies. Despite the different approaches followed by different researchers, there remains differing opinions on the origin and evolution of the chromosomes in *Brassica* (Lysak et al 2005).

Molecular studies on nuclear and chloroplast DNA (Palmer et al. 1983; Erickson et al. 1983; Warwick and Black, 1991; 1992), have indicated that there were two lineages for the origin for the diploid *Brassica* species; "*nigra*" and "*rapa*/olereacea" lineages (Fig 1.2). These groupings are in general agreement with the taxonomic classifications proposed by Takashata and Hinata (1980).

Molecular based studies in recent years, using DNA sequence data and comparing chromosomal rearrangements, have clearly demonstrated that the three *Brassica* genomes evolved from a common ancestor through polyploidization. It has been suggested that a polyploidization event occurred considerably before the estimated divergence of *Arabidopsis* and *Brassica*, between 14.5 and 20.4 M yr ago (Yang et al. 1999). Based on comparative studies with *Arabidopsis* chromosomes, Lysak et al. (2005) presumed that hexaploidization event occurred after the Arabidopsis–Brassiceae split, between 7.9 and 14.6 Mya. Subsequent to this, it has been estimated that the *B. nigra* (B) and the *rapa/oleracea* (Warwick et al. 1991) lineages (A/C) separated from each other about 7.9 Mya, followed by the splitting of the *B. rapa/oleracea* lineages (Lysak et al. 2005). The approximate

divergence time of *B. rapa* (A) from *B. oleracea* (C) was estimated to be one million years (Lagercrantz 1998).

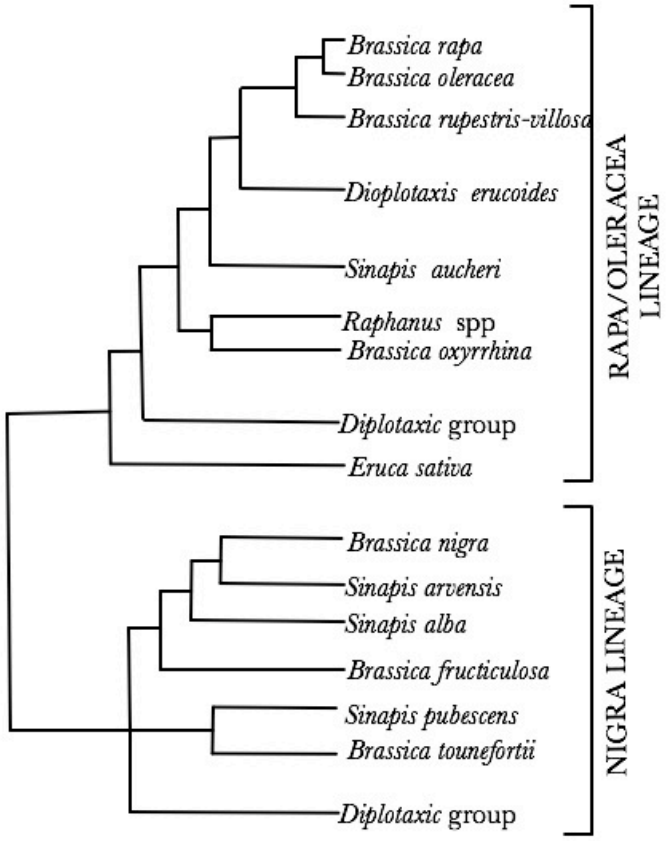

Fig 1.2. Phylogenic tree of the species of *Brassicaceae* evolved through two lineages. (redrawn from Warwick and Black, 1991).

1.3.1 Cytological evidence for the evolution of *Brassica* species

The pioneering cytogenetic research in *Brassica* started in Japan with the identification of the chromosome number of *B. rapa* (Takamine 1916; cited by Gomez-Campo and Prakash 1999). Japanese cytogenetisists were the first to reveal the relationship between the cultivated *Brassica* species almost 80 years ago. Based on the studies of chromosome pairing in interspecific hybrids, Morinaga (1934) hypothesized that *Brassica* species with high chromosome numbers like *B. napus* (2n=38, AACC), *B. juncea* (2n=36, AABB) and *B.*

carinata (2n=34, BBCC) originated as amphidiploids from combinations of pairs of species with lower chromosome numbers like *B. nigra* (2n=16, BB), *B oleracea* (2n=18, CC) and *B. rapa* (2n=20, AA). U (1935) verified this hypothesis by successfully resynthesizing *B. napus* from interspecific crosses between the diploid species *B. oleracea* and *B. rapa* and proposed the genomic relationship among *Brassica* species; which is known today as U's triangle (Fig.1.3). These findings were later verified by resynthesizing *B. juncea* (Frandsen 1943), *B.napus* (Olson 1960) and *B. carinata* (Frandsen 1947) through interspecific hybridization between diploid species followed by chromosome doubling.

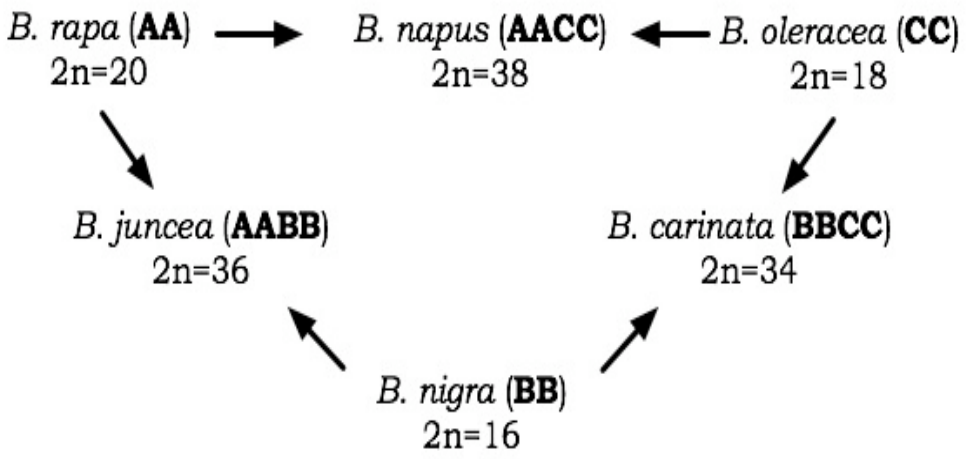

Fig. 1.3. Genomic relationships in the Brassica, known as U's triangle. U (1935).

The close relationship between the A- and C-genome has clearly been demonstrated by the observation that significantly higher amounts of chromosome pairing occur in the AC amphihaploids, compared to AB and BC amphihaploids (Attia et al. 1987; Attia and Röbbelen 1986a,b). Formation of multivalents in AC amphihaploids (haploids that contain one copy of the two diploid genomes present in the polyploid) suggests that there are structural similarities within and between chromosomes of these two genomes (Attia and Röbbelen, 1986). On the other hand, the lack of pairing of the B-genome chromosomes with the AC

chromosomes (Attia and Röbbelen 1986a; Busso et al. 1987) suggests that this genome is more distantly related to the other two genomes. It was first believed that *B. nigra* may carry a genetic factor to suppress homoeologous pairing, but no genetic or cytoplasmic factor (Busso et al. 1987) has been reported that controls pairing.

1.3.1.1. Molecular cytogenetics of *Brassica* genomes

Chromosomes of *Brassica* species are relatively small and difficult to distinguish visually due to morphological similarities (Röbbelen 1960, Olin-Fatih and Heneen 1992), and therefore cytogenetic analysis has often proven to be difficult. This problem specifically limits the ability to identify alien chromosomes in *Brassica* hybrids and to observe translocations using conventional karyotyping methods. In combination with techniques for improved cytological preparations, the modern techniques of fluorescent *in situ* hybridization (FISH) and genomic *in situ* hybridization (GISH) help in tracking the alien chromosome(s) or segments of chromosome introgressed in *Brassica* breeding research using interspecific crosses (Snowdon et al. 1997).

The genomic relationship of the allopolyploids, pairing of homoeologus chromosomes and identification of translocations has been studied in different plant species using modern molecular cytogenetic techniques such as FISH (Raina et al. 2001). This technique has provided markers for the identification of some of the chromosomes from diploid (Hasterok and Maluszynska 2000a) and allotetraploid species (Hasterok et al. 2001; Hasterok and Maluszynska 2000b). GISH, which uses total nuclear DNA as a probe, has also been widely applied to distinguish the genomes of different species (Benabdelmouna et al. 2001; Hasterok et al. 2004; Marasek et al. 2004). GISH has also been used for the detection of genomic exchanges and chromosome rearrangements (Jellen et al. 1994; Kenton et al. 1993), and can be a valuable method for tracking of alien chromatin, translocations and introgressions in interspecific hybrids (Morgan et al. 2001; Pasakinskiene and Jones 2005; Shi and Endo 2000; Snowdon et al. 1997) of different plant species. Chromosome rearrangements can be identified

and observed more specifically by using the combined method of FISH/GISH (Hasterok et al. 2005; Maluszynska and Hasterok 2005; Moscone et al. 1996). The combination of these two techniques makes use of *in situ* hybridization, with total genomic DNA as a probe to discriminate genomes followed by chromosome-specific DNA probes to identify pairs of mitotic chromosomes or visualize the pairing of homoeologous chromosomes at meiosis (Cao et al. 2000).

In case of *Brassica*, GISH has been successfully used as a tool for the identification of the diploid genomes in the B-genome containing allopolyploids. It could clearly distinguish the B-genome chromosomes from the A- or C-genome chromosomes in *B. juncea* or *B. carinata*; however not able to distinguish the A- and C-genome chromosomes due to the considerable homoeology between these two genomes (Snowdon et al. 1997). Therefore, use of this approach was initially limited in *B. napus*. Detailed study of the *Brassic*a genomes has demonstrated that the B-genome chromosomes have a different hybridization pattern compared to the A and C chromosomes (Hasterok et al. 2005; Maluszynska and Hasterok 2005). However, Howell et al. (2008) succesfully used FISH followed by GISH with probes of *B. rapa* and *B. oleracea* genomic DNA to distinguish the A- and C-genomes in *B. napus*. They also identified several specific chromosomes within a genome and showed the efficiency of a modified GISH technique (with a repetitive probe in the blocking DNA) on both mitotic and meiotic cells. The authors used *B. napus* var. Westar, which carries a known reciprocal translocation, and demonstrated the possibility of identifying intergenomic translocations involving chromosomes A7 and C6, as had been supported by genetic marker data (Howell et al. 2008). GISH has been used extensively in the analysis of the behavior of B-genome chromosomes in trigenomic hybrids, (ABC) where individual B-genome chromosomes were distinct from those of the A and C-genome chromosomes (Ge and Li 2007).

1.3.2. Cytoplasmic DNA evidence for the evolution of *Brassica* species

Our knowledge of the cytoplasmic genomes has contributed significantly to the understanding of the evolution of the *Brassica* amphidiploid species

(Palmer et al. 1988). Patterns of cpDNA restriction fragments in different *Brassica* species indicated that *B. nigra* has donated cytoplasm to *B. carinata*, while *B. rapa* has donated cytoplasm to *B. juncea* (Palmer et al. 1983; Erickson et al. 1983; Hallden et al. 1993; Pradhan et al. 1992; Warwick and Black 1991). The maternal genome of the two amphidiploid species remained conserved with no significant changes (Erickson et al, 1983 and Palmer et al., 1983). In the case of *B. napus*, no strict assignment would be made as to which diploid species function as female parent. However, chloroplast DNA of *B. napus* often shows close similarity with *B. oleracea* (Erickson et al. 1983; Hallden et al. 1993). Studies of maternal ancestors in these three amphidiploid species based on mitochondrial DNA also agree well with the results from chloroplast DNA based studies (Palmer 1988; Hallden et al. 1993).

Mitochondrial DNA is shown to have high homogeneity between the accessions within a *Brassica* species with very limited alterations from structural changes like deletions and inversions (Palmer 1988). Song and Osborn (1992) studied cpDNA and mtDNA RFLPs and identified four different types of cytoplasms in the diploid parental species, and all four types were observed in *B. napus* suggesting multiple origin for this amphidiploid species. Although it is commonly believed that mitochondria are inherited from the maternal parent, by use of cytoplasmic male sterility as a marker in *B. napus*, it has been reported that mitochondria could also be transmitted paternally in the pollen to the hybrids (Erickson and Kemble 1990; Erickson et al. 1989; Kemble et al. 1986).

1.3.3. Nuclear DNA evidence in the evolution of *Brassica* species

1.3.3.1 Use of markers in phylogeny and linkage maps

The first genetic markers were morphological markers, with allelic variation reflecting visible phenotypic differences. Llater protein markers such as isozymes and DNA markers such as RFLP and PCR-based markers were developed (Lydiate et al. 1993). Figdore et al. (1988) demonstrated the potential use of RFLP markers to study *Brassica* taxonomy, evolution and genome

structure, as well as their use in applied genetics and breeding. Maps of all of the *Brassica* species are now available (Lydiate et al. 1993), which demonstrate the significant number of chromosomal rearrangements that have occurred during the evolution of the chromosomes in the different species (Sharpe et al. 1995). A detailed review on the genome mapping studies in *Brassica* species is published by Quiros and Paterson (2004).

Examination of nuclear restriction fragment length polymorphism (RFLP) markers in a resynthesized *B. napus* mapping population revealed a predominantly disomic inheritance of these markers (Parkin et al. 1995). Disomic inheritiance is observed when each progeny (despite the fact that it contains both an A- and C-genome locus for any particular gene) carries at least one of a pair of parental alleles from both the A- and C-genome; and the A and C alleles segregate effectively as individual loci. In the hybrids of resynthesized *B. napus* x natural *B. napus*, the A-genome chromosomes of resynthesized *B. napus* derived from *B. rapa*, pair exclusively with the A-genome homologues of natural *B. napus*; similarly, the *B. oleracea* derived chromosomes of resynthesized *B. napus* pair with the C-genome homologues of natural *B. napus* (Parkin et al, 1995). In the case of the *B. juncea* map, it was possible to identify the A and B-genome linkage groups based on the same set of RFLP markers and align them with their homologues from *B. nigra* (Axelsson et al. 2000; Lagercrantz and Lydiate 1995). DNA marker based examinations of the ancestral A, B and C-genomes have demonstrated that they contain eight triplicated regions covering nearly the whole genomes (Lagercrantz and Lydiate 1996).

Although RFLP markers have been used extensively in the analysis of genome structure and the evolution of *Brassica* species (Lagercrantz and Lydiate 1996; Song et al. 1995), they are difficult to work with and detect fewer polymorphisms than microsatellite markers (Plieske and Struss 2001). Microsatellite markers, also defined as Simple Sequence Repeats (SSRs), have been extensively used in *Brassica* species. SSRs are randomly distributed sequences within eukaryotic genomes, which have high variability for repeat

number and have high levels of informative polymorphisms. These markers have a co-dominant inheritance and have been very efficient in fingerprinting and pedigree analysis of different crops (Guilford et al. 1997; Plaschke et al. 1995; Rongwen et al. 1995; Struss and Plieske 1998). In *Brassica*, due to the large and complex genome, robust markers like SSRs are required for genome mapping, gene tagging and marker assisted selection. Microsatellite markers have been developed using genomic libraries of different *Brassica* species and linkage maps of *B. napus* have been constructed successfully using these markers (Lowe et al. 2002; Lowe et al. 2004; Piquemal et al. 2005; Plieske and Struss 2001; Suwabe et al. 2002; Suwabe et al. 2003; Uzunova and Ecke 1999). Moreover, there are many additional microsattelite markers developed at Agriculture and AgriFood Canada (AAFC) and the Biotechnology and Biological Research Council (BBSRC) and are available at the *Brassica* database (http://www.*Brassica*.info/resource/markers/ssr-exchange.php). Despite the fact that Low et al. (2004) were able to develop SSR markers from *B. nigra*, however, the first evaluation of microsatellite markers for genome mapping in *B. juncea* has been reported very recently (Koundal et al. 2008) and little work has been done on mapping microsatellite markers in the B-genome.

One interesting characteristic of *Brassica* maps developed based on molecular markers is that a relatively large number of loci deviate from Mendelian segregation and are usually clusters in linkage blocks (Ferreira et al. 1994; Kianian and Quiros 1992; Landry et al. 1991; Mcgrath and Quiros 1991; Parkin and Lydiate 1997; Uzunova et al. 1995). This segregation distortion needs to be taken into consideration when constructing linkage maps to avoid biased statistical tests (Foisset et al. 1996).

1.3.3.2 Genome structure

Linkage maps of the A, B and C-genomes reveal extensive sequence duplications and have shown that about 50% of the loci in all three genomes are duplicated (Quiros et al. 1994; 1985; 1987; Quiros and Paterson 2004), which supports the hypothesis that the *Brassica* diploid species were originally

polyploids (Prakash and Hinata 1980; Quiros et al. 1994). Inter-genomic homeologous recombination has been demonstrated in *B. rapa-oleracea* monosomic addition lines using isozyme and rDNA sequences (reviewed by Quiros et al. 1994), and this knowledge have been applied in practice for the introgression of the yellow seed color gene from the A to C genome (Rahman 2001). Parkin et al. (1995) observed unusual banding patterns in mapping the nuclear genomes of *B. rapa* as well as *B. napus* and *B. olereacea*, which have been explained as resulting from nonreciprocal translocations. As demonstrated in the linkage maps of *B. olereacea*, in addition to duplications and translocations, deletions are another characteristic of the *Brassic*a genomes (McGrath and Quiros 1991; Song et al. 1991), which has also been confirmed by cytological studies in alien addition lines of the C-genome (Hu and Quiros 1991). Moreover, McGrath et al. (1990) observed that non-homologous recombination could occur at any location in the C-genome of *B. oleracea.* Inversions have also been reported in *B.oleracea* and *B. rapa* (Kianian and Quiros 1992; Song et al. 1991).

Chromosome structural rearrangements have also occurred extensively during the evolution of the allopolyploid species, although the average size of the chromosomes has not changed (Parkin and Lydiate 1997). Further genomic rearrangements have taken place subsequent to this by intra- and inter-genomic recombinations between homeologous segments shared between different chromosomes (Quiros et al. 1994), as has been demonstrated from the occurrence of homeologous chromosome pairings in a re-synthesized *B. napus* line by Parkin et al. (1995).

1.3.3.3 Genome homology and ancestral chromosome number

Even though there is considerable conservation in certain chromosome regions within and between the three diploid genomes, gene reordering has occurred during the evolution of *Brassica* species. While the ancestral genome of *Brassica* had fewer chromosomes (Kowalski et al. 1994; Lagercrantz et al. 1996; Truco et al. 1996), it has undergone broad alterations through chromosomal

rearrangements and changes in chromosome number (Kianian and Quiros 1992; Quiros et al. 1987).

Comparisons of genomes between related species with different chromosome numbers are informative for genome homology. These include comparisons between *Brassica* species or between *Brassica* and *A. thaliana* (Kowalski et al. 1994). Truco et al. (1996) compared the homologous regions of the three cultivated *Brassic*a genomes and found intergenomic conserved regions, but with extensive reordering among the genomes. Their findings supported the hypothesis that the diploid *Brassica* species are derived form an ancient polyploid (Quiros et al. 1994). Truco et al. (1996) constructed the RFLP maps of *B. rapa*, *B. oleracea* and *B. nigra* and studied the chromosomal relationships between the *Brassica* A, B and C-genomes. Based on this study they drew a phylogenetic pathway showing the hypothetical origin of the three *Brassica* genomes from an ancestral genome of x=6 (Fig. 1.4), where the B-genome initially separated from the C-genome and the C-genome subsequently gave rise to the A-genome. These phylogenetic studies demonstrated the presence of intergenomic conserved regions which have been involved in extensive duplication and rearrangements during the evolution of the *Brassic*a genomes, from a smaller ancestral genome whose chromosome number is unknown (Teutonico and Osborn 1994; Truco et al. 1996). While Truco et al. (1996) suggested that the ancestral genome had at least five and no more than seven chromosomes; and based on karyotype analysis Röbbelen (1960) suggested that the ancestral genome had x=6 chromosomes. However, based on RFLP probes and the fraction of duplicated loci, Lagercrantz and Lydiate (1996) proposed that the ancestor genome was a hexaploid (Fig. 1.4). In addition, Parkin et al., (2005) provided strong support for a hexaploid ancestor by considering the segmental structure of the *B. napus* chromosomes in a comparative mapping study with *A. thaliana*. However, whole genome comparison provides little evidence of genome triplication, although it does provide strong evidence of genome replication and rearrangement (Lukens et al. 2004). Thus the hypothesis of the evolution of the diploid *Brassica* species through a hexaploid ancestor still remains controversial.

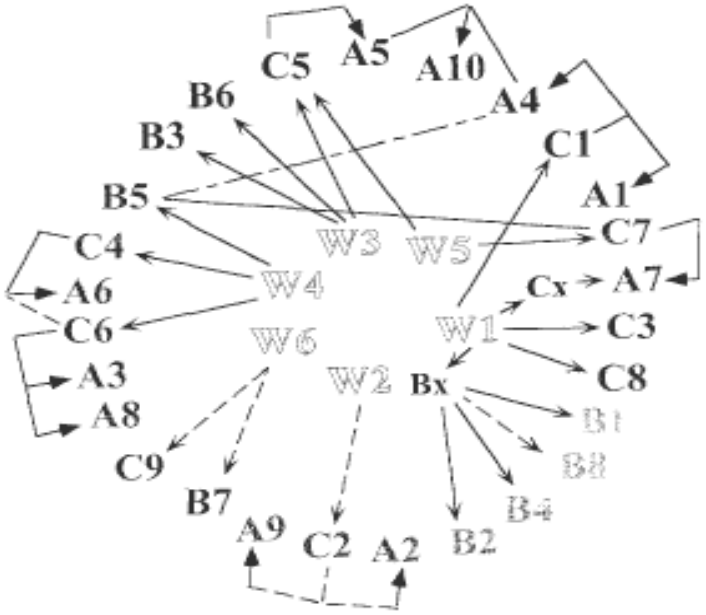

Fig. 1.4. Hypothetical origin of chromosomes of the A, B and C *Brassica* genomes from a hexaploid ancestor, w1-w6 represents the ancestral chromosomes (from Truco et al. (1996).

1.3.3.4. A and C-genomes

Parkin et al. (1995) constructed a linkage map of *B. napus* and identified the 10 chromosomes of A-genome (N1-N10) and the 9 chromosomes of C-genome (N11-N19). Most researchers currently use this nomenclature of the linkage groups. These two *Brassica* genomes show high homoeology as reported by different researchers based on cytogenetic analysis (Attia and Röbbelen 1986a, 1986b) and more recently through molecular analysis (Parkin and Lydiate 1997; Sharpe et al. 1995). Almost every mapped location on the A-genome detects a homoeologus locus in the C-genome (Parkin et al. 2003), and a high proportion of duplicated loci were detected within these diploid genomes (Parkin et al. 2003, 2005). This is not surprising considering that these diploid genomes evolved from a polyploid ancestor. Molecular marker data has shown that *B. rapa* and *B. oleracea* chromosomes pair exclusively with their respective A- and C-genomes

homologues of natural *B. napus* (Parkin and Lydiate 1997), indicating that the two progenitor diploid genomes remained quite unaltered in the amphidipoloid *B. napus* since its evolution in nature (Parkin et al. 1995)(Fig. 1.5). Comparative genome mapping studies have identified conserved genomic regions in the A- and C-genomes of *B. napus* (Parkin et al. 2005), and the duplicated loci have undergone rearrangements such as inversions and translocations, presumably due to co-linear arrangements and homoeologous recombinations (Parkin and Lydiate 1997; Parkin et al. 2005; Parkin et al. 1995; Sharpe et al. 1995). Most of the duplicated loci within the diploid genomes have been reported to be located on separate linkage groups as collinear blocks of linked loci, some of which have undergone a variety of rearrangements like inversions and translocations (Parkin et al. 2003). Some of these rearrangements were identical in the two diploid genomes, suggesting they had occurred before the divergence of the two species, and at least 16 large chromosomal rearrangements have differentiated the A- and C-genomes during their divergence from the common ancestor (Parkin et al. 2003).

Cheung et al. (1997b) constructed RFLP maps of *B. juncea* and compared it with maps of *B. napus* and reported homologies between the two A-genomes, with the conserved sequences from *B. rapa*. Cheung et al. (1997a) built genetic maps of *B. napus* and *B. oleracea* with RFLP and RAPD markers and demonstrated high similarity between the chromosomes of *B. oleracea* with C-genome chromosomes of *B. napus*. They also demonstrated homology of the *B. oleracea* chromosomal regions with more than one genomic region of *B. napus* and thereby supporting close homoeology between the A- and C-genomes. Similar results of colliniarity between the C-genome of *B. oleracea* and the C-genome of *B. napus* have also been reported by Bohuon et al. (1996).

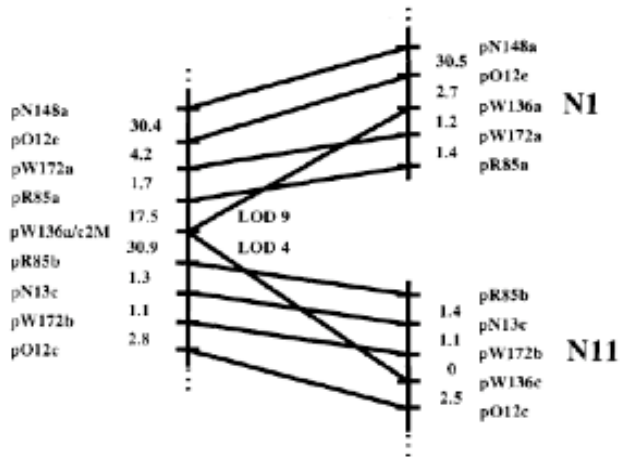

Fig. 1.5. Homogenization of the A- and C-genomes as a result of non-reciprocal homoeologous translocations. From Sharpe et al. (1995).

1.3.3.5 The B-genome

The *Brassica* B-genome has diverged significantly from the A- and C-genomes (Warwick et al. 1991) and is considered to have very limited homeology with the A- and C-genome chromosomes (Axelsson et al. 2000; Warwick et al. 1992). As such, the *Brassica* B-genome chromosomes do not tend to pair homoeologously with chromosomes of the A- or C-genomes in *Brassica* interspecific hybrids (Attia and Röbbelen 1986a; Busso et al. 1987). Molecular systematic studies suggests that *B. nigra* is more closely related to *Sinapis arvensis* (Song et al. 1988a) and high numbers of homeologous bivalents occur in interspecific hybrids of *B. nigra* and *Sinapis arvensis* (Gupta et al. 1992; Mizushima 1980) (Fig.1.2). However, based on RFLP markers, it has been demonstrated that the B-genome shares some homoeology with the A- and C-genomes (Lagercrantz and Lydiate 1996; Panjabi et al. 2008).

Comparative studies between *Brassica* and Arabidopsis can be applied to aid in understanding the *Brassica* genome. Sadowski and Quiros (1998) studied a well defined gene complex of the *Arabidopsis* in the *B. nigra* genome and found a

highly conserved region on linkage group B1 of the B-genome, whereas linkage group B4 showed a complex arrangement when compared to the *Arabidopsis* genome. They concluded that despite the complex and duplicated *Brassica* genomes it is still possible to identify ancestrally related chromosome segments, such as that of the genome of *B. nigra*, allowing one to draw conclusions of its origin and evolution. Struss et al. (1996) studied the alien B-genome chromosomes of the three species *B. nigra, B. carinata* and *B. juncea* in *B. napus* background and detected translocations and recombination between the non-homeologous chromosomes (Table 1.3).

Table 1.3. Summary of major genetic studies done on the Brassica B-genome.

Publications	Species used as B-genome source	Cyto-Genetic study	FISH study	Molecular markers	Comparative mapping information	Type of population used	Number of B-genome LGs targeted	Mapping information availability	Chromosome counts
This et al. (1990)	*B.nigra*	YES	NO	Isozyme-RFLP	YES: B vs C	intra specific BC	Eight	YES	2n=15-16
Truco and Quiros (1994)	*B.nigra*	NO	NO	isozyme-RFLP	NO	F2	Eight-lots of duplicated loci	YES	na
Struss et al. (1996)	*B. nigra, B. juncea and B. carinata*	NO	NO	isozyme-RFLP	NO	Monosomic addition lines	Eight	YES	na
Chevre et al. (1996)	*B. nigra*	NO	NO	isozyme	NO	Addition lines	Five	YES	na
Chevre et al. (1997)	*B. juncea*	YES	NO	RAPD	NO	BC of RIL and addition lines	Two	NO	4n=36-38
Schelfhout et al. (2004)	*B.nigra-B. juncea*	YES	YES	496 bp seq-PBNBH35 B specific	NO	U's triangle Brassica varieties	Eight	NO	na
Schelfhout et al. (2006)	*B. juncea*	YES	YES	496 bp seq-PBNBH35 B specific	NO	interspecific BC	Eight	NO	4n=36-38
Ge and Li (2007)	*B. carinata- B. nigra*	GISH	NO	NO	ABC genome homology	2n x n interspecific crosses	Eight	NO	2n=12-20
Schelhout et al. (2008)	*B. juncea*	NO	NO	NO	NO	interspecific BC	Eight	NO	na
Chevre et al. (2008)	*B. juncea*	YES	NO	Specific marker 11-*Hae* III	NO	Interspecific RILs	LG carrying *Rlm6*	NO	2n=38-39 + I

Many cytological and molecular studies have been conducted on the *Brassica* B-genome, however further research is still required to explain the evolution and chromosome characteristics of this genome. In summary, these studies reveal that B-genome chromosomes, although sharing some homologous regions with the A-and C-genome chromosomes, have very little homoeology with the A- and C-genome chromosomes(Busso et al. 1987).

1.4 Comparative mapping and its significance for evolutionary studies

Arabidopsis thaliana has become a model plant for genetic studies, due to its small size, rapid generation time, small genome and ease of transformation with *Agrobacterium tumefaciens*. It was the first plant to have its genome sequenced completely (Tabata et al. 2000). Although the genome of the diploid *Brassica* species is 3-5 times bigger than that of *Arabidopsis* (Arumuganathan and Earle 1991), the study of the genome of members of the *Brassica* genus has been able to benefit from this knowledge. However, significant amount of genome duplication and rearrangements, insertions and inversions have occurred after the separation of the *Arabidopsis* and *Brassica* lineages that lead to the formation of the Arabidae and Brassiceae tribes. Comparison of coding region sequences between *A. thaliana* and *B. napus* show 87% similarity between the two genomes (Cavell et al. 1998), and gene for gene alignment of the genomes from *Brassica* and *Arabidopsis* have been extensively studied (Cavell et al. 1998; Parkin et al. 2002; Quiros et al. 2001). Given the close similarity between these two species at the genomic level, it should be possible to relate findings in one species to the other species. Genomic and EST sequences available for *A. thaliana*, in particular, can be useful in characterizing the genome sequences from *Brassica*.

Parkin et al. (2005) compared over 1000 genetically linked RFLP loci of *B. napus* with the *A. thaliana* genome on the basis of sequence similarity and found that at least 21 conserved genomic units within the *Arabidopsis* genome which were duplicated and rearranged during the evolution of the *B. napus* genome. More recently, Panjabi et al. (2008) conducted a comparative mapping study by using Intron Polymorphism (IP) markers and also reported a high degree

of co-linearity between the A- and B-genomes of *B. juncea* with A- and B-genomes of *B. napus* and *B. nigra*, respectively. They demonstrated that ancestral block arrangements occurred during the evolution of the *Brassica* genomes, as well as a found high degree of colinearity between three B-genome linkage groups (B4, B5 and B6) and three A-genome linkage groups (A4, A5 and A6). On the basis of homoeology among the three genomes, they introduced a re-designation for the B-genome linkage groups and suggested a new nomenclature for *Brassica* linkage groups to ensure uniformity.

1.5 Characteristics of blackleg disease

1.5.1. Life cycle and symptoms

Blackleg is a fungal disease caused by *Leptosphaeria maculans* that attacks the leaves and stems of many species within the family *Brassicaceae*. This pathogen produces stem cankers, a common symptom of blackleg (Fig.1.6). As such, lesions form at the base of the plant prior to flowering, causing the plant to be cut off from its root system. This results in shriveled seeds and yield losses (Agrios 1997).

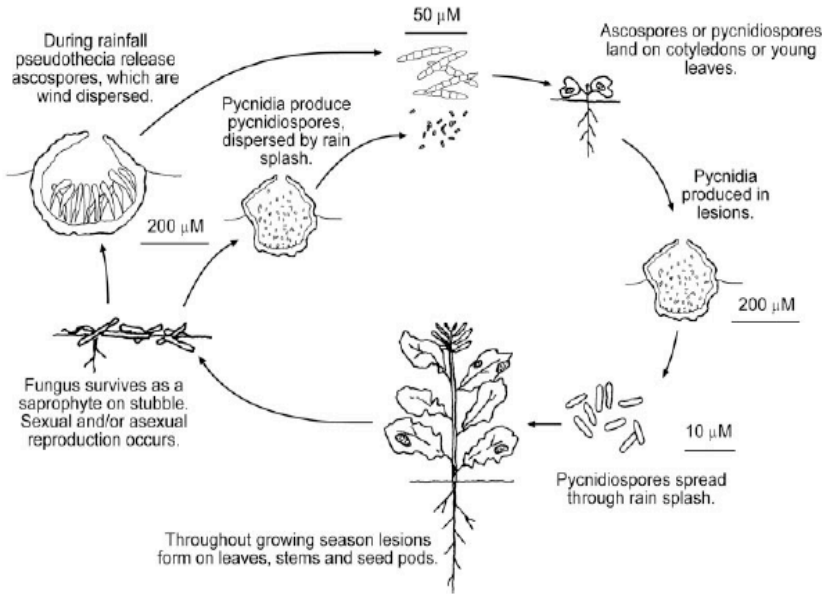

Fig. 1.6. Life cycle of *Leptosphaeria maculans* (Howlett et al. 2001).

1.5.2 Genetics of resistance to blackleg

Different genetic sources of resistance are reported for blackleg. Many race specific major genes and several minor genes are involved in seedling and adult plant resistance for *B. napus* (Ballinger and Salisbury 1996; Bansal et al. 1994; Mcnabb et al. 1993; Pang and Halloran 1996; Stringam et al. 1995). These include *Rlm1*, *Rlm3*, *Rlm4*, *Rlm7* and *Rlm9* (Ansan-Melayah et al. 1998; Balesdent et al. 2001; Balesdent et al. 2002), which all mapped to N7 (Delourme et al. 2004), and *Rlm2* on N10 (Ansan-Melayah et al. 1998; Delourme et al. 2006; Delourme et al. 2004). All blackleg resistance genes in *B napus* are located on the A-genome (Table 1.4), where *Rlm8* is from the A-genome of *B. rapa* (Balesdent et al. 2002). Most of the B-genome containing *Brassica* species also carry blackleg resistance genes, such as *Rlm5* and *Rlm6*, originally identified in *B. juncea* (Balesdent et al. 2002; Christianson et al. 2006; Rimmer and Vandenberg 1992) and *Rlm10* from the B-genome of *B. nigra* (Table 1.4) (Chevre et al. 1996).

Table 1.4. Resistance genes to blackleg identified in the Brassica.

***Leptosphaeria maculans* resistant gene**	**Linkage group mapped to**	**Brassica species**	**Reference**
Rlm1	N7	*B. napus*	Ansan-Melayah et al. 1998;
Rlm2	N10	*B.napus*	Ansan-Melayah et al. 1998; Delourme et al. 2006; Delourme et al. 2004
Rlm3	N7	*B. napus*	Delourme et al. 2004, Balesdent et al. 2002
Rlm4	N7	*B. napus*	Delourme et al. 2004, Balesdent et al. 2002
Rlm5	?	*B juncea*	Balesdent et al. 2002; Christianson et al. 2006; Rimmer and Vandenberg 1992
Rlm6	?	*B. juncea*	Balesdent et al. 2002; Christianson et al. 2006; Rimmer and Vandenberg 1992
Rlm7	N7	*B. napus*	Delourme et al. 2004, Balesdent et al. 2002
Rlm8	?	*B. rapa*	Balesdent et al. 2002
Rlm9	N7	*B. napus*	Delourme et al. 2004, Balesdent et al. 2002
Rlm10		*B. nigra*	Chevre et al. 1996

Efforts to transfer *B. juncea* B-genome resistance into *B. napus* as a result of the introgression of large chromosomal segments (Barret et al. 1998; Chevre et al. 1997; Saal et al. 2004) has presumably failed, since no recombination occurred between the B-genome introgressed fragments and the homeologous region (or any nonhomeologus region) in *B. napus*, due to the very low levels of homology between these linkage groups (Leflon et al. 2007).

1.6 Characteristics of stem rot disease

1.6.1 Symptoms and life cycle *of Sclerotinia sclerotiorum*

Sclerotinia sclerotiorum is a necrotrophic homothallic pathogen. Since it has a large host range, there are no unique symptoms that belong to all plants infected by this fungus. Infected leaves usually show water-soaked lesions that expand rapidly and move down the petiole into the stem. On the stem the initial indication is the appearance of water-soaked lesions that usually develop into necrotic tissues and subsequently develop patches of fluffy white mycelium (Bolton et al. 2006).

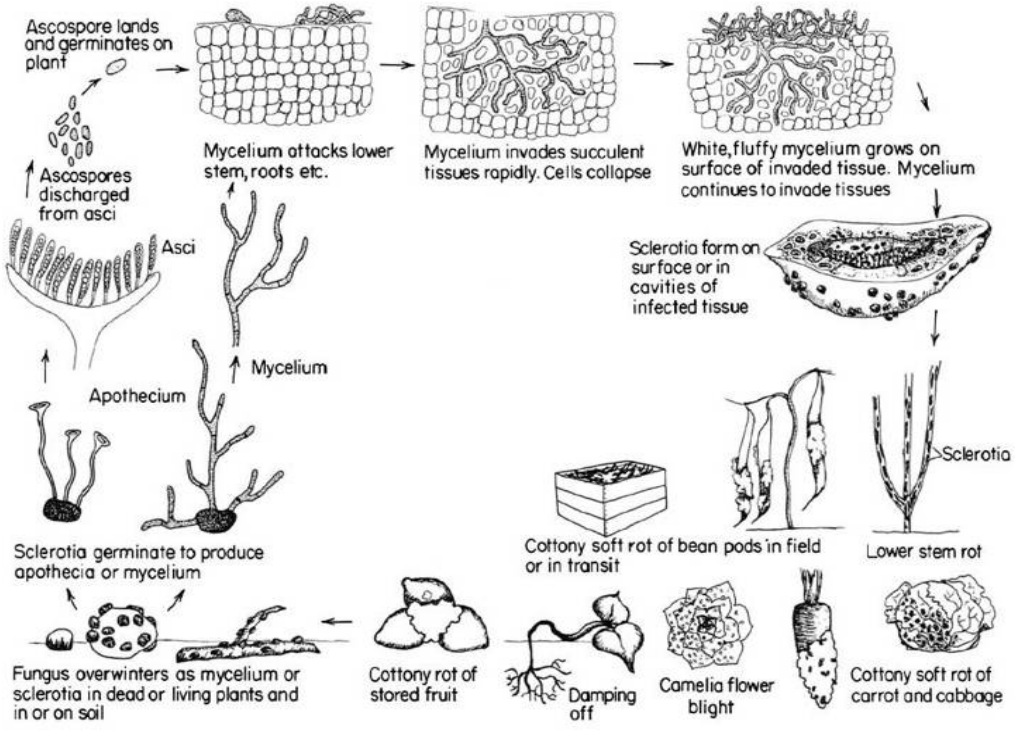

Fig. 1.7. Life cycle of *Sclerotinia sclerotiorum* (from Agrios, 1997).

Sclerotia can germinate carpogenically or myceliogenically, depending on environmental conditions, resulting in two distinct categories of diseases (Fig. 1.7). Sclerotia that germinate myceliogenically produce hyphae that can directly attack plant tissues (Bardin and Huang 2001). Sclerotia that germinate carpogenically produce apothecia and subsequent ascospores that infect over-ground portions of the host plants. Hyphae resulting from either germination type are hyaline, septate, branched, and multinucleate, with mycelium appearing white to tan in culture and in planta (Fig. 1.7). *Sclerotinia sclerotiorum* does not produce any asexual conidia (Bolton et al. 2006).

1.6.2 Genetics of resistance to *Sclerotinia sclerotiorum*

Diseases caused by *S. sclerotiorum* occur worldwide, cause considerable damage, and have proven difficult to control culturally or chemically (Dickman 2007). While there are no genetic sources of resistance identified to date with the *Brassica* B-genome, the most effective way to control this disease would be to develop genetically resistant cultivars.

In *Brassica* species in general, multiple sources of genetic resistance to *S. sclerotiorum* have been reported. Zhao and Meng (2003) identified a winter rapeseed (*B. napus*) accession resistant to this disease. By use of an F_2 population of *B. napus*, they mapped six QTLs for *S. sclerotiorum* resistance, where three QTLs explained 40.7% of the phenotypic variation for leaf resistance at the seedling stage. One of these QTLs was assigned to N3 of the A-genome, while the other two were assigned to N12 and N17 of the C-genome. The other three QTLs explained a total of 49.9% of the variation for stem rot resistance at the adult-plant stage, and were assigned to chromosomes N15 and N10 of the C-genome and N7 of A-genome. Both single locus QTLs and epistatic interactions were found to play important roles in *S. sclerotiorum* resistance, and the authors suggested that different resistance loci might be involved at different developmental stages or in different parts of the plant (Zhao and Meng 2003). In subsequent studies (Zhao et al. 2006; Zhao et al. 2007) used two DH populations

and found a number of QTLs involved in resistance of *S. sclerotiorum*, five of which were identified in both populations. One QTL on N2 explained 22% of the variation while a second QTL on N12 explained 10% of the variation and mapped to a region containing a homeologous non-reciprocal translocation. They suggested that these two QTLs enhanced the resistance by increasing the dosage of resistance genes (Zhao et al. 2006). Gene expression studies of the candidate genes using a microarray of the whole genome of *Arabidopsis* could successfully align these loci to chromosomes At5 and At3 of *Arabidopsis* (Zhao et al. 2007). Some of these QTLs are associated with other traits like days to flowering and glucosinolate content (Zhao and Meng 2003; Zhao et al. 2006).

1.8. An example of the successful introgression of CMS in *Brassica*

A well studied, commercially used example of introgression of traits from allied species into the *Brassica* is the development of cytoplasmic male sterility (CMS) systems in *B. napus*. CMS, and the corresponding nuclear restorer locus, *Rfo*, have been introgressed from radish (*Raphanus sativus*) into *B. napus* (Heyn 1976; Delourme et al 1992; Delourme and Eber 1992). However, as is often the case with intergeneric hybridizations, the introgression was also accompanied by deleterious genetic characteristics from the radish genome around the *Rfo* gene (Delourme et al. 1998). To improve the female fertility of the restorer lines and reduce glucosinolate content, extensive backcross and pedigree breeding were performed but due to tight linkage and impaired meiotic recombination between radish and rapeseed DNA, it was not possible to improve the agronomic and seed quality properties of the genetic material containing the *Rfo* gene. The introgression of *Rfo* gene was localized on the C-genome of *B. napus* genetic map, through homeologous recombination and replacement of a non-distal *B. napus* region of around 50 cM (Delourme et al. 1994; Delourme et al. 1998). In order to induce recombination between the introgressed radish genetic region and the homeologous *B. napus*, gamma ray irradiation was applied to the *Rfo* line to induce chromosome breakage and reunion from which the *Rfo* containing line

R2000 was selected, which had improved agronomic properties and a low glucosinolate content (Primard et al. 2005)

Due to the difficulties in the development of agronomically useful *Rfo* restorer lines by traditional breeding methods, a positional cloning approach was also initiated to isolate the *Rfo* gene from radish. Using a radish mapping populations segregating for the *Rfo* gene, *Raphanus-Arabidopsis* microsynteny in that region was exploited and the locus was genetically and physically delimited. The *Rfo* gene was cloned independently and shown to be a member of the pentatricopeptide-repeat protein family (Brown et al. 2003; Desloire et al. 2003). It was shown that transformed *B. napus* plants, carrying the cloned *Rfo* gene from radish, were not noticeably different from fertile rapeseed plants and did not have elevated levels of glucosinolates, indicating that the transgenic approach is likely to be successful for the commercial use (Brown et al. 2003). This example illustrates the challenges such as linkage drag, reduced recombination rates and complicated mapping analysis, involved in introgessing gene(s) of interest from different allied species.

1.9. Objectives of this thesis

Brassica B-genome chromosomes carry many valuable traits that are not be found in the A and C-genomes, including blackleg resistance (Schelfhout et al. 2006), heat and drought tolerance (Kumar et al. 1984), aluminum tolerance (Huang et al. 2002) and tolerance to salinity conditions (Malik 1990). Transferring blackleg resistance (Chevre et al. 1997; Dixelius and Wahlberg 1999; Gerdemannknorck et al. 1995; Roussel et al. 1999; Roy 1984) and siliques shatter resistance (Prakash and Chopra 1988) from the B-genome of *B. juncea* or *B. nigra* to *B. napus* has been attempted, but these traits have not been successfully introduced into commercial germplasm. As explained earlier using the example of the CMS/*Rfo* gene, lack of adequate recombination in wide hybridization usually limits the transfer of traits from related species. Problems are also associated with unstable introgressions and incorporation of agronomically undesirable traits as linkage drag. *Brassica carinata*, an

amphidiploid species carrying the B- and C-genomes, has been used as a source of the B-genome in interspecific crossings of *B. napus* x *B. carinata* to generate the plant material used in this study. The initial goal was to transfer disease resistance from the B-genome into the AC-genome background.

In this study I tested the following hypotheses:

1- That in the backcross interspecifc hybrids, B-genome chromosomes can be maintained and the specific linkage group can be identified using DNA markers.
2- Second, that segments of the B-genome chromosomes can be introgressed into either the A or C linkage groups.
3- Third, that there is significant variation for both morphological traits and disease resistant traits associated with the B genome linkage groups.
4- Finally, I tested the hypothesis that the morphological and disease resistant traits could be mapped to specific B genome linkage groups.

This study builds on previous work in which two interspecific populations of *B. napus* x *B. carinata* were developed to introgress B-genome genetic traits into *B. napus*. The fundamental objectives of this research were:

a) to study the inheritance of the B-genome chromosomes in progenies of two interspecific crosses using SSR markers.
b) to study the B-genome introgressions using GISH.
c) to characterize the interspecific populations for morphological traits and resistance to Scletotinia stem rot and blackleg.
d) to identify the effect of the introgressed B-genome chromosome(s) on the agronomics and disease resistance of this material.

In Chapter 2, a doubled haploid interspecific population was used to study the B-genome chromosomes and traits associated with them. Chapter 3 describes the

study of the DH lines for resistance to Sclerotinia stem rot and its association with the *Brassica* B-genome, and Chapter 4 took advantage of a number of advanced backcross families that had previously been developed to study the inheritance of the B-genome chromosomes. In both populations, microsatellite markers and a GISH assay were used to study the B-genome chromosomes.

The doubled haploid population used in Chapters 2 and 3 was originally developed by Dr. Vipan Bansal in the Canola breeding program at the University of Alberta. I was provided with seed packets and I completed all the subsequent work including phenotypic measurements, genotyping and the cytogenetic studies. The field trials were seeded and harvested with the assistance of the members of the Canola breeding program.

Microsatellite markers were provided by Agriculture and Ari/Food Canada, (Saskatoon Research Centre), and the work was done in collaboration with Dr. Isobel Parkin's lab.

The genetic material used in Chapter 4 was generated in Dr. Good's lab in the Department of Biological Sciences, by a previous Ph.D. student, Kiersten Stead. I used seeds from the BC3 plants described in her thesis, to evaluate their phenotype, genotype and in cytogenetic studies.

I performed the cytogenetics work in Dr. J. Chris Pires lab, at the University of Missouri-Columbia. The cytogenetics figures were developed with the assistance from Zhiyong Xiong, a post doctoral fellow in Dr. Pires lab.

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2. INTROGRESSION OF B-GENOME CHROMOSOMES IN A DOUBLED HAPLOID INTERSPECIFIC POPULATION OF *Brassica napus* × *B. carinata*

2.1. Introduction

The Brassicacea family, with more than 3000 species, includes a number of important crop plants. Genome homoeology of the different *Brassica* species has been characterized and detailed genetic and physical maps of *Arabidopsis* have been aligned with genetic maps of the different *Brassica* species (Lagercrantz and Lydiate 1996; Lagercrantz et al. 1996; O'Neill and Bancroft 2000; Parkin et al. 2002; Parkin et al. 1995; Sharpe et al. 1995).

Brassica species containing the B-genome (*B. nigra*, *B. carinata* and *B. juncea*) possess many valuable agronomic traits not found in the A- and C-genomes, including resistance to blackleg disease caused by *Leptosphaeria maculans* (Schelfhout et al. 2006), heat and drought tolerance (Kumar et al. 1984), aluminum tolerance (Huang et al. 2002) and tolerance to salinity (Malik 1990). Transferring blackleg resistance (Chevre et al. 1997; Dixelius and Wahlberg 1999; Gerdemannknorck et al. 1995; Roussel et al. 1999; Roy 1984) and silique shattering resistance (Prakash and Chopra 1988) from the B-genome of *B. juncea* to *B. napus* has been attempted, but these traits have not been successfully introduced into commercial germplasm. Interspecific hybridization for the transfer of traits from related species is often associated with problems of unstable introgressions and the incorporation of agronomically undesirable traits due to linkage drag (Parkin and Lydiate 1997).

Blackleg is a fungal disease caused by the pathogen *Leptosphaeria maculans* that attacks the leaves and stems of many species within the family Brassicaceae, resulting in shriveled seeds and yield losses (Howlett et al. 2001). There are different sources of resistance reported for blackleg (Table 1.5). All blackleg resistance genes in *B. napus* mapped to date are located on the A-

genome. Most B-genome containing *Brassica* species also carry resistance genes to blackleg, of these, the ones that have been genetically mapped were originally identified in *B. juncea* (Balesdent et al. 2002; Christianson et al. 2006; Rimmer and Vandenberg 1992) with one being identified in the B-genome of *B. nigra* (Chevre et al. 1996). Transfer of B-genome derived resistantce genes to *B. napus* by incorporating large introgressions (Barret et al. 1998; Chevre et al. 1997; Saal et al. 2004) has failed, presumably because of a lack of recombination between B-genome introgressed fragments and the A or C genome chromosomes of *B. napus*, due to the very low levels of homoeology between linkage groups (Leflon et al. 2007).

One of the goals of *Brassica* oilseed research programs is the successful and stable introgression of genes from wild or closely related species into cultivated canola plants through inter- and intra-specific crosses, in order to introduce novel traits (Ky et al. 2000). The objective of this study was to use an advanced backcross approach (Tanksley and Nelson 1996) to transfer traits of economic interest into adapted *Brassica* germplasm, while at the same time identifying the loci coding for the specific trait(s).

2.2. Materials and methods

2.2.1. Plant material

An accession of *Brassica carinata* (Acc. 17-009) from the canola breeding program at the University of Alberta, which carries resistance to blackleg, stem rot and black spot (caused by *Alternaria brassicae*) fungal diseases (Bansal et al. 2000), was crossed with *Brassica napus* cv. Westar. The pedigree of the interspecific cross is shown in Figure 2.1. Due to the intention to eventually introgress this genetic material into canola quality *B. napus*, in each generation, selection was applied for blackleg resistance and *B. napus* morphology and optimal seed set. The evaluation of blackleg resistance was performed using a cotyledon based test, as described by Bansal et al. (1999). Six hundred DH lines

were produced from the BC_3S_1 plants and 60 DH lines were selected randomly and used in this study.

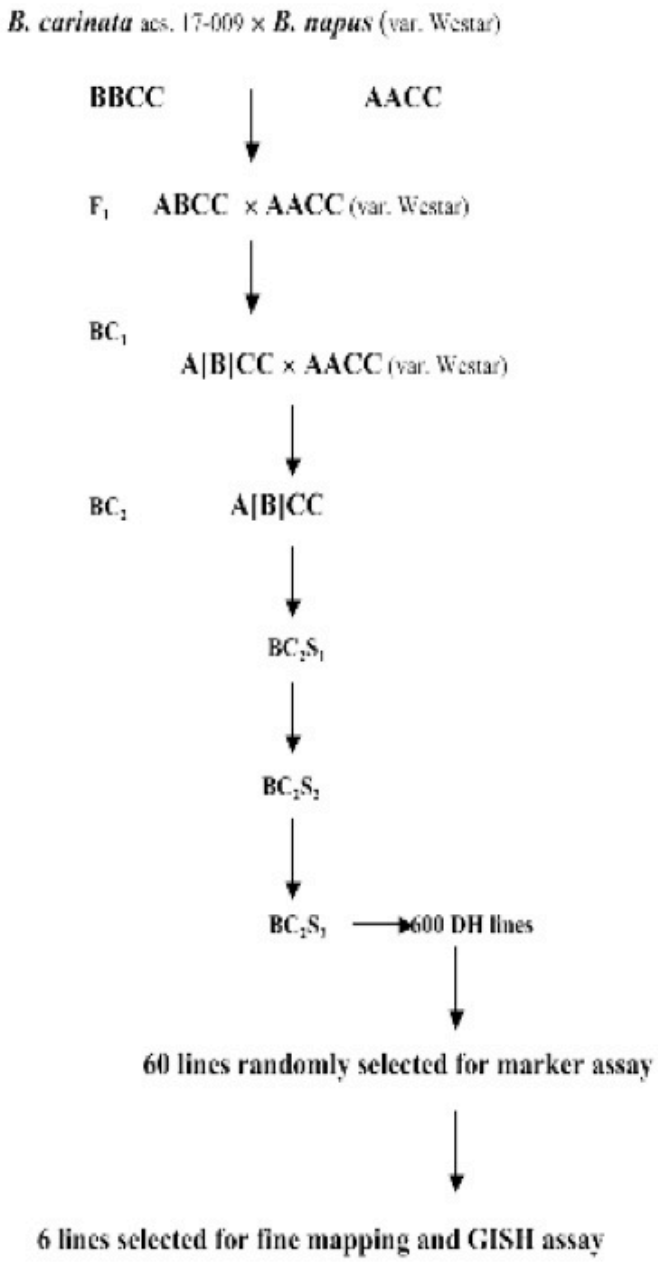

Fig. 2.1. Pedigree and crosses used for the development of the DH lines used in this study. Selection for disease resistance and *B. napus* morphological phenotypes.was performed in each generation

2.2.2. Phenotyping

Sixty DH lines with parental controls were grown in Metro Mix® 290 (Grace Horticultural Products, Ajax, Ontario, Canada) in the greenhouse at 21°C/day and 18°C/night with 16 hour photoperiod. Plants were fertilized every two weeks with 200 ppm Peres 20-20-20 (N-P-K) complete fertilizer with micronutrients (Plant Products, Brampton, Ontario). Fresh leaf tissue was collected from seedlings at the 4-5-leaf stage for DNA extraction using a GenElute™ Plant Genomic DNA Miniprep Kit (Sigma).

The DH lines, parental lines and four controls (*B. carinata* lines 070 and 065; *B. napus* line MBX and Topas) were evaluated in field plot trials at the University of Alberta Edmonton research station for two consecutive years (2006 and 2007). An 8 x 8 unbalanced square lattice design (8×8) with two replications was used. Each plot consisted of four rows, two meters long with 20 cm row spacing. The morphological characteristics, leaf shape, number of lobes, days to flowering, days to maturity, pod attitude, pedicel length, silique length, beak length, and number of seeds per pod were recorded following the Canola/rapeseed Objective descriptions for plant breeders, according to the Canadian Plant Breeders Rights Office (http://www.inspection.gc.ca/english/plaveg/pbrpov/pbrpove.shtml). Oil profile was determined on the seed harvested from each plot in each year, following the protocol of Daun et al. (1989). Seed yield was not measured due to the variability in number of plants present in each plot. Blackleg cultures of the isolate V77 (which belongs to the pathogenicity group, PG2 and was kindly provided by Dr. Stephen Strelkov, University of Alberta), were grown on V8 agar plates at room temperature under 12 hours photoperiod to enhance pycnidia production. After two weeks, a suspension of pycnidiospores was prepared and adjusted to 1×10^6 spores/ml in water. Inoculations were performed for all sixty lines randomly planted in two replications with four plants in each, on needle-wounded stems before the flowering stage following Bansal et al. (1994). Three weeks after

inoculation, the length of the internal and external symptoms was scored individually as was their ratio to plant height (Bansal et al. 1994).

2.2.3. Statistical analysis

Data from each season was subjected to analysis of variance following the linear model $Z_{ijkl} = \mu + b_i + I_j(b_i) + T_k + e_{ijkl}$, where for each trait Z_{ijkl} is the phenotypic value of each DH line in each replication, μ is the grand mean, I_j is the effect of incomplete block nested in block (b_i), T_k is the effect of treatment and e_{ijkl} stands for the experimental error.

A mixed model analysis (Littell et al. 1996) was performed using PROC MIXED in SAS® (SAS Institute Inc. 1989) in which genotype was considered as a fixed effect and block and incomplete block (block) were considered random effects. Based on the marker data, the DH lines were divided into two groups: the first group consisting of three lines carrying B-genome chromosomes, hereafter referred to as "B+", and the other one consisting of three lines with no B-genome content, referred to as "B–". Single degree of freedom contrasts were performed using the ESTIMATE statement in PROC MIXED to make comparisons between these two groups. A two-tailed t-test was performed in order to compare individual lines with different chromosomal contents. A combined analysis of variance was also performed using PROC MIXED, in which genotype was considered fixed while the year, block, incomplete block (block) and all corresponding interactions were considered random. Combined analysis was also followed by single degree of freedom contrasts and the t-tests were conducted as described for single year analyses.

2.2.4. Genotyping

From a total of 1242 B-genome microsatellite (SSR) markers, developed at the Agriculture and Agri-Food Canada (AAFC) Saskatoon Research Station, 220 were selected based on their location on the B-genome linkage groups and their ability to amplify strong bands, in the AAFC *B. juncea* reference mapping

population. Furthermore, these markers had been used for mapping other populations and therefore provided the opportunity to cross reference with other map data. Of these, 48 are publicly available; their location, and the size of the allele in *B. carinata* is reported in Appendix 2.2. Initially, all 60 DH lines and parental controls were genotyped with these 48 markers in a nested PCR reaction (Schuelke 2000). The PCR mix contained 2 pmoles of forward primer, 8 pmoles each of reverse and labeled M13 primer (5'-TGTAAAACGACGGCCAGT-3'), 1 U of Taq enzyme, 0.2 mM dNTPs, 10ng template DNA, 1.6 mM $MgCl_2$ and 1.5 µl 10X buffer in a total volume of 15 µl. The PCR reaction consisted of 5 min at 94ºC of initial denaturation, followed by 30 cycles of 94ºC (30 s), 57ºC (45 s) and 72ºC (45 s), nested with a second round of PCR with 8 cycles of 94ºC (30 s), 55ºC (45 s) and 72ºC (45 s) and a final extension step of 10 min at 72ºC. Subsequently PCR products were diluted 10 times in formamide solution with GS 500 TAMRA 3730 (Applied Biosystems) as the size standard, dissolved on an ABI 3735 DNA analyzer and sized using GeneMapper® software (Applied Biosystems).

The remaining 172 SSR primers (kindly provided by AAFC Canada), unlike the publicly available ones, were already fluorescently labeled. PCR assay preparation for these primers involved adding 0.5µl of each forward and reverse primer (5µM) to 5.0 µl ABI Amplitaq Mastermix. forty ng of genomic DNA was used in a total volume of 10 µl for a 384 well plate. The PCR conditions were as follows: 95ºC (10 min), followed by 8 cycles of 94ºC (15 s), 50ºC (15 s) and 72ºC (30 s). In the next 27 cycles, the melting point decreased to 89ºC (15s) while annealing and extending temperatures remained the same at 50ºC (15s) and 72ºC (30s), with a final extension at 72ºC for 10 min. Pooled PCR products, labeled with three different fluorescent dyes, were diluted 10 times in 0.1% TWEEN 20, dissolved on a ABI 3700 DNA Analyzer (Applied Biosystems Inc.) with ET ROX 550 size standard (Applied Biosystems Inc.), and analyzed using Fragment Profiler® (MegaBACE™) software.

To identify B-genome alleles, a panel of four *B. napus* lines (Westar, Delta, Topas, MBX) and three *B. carinata* lines (17009, 070 and 065) were used (Appendix 2.1). A second panel of four parental and 60 DH lines was used to detect those DH lines carrying B-genome chromosome segments (data not shown). Based on the preliminary marker data, six of the 60 DH lines were subjected to fine mapping, three of which were shown to carry whole, or segments of some of the B-genome chromosomes.

2.2.5. Cytology and, GISH analysis

Three positive lines, having at least one B-genome chromosome (DH45, DH51 and DH39) and three negative lines (DH21, DH31 and DH107) were selected for GISH assay. Immature flower buds were collected from one plant of each of the DH lines for mitotic and meiotic chromosome spreads. Flower buds were treated with nitrous oxide gas for one hour followed by ice-cold 90% acetic acid treatment for 10 min to fix the chromosomes and stored in 70% ethanol at -20°C until use. Slides were prepared following enzyme maceration (Kato et al. 2004). *Brassica nigra* and *B. oleracea* genomic DNA and repeated sequences were labeled with Fluorescein-12-dUTP, Cy3-dCTP, Cy5-dUTP or together with Fluorescein-12-dUTP and Cy3-dCTP (Perkin Elmer Life Sciences, Boston, MA) using a nick translation procedure (Kato et al. 2004). We also used a 45S DNA probe to detect Nuclear Organizing Regions (NORs). Fluorescent *in situ* hybridization was performed following the method of Kato *et al*. (2004) with slight modifications as described by Lamb and Birchler (2006). After hybridization and washes, a drop of Vectashield® mounting medium containing DAPI (4'-6-Diamidino-2-phenylindole; Vector Laboratories Inc., Burlingame, CA) was applied, and the cells were covered with a 24 X 50 mm cover glass. Visualization was performed using an Olympus® BX61 fluorescent microscope with a 60X plan apo oil immersion lens, and digital images were captured using the Olympus® Microsuite™ 5 software package. Images were cropped, size adjusted, and contrast optimized using only functions affecting the whole image with Adobe® Photoshop® 9.0.2 (Adobe Systems Inc.).

2.3. Results

An interspecific cross of *B. carinata* and *B. napus* was performed with the initial intention of introgressing B-genome derived blackleg resistance into a *B. napus* background. Sixty DH lines were used in this study for phenotyping and genotyping using 48 publicly available SSR markers, out of which six lines were selected for detailed genotyping (Fig 2.1).

2.3.1. Genotypic evaluation of the DH lines

Out of 220 microsatellite markers, 32 primer pairs failed to amplify and four were not polymorphic between the parents. Of the 184 informative primers, 85 also amplified an allele in the *B. napus* controls, in some cases producing a stronger signal than in the B-genome containing lines. Therefore, it was not possible to assign these alleles to the A- or C-genome owing to the low number of markers and the nature of the backcross.

Table 2.1. B-genome chromosome content of the parental and DH lines.

Linkage group	Westar *B. napus*	98-17-009 *B. carinata*	O70	DH 21	DH 31	DH 39	DH 45	DH 51	DH 107	"B-"[d] Group
J11	-	+[a]	+	-[b]	-	-	-	-	-	-
J12	-	+	+	-	-	74%[c]	-	-	-	-
J13	-	+	+	-	-	+	71%	71%	-	-
J14	-	+	+	-	-	+	17%	-	-	-
J15	-	+	+	-	-	-	-	-	-	-
J16	-	+	+	-	-	-	-	-	-	-
J17	-	+	+	-	-	+	-	-	-	-
J18	-	+	+	-	-	+	65%	-	-	-

[a] (+) Indicates that all of the markers along the linkage group amplified in the line, indicating the presence of the complete linkage group.
[b] (-) shows the absence of the linkage group in the lines.
[c] The percentage of the LG present (in cM), are represented for linkage groups that are not entirely present in the DH lines in which either the tip or the middle of the chromosome is lost.
[d] (B-) is a group of three DH lines with no B-genome content.

In the advanced backcross population of BC_2S_3 generation, there were two DH lines containing multiple B-genome chromosomes and in most cases these chromosomes were present as whole B chromosomes (Table 2.1, Fig 2.2). After two generations of backcrosses there was preferential maintenance of five specific B chromosomes (J12, J13, J14, J17 and J18). DH39 carried J12, J13, J14 and J18 (but with deleted terminal and internal segments), and also carries most of J17 (not shown in Fig. 1). DH45 carried the majority of J13 as well as segments of J14 and J18. DH51 carried J13 but was missing the terminal end of the chromosome (Table 1, Fig 2). J13 was the only chromosome maintained in all three lines, J18 and J14 were maintained in two lines and segments of J12 and J17 were maintained in one line, out of sixty. Figure 2 illustrates that these lines generally tend to lose the terminal segments of B-genome chromosomes, presumably through terminal deletions. Interestingly the breakpoint on J13 in both DH45 and DH51 appears to be conserved, while DH39 appears to have lost an internal segment of J13.

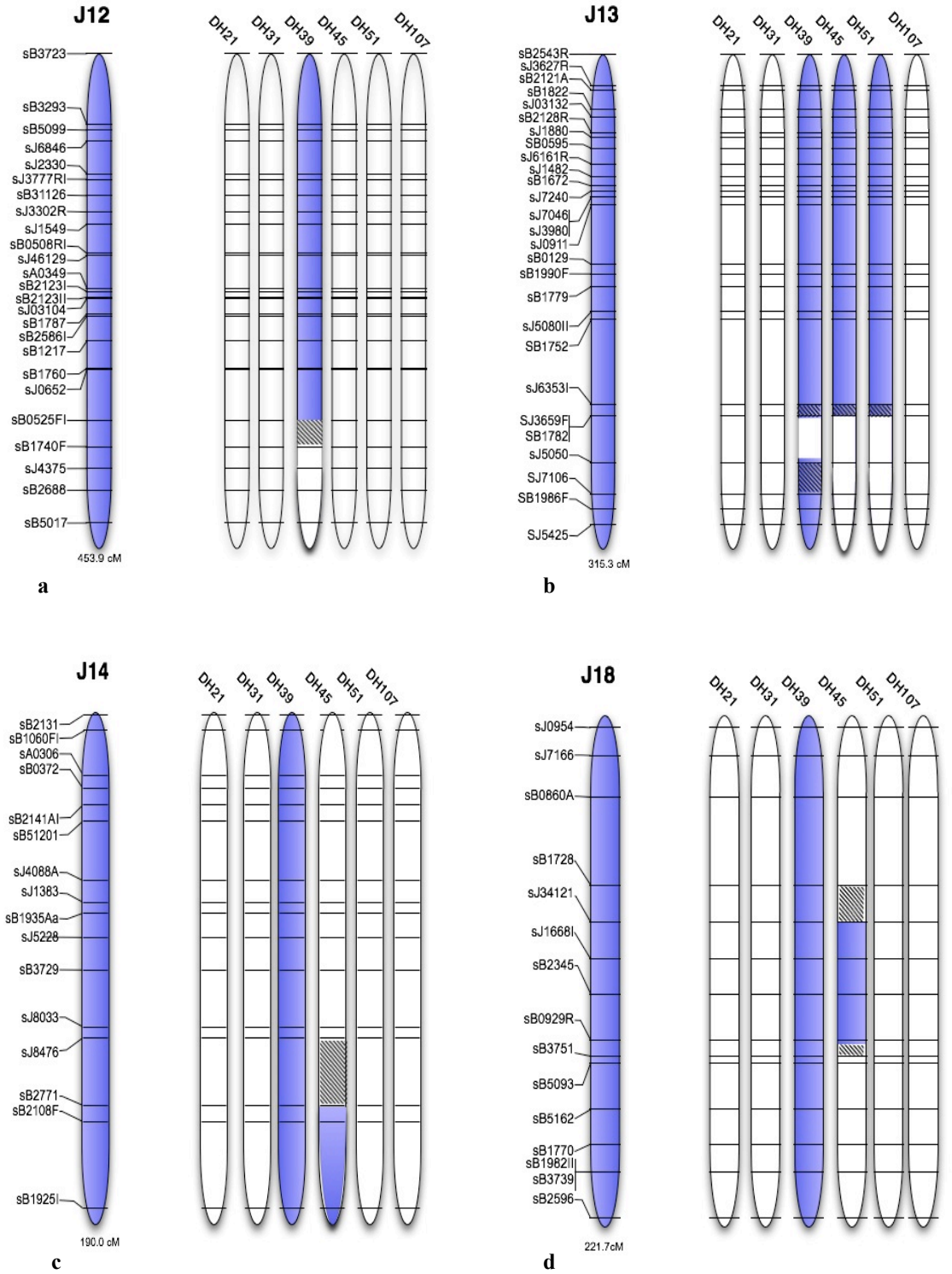

Fig. 2.2. Physical representation of the B-genome linkage groups maintained in the DH lines. Location of the markers selected on the four B-genome linkage groups is based on the AAFC reference maps. Shaded segments show the presence of B-genome chromosomes and hatched segments shows the area of possible recombination.

2.3.2. GISH analysis

Figure 2.3 shows the images from the GISH assay. *Brassica carinata* displayed eight pairs of chromosomes that were painted red (Fig. 2.3a), while *B. napus* (2n=38, Fig. 2.3b) showed 19 pairs of green signals, indicating that genomic DNA from *B. nigra* is capable of detecting B-genome chromosomes in allopolyploid species, while genomic DNA from *B. oleracea* can paint both A- and C-genome chromosomes, but cannot distinguish between them. I was able to count 34 chromosomes in the *B. carinata* (2n=34) parent, sixteen of which emitted a red signal characteristic of the B-genome chromosomes (Fig 2.3a). These signals are very strong around the centromere, but difficult to visualize on the chromosome arm, and therefore, it was not possible to detect translocations. For DH51 and DH45, it was possible to count 38 chromosomes, but two of them had strong red signals indicating they were B-genome linkage groups. Therefore, it can be inferred that these lines contain a pair of additional J13 chromosomes (2n=36+II (J13) (Fig. 2.3, c,d). Based on the original marker data, DH 39 appeared to be carrying four B-genome chromosomes (J12, J13, J14, J18). However, selfed seed from the DH39 plants used for marker analysis did not germinate, nor did any of the seed from DH39's sibling plants, therefore it was not possible to include this line in GISH assay. For the DH lines with no B-genome chromosomes, DH17 carried 19 pairs of chromosomes, 2n=38, with no red signals (Fig. 2.3e), while DH21 was missing one chromosome, i.e. it had 2n=38 - 1 chromosome (Fig. 2.3f).

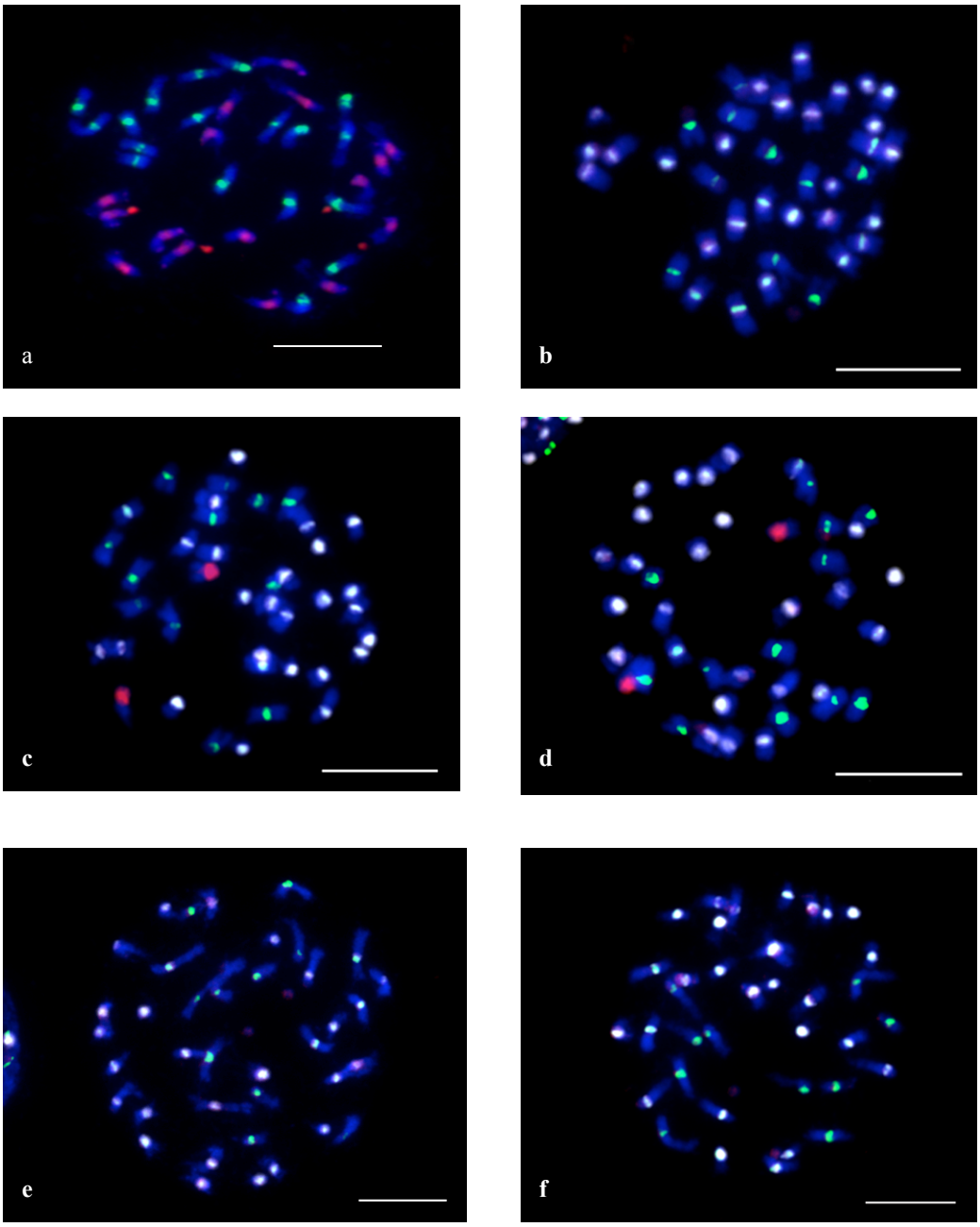

Fig. 2.3. Chromosome painting at prophaseII, using 60x magnification, using the GISH technique. *Brassica nigra* genomic DNA is fluorescently labeled in red, *B. oleracea* genomic DNA in green and 45 S DNA in white. a) *B. carinata*, 2n=34 b) *B. napus*, 2n=38 c) DH 51, 2n=36+2B, d) DH45, 2n=36+2B, e) DH17, 2n=38, f) DH21, 2n=37. Each bar is equal to 10 µm.

2.3.3. Phenotypic evaluation of the DH lines

Disease variation within the DH lines followed a normal distribution for blackleg symptom length and for the following agronomic traits: silique length, pedicle length, days to flowering, days to maturity, number of seed per silique and beak length (Table 2.2). However, while the distribution of phenotypic traits followed a normal distribution, there was also significant transgressive segregation for all of these traits (data not shown). There are significant differences ($p < 0.01$) in the DH population for blackleg symptom length, both internally and externally. The *B. napus* cultivar, Westar is susceptible to blackleg and showed significant difference from the *B. carinata* parent 98-17-009 ($p < 0.01$). However, most of the DH lines used in this study were not significantly different from Westar, except for the line DH39, which was not significantly different from its *B. carinata* parent. DH45 was not significantly different from Westar for either internal or external symptom length, confirming that is susceptible to blackleg disease; and DH51 showed moderate resistance for internal symptom length with a value of 5.5 ± 1.71cm (Table 2.3).

The DH population was evaluated for agronomic and seed quality traits in two seasons of field trials and the data are presented in Tables 2.2 and Appendix 2.3, respectively. Significant differences were observed between the parental controls and the DH lines for all of the traits studied (Tables 2.2 and Appendix 2.2). The two parents, Westar and *B. carinata* (98-17-009) were different for all traits ($p < 0.01$) except for pedicel length, leaf margin and myristic acid (C14:0) content. In the two seasons of measurements, year did not have significant effect on any of the morphological or agronomic traits (Table 2.2 and Appendix 2.3).

Table 2.2. Statistics and comparison of the DH lines and the two parental liens; westar (*B. napus*) and 98-17-009 (*B. carinata*) in two field trial seasons for key agronomic traits.

Trait	Leaf Margin	Days to flowering	Days to maturity	Pod attitude	Silique length	Beak length	Number of seed/silique
Population mean	2.8(0.02)[a]	40(0.5)	97(1.1)	3.0(0.05)	51.5(0.36)	5.3(0.10)	22(0.29)
Mean of DH lines	2.8(0.02)	39(0.5)	96(1.1)	3.0(0.05)	52.0(0.34)	5.3(0.10)	22(0.28)
98-17-009	2.2(0.18)	52(5.9)	114(15.4)	1.0(0.48)	42.1(2.35)	3.8(0.85)	13(1.7)
Westar	2.4(0.18)	43(5.9)	98(15.4)	4.5(0.48)	53.2(2.35)	7(0.85)	19(1.7)
DH45	1.7(0.18)	48(5.9)	99(15.4)	2.5(0.48)	47.9(2.35)	8.7(0.85)	23(1.7)
DH 51	1.9(0.18)	40(5.9)	99(15.4)	3.0(0.48)	43.8(2.35)	7.8(0.85)	17(1.7)
B-	2.9(0.08)	38(1.6)	96(4.9)	3.3(0.22)	52.1(1.46)	5.1(0.31)	23(0.98)
B+[b] vs B-[c]	**	**	ns	ns	ns	**	**
B- vs 39 [d]	ns	**	ns	ns	ns	ns	**
B- vs 45	**	**	ns	ns	ns	**	ns
B- vs 51	**	ns	ns	ns	**	**	**
DH45 vs DH51	ns	**	ns	ns	ns	ns	*
98-17-009 vs Westar [e]	ns	**	**	**	**	**	**
98-17-009 vs DH39	*	ns	ns	*	**	ns	ns
98-17-009 vs DH45	ns	ns	*	*	ns	**	**
98-17-009 vs DH51	ns	**	*	**	ns	**	ns
Westar vs DH39	ns	*	ns	ns	ns	ns	**
Westar vs DH45	**	ns	ns	**	ns	*	ns
Westar vs DH51	ns	ns	ns	*	**	ns	ns
year	ns	ns	ns	ns	ns	ns	.
line	**	**	**	*	**	**	**
line*year	*	*	**	**	**	ns	.

[a] Least square means (standard error) for parental and the DH lines.
[b] (B+) a group of lines that carry at least one B-genome linkage group.
[c] (B-) includes a group of three DH lines that fine mapping proved them without any B-genome content.
[d] Single degree of freedom contrasts between lines with and without B-genome content.
[e] One tailed t-test comparison for individual lines.

2.3.4. The effect of the B-genome linkage groups on traits

The two groups of DH lines, with, "B+", and without, "B-", B-genome chromosomes, were statistically different for the following traits, using a contrast of single degree freedom : leaf margin, days to flowering, beak length, and number of seed per silique, C14:0 (Myristic acid), C18:0 (Stearic acid), C18:1 (Oleic acid), C18:2 (Linoleic acid), C20:1 (Arachidic acid), C20:1 (Gadoleic acid), C20:2 (Eicosadienic acid), C22:0 (Behenic acid), C22:1 (Erucic acid), C24:0 (Lignoceric acid), C24:1 (Nervonic Acid) and saturated fatty acids. These values changed slightly when the "B-" group was compared with lines of "B+" group individually (Table 2.2 and Appendix 2.3). More specifically, it was found that DH45 differed ($p < 0.01$) from the "B-" group for days to flowering (Table 2.3); however this line was not significantly different from its *B. carinata* parent but was different from its *B. napus* parent ($p < 0.05$) for this trait. DH51 was also significantly different from the "B-" group, for number of seeds per silique and not different from its *B. carinata* parent but had comparatively lower seed set (17±1.7 seeds) compared to the other DH lines. For all measured traits except days to flowering and number of seed per silique, DH45 and DH51 were not significantly different from each other but were different from the "B-" group ($p < 0.01$).

Table 2.3. Analysis of blackleg resistance in the DH population and a sub set of DH lines.

	Internal Blackleg Symptom Length (cm)	**Relative internal Symptom length (cm)** [a]	**External Symptom length (cm)**	**Relative external symptom length (cm)**
Max	21	0.27	16.16	0.29
Min	1.5	0.02	0.5	0.008
Mean	7.14	0.11	5.2	0.08
F value	**	**	*	**
Westar	9.5a	0.15a	7a	0.11a
98-17-009	3.75b	0.05b	2.6b	0.033b
DH39	3.7 b (1.56)	0.04 b (0.03)	2.6 b (2.11)	0.03 b (0.02)
DH45	9.5 a (1.56)	0.16 a (0.03)	7.0 a (2.11)	0.11 a (0.03)
DH51	5.5 c (1.71)	0.20 a (0.03)	6.7 a (2.59)	0.20 a (0.03)
DH21	10.1 a (1.56)	0.17 a (0.03)	7.8 a (2.11)	0.13 a (0.03)

2.4. Discussion

The choice of *B. carinata* as a source of B-genome traits was based on several criteria. First, since parts of the B-genome have higher homoeology to the A- than C-genome (Panjabi et al. 2008), it was hypothesized that by using *B. carinata* as a parent it would be possible to increase the chance of the B-genome segments recombining with their corresponding A-genome linkage groups. Second, *B. carinata* has not yet been used in an interspecific cross for the purpose of transferring blackleg resistance, despite the fact that this species has been shown to have very high levels of field resistance in Australia (Marcroft et al. 2002). Finally, all the blackleg resistance genes that have been mapped to date are located on N7 (Delourme et al. 2006). Therefore, by using *B. carinata* rather than *B. juncea*, it would be likely to have two potential resistance sources, one from the B-genome and one from N7.

The microsatellite markers used in this study were specifically chosen to amplify the B-genome alleles. Despite this, due to the nature of the *Brassica* genome and the fact that the A-, B- and C-genomes are related, it was found that 46% of these primers also generated PCR products from the A- or C-genome. Amplification of these markers in the A-genome in the original screening of the *B. juncea* mapping populations was also observed (Isobel Parkin, personal communication). I found that most of these loci amplified in the A-genome, based on observing the behavior of these primers in DNA samples from different *Brassica* species (Apendix 2.1). The amplification of B-genome markers in the A-genome may be explained by their partial homology. Panjabi et al. (2008) have shown that three B chromosomes, B4, B5 and B6, have regions of homology with three A-genome linkage groups, A4, A5 and A6, respectively. Additionally, specific blocks of A- and B-genome chromosomes of *B. napus* and *B. juncea* have been identified in alignment with blocks of the *Arabidopsis* genome (Panjabi et al 2008).

The observation of the lack of an amplified product in a DH line is often difficult to interpret, since it may result from a negative score or a failed PCR

reaction in that individual sample. However, all of the DNA samples from the different DH lines could amplify other known alleles, both within the B-genome or the AC-genome. Some of our DH lines possessed alleles which differed in size from the B-genome parent, often by 10-100 bp, which can be explained by either the slippage of DNA polymerase enzyme on SSR repeats or the fact that these alleles may belong to the C-genome (Appendix 2.1).

There are considerable difficulties in mapping the introgressions in the *Brassica* genomes due to the occurrence of sequence repetition, chromosomal rearrangements and insertion–deletions altering chromosomal structure, gene complement and abnormal recombination frequencies (Mayerhofer et al. 2005; Parkin et al. 2005). In this study I was able to overcome these problems to some extent by using the AAFC reference maps and by knowing the size and location of each allele on the B-genome linkage maps.

One of the benefits of the GISH assay with *B. nigra* genomic DNA was that it allowed me to visualize and distinguish the B-genome chromosomes from the A- and C-genome chromosomes (Ge and Li 2007; Hasterok et al. 2005; Maluszynska and Hasterok 2005; Snowdon et al. 1997; Figure 2.2). Application of this technique allowed tracking of the B-genome chromosomes, while at the same time, determining the number of chromosomes in the DH lines. It was also possible to determine the DH lines carrying a pair of B-genome chromosomes. The presence of a pair of intogressed chromosomes in a line was not surprising, given the doubled haploid nature of these lines. However, the GISH technique did not allow targeting of the chromosome arms because of preferential painting of the centromeres. Therefore, cytological confirmation of the segments that are translocated would require chromosome-specific DNA probes, which was beyond the scope of this study.

The cytology study also provided information on the chromosome number in each of the six DH lines. In most cases, as expected, the number of chromosomes in the negative controls (DH lines without B-genome chromosome) was the same as their *B. napus* parent (2n=38) except for line DH21, which was

randomly selected to be a negative control; however, this line was found to have lost a single A or C chromosome. In multiple spreads and chromosome counts, this line was always observed to have 37 chromosomes (2n=38-1). Of the total 60 DH lines only three lines (5%) contained B-genome chromosomes. Moreover, of the eight B chromosomes, only five were maintained in the DH lines derived from BC_2S_3 plants; and the two lines (DH45 and 51) carrying a single linkage group had J13. The low frequency of lines carrying B-genome linkage groups is not surprising, given that in each generation, selection was applied for *B. napus*-type plants. Furthermore, natural selection may have also played a role against the gametes carrying a B-genome linkage group.

Chevre et al. (2007) proposed that selection pressure could significantly affect the presence of additional chromosomes in interspecific hybrid plants. In this study, I observed the presence of J13 as an additional chromosome in three of the DH lines. The donor BC_3S_3 plant of the DH lines was generated by backcrossing and selfing (Fig 2.1), with selection for resistance to blackleg disease. Christianson et al. (2006) reported a blackleg resistance gene to be located on J13 of *B. juncea.* The E segment of the *Arabidopsis* genome, which aligns with J13 also aligns with a segment of N7 from *B. napus* (Panjabi et al. 2008), where another blackleg resistance gene has been mapped (Mayerhofer et al. 2005). Based on this knowledge and the observation that the blackleg resistant DH lines analyzed in this study also contains J13, it can be proposed that the B-genome resistance to blackleg is located within the E segment of J13, which corresponds to Chromosome 1 of *Arabidopsis*. However, in the present study, no significant correlation between the presence of J13 in DH39, DH45 and DH51 and their resistance to blackleg disease was found. This may be due to the reason that there are multiple sources of resistance for this disease (Delourme et al. 2006; Ferreira et al. 1995; Pilet et al. 1998; Pilet et al. 2001) and the *B. carinata* parent used in this study might be lacking the resistance allele in J13.

From this study, it was not possible to detect the effect of B-genome chromosomes on morphological and seed quality traits, which is due to the small

population size of the "B+" group. The DH population showed a significant transgressive segregation for the following traits: days to flowering, number of seeds per silique, oleic acid (C18:1) and gadoleic acid (C20:0) content (Tables 2.2 and Appendix 2.3). As the DH population can potentially carry C-genome chromosomes from the *B. carinata* parent, this variation cannot therefore be attributed exclusively to the presence of the B-genome. More detailed investigation would be needed to reveal the chromosome architecture of these lines.

In summary, in this study I evaluated a series of DH lines generated from a backcross population derived from *B. carinata* and *B. napus* cross, and used this population to study the inheritance of the B-genome chromosome(s) and association of specific traits with specific B-genome chromosome(s). I also generated a molecular karyotype of the B-genome linkage groups in each of these DH lines, using a set of 184 informative SSR markers (approximately 23 markers per linkage group). To the best of my knowledge, this is the very first successful development of a series of doubled haploid lines from a *Brassica* interspecific cross, particularly one that includes the B-genome. I have been able to identify the specific B-genome linkage groups that are maintained in these interspecific hybrid progeny and was able to evaluate whether these lines were substitution lines. Despite the general selection pressure against B-genome chromosomes, we have identified lines which have retained B-genome chromosomes in a "stable" manner, due to their DH nature.

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3. EFFECT OF THE *Brassica* B-GENOME ON RESISTANCE TO STEM ROT (*Sclerotinia sclerotiorum*) IN A DOUBLED HAPLOID POPULATION OF *Brassica napus* × *Brassica carinata*

3.1 Introduction

Stem rot is a serious disease caused by the fungus *Sclerotinia sclerotiorum* (Lib) de Bary, which is a generalist pathogen that infects important crops such as canola, common bean, sunflower, alfalfa, soybean, and peanut worldwide (Bolton et al. 2006). The pathogen is a serious problem in western Canada, causing the disease generally known as Sclerotinia or white stem rot of canola plants (Sedun et al. 1989). Infection by the pathogen can occur on the leaves, stems, and pods at different developmental stages, causing yield losses of up to 80% and significant reductions in the seed oil content and quality in *B. napus* (Canola Council of Canada 2006). *Sclerotinia sclerotiorum* is a necrotrophic, homothallic pathogen. It has a broad host range, however; there are no symptoms that are unique to all species infected by this fungus. Infection of canola leaves usually leads to the development of water-soaked lesions that expand rapidly and move down the petiole into the stem. The initial indication of infection on the stem is the appearance of a water-soaked lesions. The initial lesions usually become bleached and necrotic and subsequently develop patches of fluffy white mycelium, which are the most obvious signs of infection by *S. sclerotiorum* (Bolton et al. 2006).

Conventional methods for the control of Sclerotinia diseases include chemical and cultural control. Although cultural practices can reduce the impact of *Sclerotinia* diseases, they are often not sufficient to control the pathogen. Crop rotations, which include the growing of non-host species, represent the most common cultural control strategy, but extended rotations are often required for this approach to be effective. A 3 to 4 year rotation has been reported to be ineffective in reducing the incidence of white stem rot in canola (Williams and Stelfox 1980). Therefore, fungicides have been extensively used for the control of *S. sclerotiorum* in canola, as well as in other crops like soybean, dry bean, canola

and some vegetables (Bailey et al. 2000; Budge and Whipps 2001). In canola, fungicides are often applied at the full bloom stage, to prevent infection of the senescing petals, which can fall on the leaf axils and lead to infection of the stem (Rimmer et al. 2003). The application of fungicides can be expensive and also has negative environmental impacts (Gossen et al. 2001). Moreover, the development of resistance to fungicides in the pathogen population always remains a threat (Gossen et al. 2001). Concerns associated with the use of chemical pesticides have made biological control of *Sclerotinia* diseases an area of research interest amongst plant pathologists (Bardin and Huang 2001). Whilst various mycoparasites, such as *Coniothyrium minitans* and *Sporidesmium sclerotiorum*, have been tested as biocontrol agents for *S. sclerotiorum* (Ayers and Adams 1981; McQuilken et al. 1995), none have yet to be adopted to control this disease in commercial canola crops.

One of the most effective, economical and environmentally friendly approaches to control plant diseases is the development of genetically resistant cultivars. Most of the Canadian canola cultivars, howerver are susceptible to stem rot. Breeding programs aimed at developing increased physiological resistance have had limited success possibly due to the polygenic nature of the resistance, as has been reported in the case of the common bean (Fuller et al. 1984). In white bean, resistance is known to be associated with lower rates of diffusion of oxalic acid, a phytotoxin produced by *S. sclerotiorum*, in the infected leaf tissue (Tu 1985, 1989). In soybean, some plant characteristics such as early maturity and greater resistance to lodging are often associated with less disease, which primarily a result of the unfavorable microclimate conditions for the pathogen (Boland and Hall 1987; Buzzell et al. 1993). High levels of resistance to Sclerotinia stem rot are absent in most major crops. Soybean breeders utilize partial resistance for the development of cultivars, as no soybean genotype with complete resistance to this pathogen has yet been reported. Partial resistance can still provide an economically useful disease control measures for the growers (Boland and Hall 1987).

Significant variation for Sclerotinia stem rot occurs between *Brassica* species as well as between accessions within these species (Sedun et al. 1989). Zhao and Meng (2003b) identified three quantitative trait loci (QTLs) on the linkage groups N3, N12 and N17 of the A- and C-genomes of *B. napus*, which are involved in the control of resistance to Sclerotinia stem rot at seedling stage; while three QTLs on N7, N10 and N15 control resistance at the adult plant stage. Zhao et al. (2006) identified eight regions on N2, N3, N5, N12, N14, N16 and N19 affecting resistance to this disease. Thus, it is apparent that at least 11 of the 19 *B. napus* chromosomes carry QTLs for resistance, and the phenotypic variation explained by these QTLs varied from 5.9% to 39.8%. One of these loci was found to be linked with a 3-indolyl-methyl glucosinolate locus (Zhao and Meng 2003a). Molecular markers linked to these QTLs would allow screening for resistance at the genotypic level, thereby increasing the effectiveness of selection for this trait. In the case of soybean, three QTLs for resistance to *S. sclerotiorum* were identified. However, two of these loci were found to be associated with disease escape mechanisms, while the third QTL appeared to contribute to host resistance at the physiological level (Kim and Diers 2000). QTLs for resistance to *S. sclerotiorum* have also been reported in several other important crops, such as sunflower, common bean and snap bean (Arahana et al. 2001; Bert et al. 2004; Kolkman and Kelly 2003; Miklas et al. 2003; Zhao and Meng 2003a; Zhao and Meng 2003b).

Most of the resistance reported so far in *Brassica* is from the A- and/or C-genomes, while research on B-genome resistance is very limited. The objective of this study was to characterize a doubled haploid (DH) population, derived from an interspecific cross of *B. carinata* × *B. napus* for resistance to *S. sclerotiorum,* with the aim of introgressing resistance from the B and/or C-genome of *B. carinata* into offspring oilseed *B. napus.* Furthermore, the response of the *Brassica* diploid and amphidiploid species to Sclerotinia stem rot was examined for comparisons with the DH lines carrying introgressed B-genome chromosome(s).

3.2. Materials and Methods

3.2.1. Plant materials

An accession of *B. carinata* (Acc. 98-17-009) from the Canola Breeding Program of the University of Alberta, carrying resistance to multiple fungal diseases (Bansal et al. 2000), was crossed with *B. napus* cv. Westar. The F_1 was backcrossed to 'Westar' twice, and self-pollinated three times with selection for blackleg resistance, *B. napus* morphology and optimal seed set, and BC_2S_3 families were generated as described in Chapter 2 of this thesis. Six hundred DH lines were generated from these BC_2S_3 families, of which 58 DH lines were selected randomly and used in this study. Based on the presence or absence of B-genome chromosomal segments established by molecular marker and cytological tools (Chapter 2), the DH lines were classified into two groups: one group, designated "B-", possessed no detectable alleles from the B-genome chromosome(s) while the second group consisted of two lines carrying chromosome J13 of the B-genome (Chapter 2) and was designated "B+". . In addition to the segregating DH population, the diploid and amphidiploid species of U's Triangle (U 1935), including the two parental lines, were also used in this study as "reference lines" (Table 3.1).

Four plants of each of the 58 lines were grown in 9 cm^3 plastic inserts filled with Metro Mix® 290 potting mixture (Grace Horticultural Products, Ajax, Ontario, Canada), and were arranged in a complete randomized design (CRD) in flats consisting of 18 cells. The plants were kept in a growth chamber at 21°C/18°C (day/night) with a 16 hour photoperiod, and were fertilized every second week with 200 ppm 20-20-20 (N-P-K) complete fertilizer with micronutrients (Plant Products, Brampton, Ontario). The plants were inoculated at the early flowering stage as described below. The experiment was repeated once to provide two replications for statistical analysis.

3.2.2. Pathogen and inoculation

A strain of *S. sclerotiorum* isolated from a canola field and maintained at the Plant Pathology Laboratory of the University of Alberta was used in this study (graciously provided by Dr. Stephen Strelkov). Fungal cultures were grown on Potato Dextrose Agar (PDA) medium in 9 cm diameter Petri dishes. Cultures were incubated in darkness at room temperature for 4-6 days. Agar plugs of 0.5 cm diameter were excised with a sterile cork borer from the leading edge of the fungal colonies and transferred to fresh PDA medium. After three days, the agar plugs were again excised and used for inoculation. Agar plugs of *S. sclerotiorum* were placed directly on the stem at the 3rd or 4th leaf position, where the leaf was excised from the base to make a wound to ease penetration of the pathogen. Parafilm strips were used to tie and hold the agar plug on the stem. Inoculated plants were sprayed with water to increase relative humidity and were incubated in a growth chamber under the same conditions as described above.

3.2.3. Disease assessment and statistical analysis

Disease symptoms were assessed 7, 14 and 21 days after inoculation (DAI). The lengths of the stem lesions were measured and the lesion sizes were scored on a scale of 1 to 6 based on the phenotype, where:

1 = no symptoms or lesions <5 cm long with a water-soaked apearance;
2 = lesions <5 cm long with growth of feathery mycelium on the stem;
3 = lesions 5-15 cm long with dry feathery mycelium on the stem, usually no seed set on the plant;
4 = lesions 5-15 cm long with dry feathery mycelium on the stem; shredding of stem, resulting in lodging and no seed set;
5 = lesions 15-25 cm long, dried stems, plants usually dead;

6 = lesions > 25cm long, shredding of stem, stem breakage, entire plant is dead and dry.

A Mean Disease Severity (MDS) index was calculated for each line, using the following formula (R. Lange, Alberta Research Council, Vegreville, AB, personal communication):

$$MDS_{ij} = \sum r_{ij} n_i / N$$

where r_i is the score from 1 to 6 recorded for the individual plants of a DH line, n_i is the number of plants within each category of r_i and N is the total number of plants for each DH line in the experiment.

Multiple measurements over time (7, 14 and 21 DAI) allowed calculation of the area under the disease progress curve (AUDPC) for each experimental unit, using the formula:

$$AUDPC = \sum_{i=1}^{n-1} [(x_i + x_{i+1})/2] (t_{i+1} - t_i)$$

where x_i is symptom length on the i^{th} day, t_i is time in days on the i^{th} day and n is the number of measurements (3 in this study) (Das et al. 1992).

Analysis of variance for the unbalanced data was performed using PROC MIXED of SAS® software (SAS Institute Inc. 1989) for the following parameters: lesion length at 7, 15, and 21 DAI, disease symptom score (1-6) and the AUDPC. Mixed model analysis followed the linear model:

$$Z_{ij} = \mu + T_i + L_j + t_i L_j + e_{ij}$$

where Z_{ij} is the phenotypic value of the j^{th} line in the i^{th} test, μ is the grand mean, T_i is the effect of the i^{th} test, L_j is the effect of j^{th} line, $t_i L_k$ is the interaction of the j^{th} DH line and the i^{th} test and e_{ij} is the residual effect.

Analysis was performed, in which the effects of line and test and their interaction effect were considered fixed. Single degree of freedom contrasts were performed using the ESTIMATE statement in PROC MIXED to make comparisons between the "B-" and "B+" groups of the DH lines. A two-tailed t-test was performed to compare the individual reference lines as well as the two "B+" lines. Least square means (LSmeans) were computed for each of the DH lines using the LSMEANS statement and a pair-wise test of significance was

conducted on LSmeans using the PDIFF option (SAS Institute Inc. 1989) with PROC MIXED using the REPEATED statement.

A second mixed model analysis was performed, in which all effects were considered random. The COVTEST option was used in PROC MIXED (SAS Institute Inc. 1989) to compute the variance components for the effects in the model. An estimate of heritability was computed using the following formula:

$$H = \sigma^2_l / \sigma^2_l + \sigma^2_t + \sigma^2_{lt}$$

where H is the broad sense heritability, and σ^2_l , σ^2_t and σ^2_{lt} are the variance components of line, test, and line by test interaction, respectively.

3.3. Results

3.3.1. Disease symptoms and severity

In this experiment none of the genotypes were found to be immune to *S. sclerotiorum*. The most resistant genotypes developed a dark greyish brown, round lesion on the stem, about the size of the agar plug. In the case of the susceptible genotypes (e.g., *B. napus* cv. Westar), feathery mycelia tended to grow on the water soaked lesions on the stem (Fig. 3.1). However, in most susceptible genotypes, such as *B. oleracea* var. *alboglabra*, the fungus started to form black sclerotial structures on the stem (Fig.3.1). Later, the stems dried out and the plants failed to set any seeds. In some cases, the stem of the flowering plant wilted soon after inoculation, causing the entire plant to rapidly die.

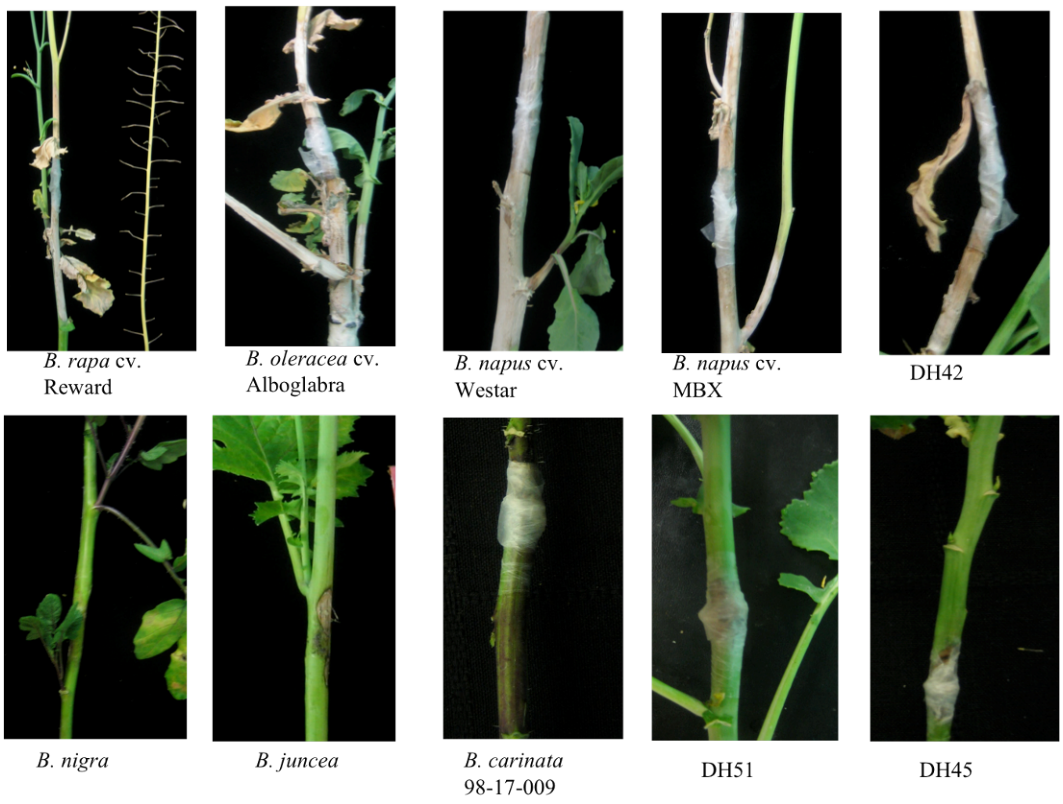

Fig. 3.1. Typical symptoms caused by *Sclerotinia sclerotiorum* in *Brassica*. Accessions on the top row belong to the group "B-" (without B-genome chromosomes) and on the bottom row belong to the group "B+" (with B-genome chromosomes).

The repeated measurements scored for each experimental unit at 7, 15 and 21 DAI revealed that time had a significant effect ($p < 0.01$) on disease progress, with increasing severity of disease symptoms as time progressed (data not shown). In general, *Brassica* genotypes with B-genome chromosomes and the DH lines carrying B-genome introgression had significantly lower MSD indices compared to the genotypes lacking the B-genome. The *B. carinata* and *B. nigra* genotypes had the lowest MDS values; the DH lines with introgressed B-genome chromosome segments also had low MDS values (Table 3.1).

Table 3.1. General mean disease severity response to Sclerotinia stem rot in different *Brassica* species and in interspecific cross (*B. napus* x *B. carinata*) derived doubled haploid lines with or without B-genome introgression.

Parental, DH and reference lines	Species	Group[a]	Genome	Disease score index MDSij=∑rijni/N [b]
Alboglabra	*B. oleracea*	B-	CC	5.22
Reward	*B. rapa*	B-	AA	4.86
Nigra	*B.nigra*	B+	BB	1.00
MBX	*B. napus*	B-	AACC	5.33
Westar	*B. napus*	B-	AACC	5.14
O70	*B. carinata*	B+	BBCC	1.00
98-17-009	*B. carinata*	B+	BBCC	1.00
Juncea	*B. juncea*	B+	AABB	1.43
JO65	*B. juncea*	B+	AABB	1.40
DH107	DH line	B-	AACC	4.75
DH42	DH line	B-	AACC	4.50
DH45	DH line	B+	AA[B]C	1.86
DH51	DH line	B+	AA[B]C	2.38

[a] B- and B+ indicates absence or presence of B-genome chromosome(s), respectively.
[b] Mean severity of the disease based on the 1-6 scores is tabulated.

3.3.2. Reaction of parental and reference lines

Among the *Brassica* species evaluated in this study, the species carrying the B-genome consistently had a lower LSmeans value for all five parameters (disease symptoms at 7, 15 and 21 DAI, disease score on the 1-6 scale and AUDPC), compared to the species without the B-genome. The two *B. carinata* accessions, 98-17-009 and O70, had the lowest LSmeans (0.1 cm) for each of the three symptom length measurements, while the *B. napus* line 'MBX', had the highest values, 14.3 ± 1.24 cm, 19.1 ± 1.77 cm and 24.4 ± 2.47cm , respectively, at 7, 15 and 21 DAI. The highest AUDPC of 46.9 ± 5.61 was recorded for the *B. napus* line 'MBX' and the lowest (0.02 ± 3.63) for *B nigra* (Table 2). The accessions of *B. carinata* (98-17-009 and O70), *B. juncea* and *B. nigra* were not

significantly different from each other; but were significantly different ($p < 0.01$) from *B. oleracea* var. *alboglabra*, *B. rapa* (Reward) and *B. napus* ('MBX' and 'Westar') for all five disease severity parameters used in this study (Table 3.3). All accessions of the *Brassica* species without the B-genome were statistically similar with respect to the five disease severity parameters (Table 3.3).

Table 3.2. Factors and comparison of different *Brassica* species and DH lines for *B. napus* x *B. carinata* resistance to Sclerotinia stem rot.

	7 DAI (cm)[a]	**15 DAI (cm)**	**21 DAI (cm)**	**Score (1-6)**	**AUDPC (cm^2)**
Line	**	**	**	**	**
Block	**	**	**	**	**
Line*Block	*	**	ns	ns	**
B- vs B+	**	**	**	**	**
DH45 vs B-	**	**	**	**	**
DH51 vs B-	**	**	**	**	**
Albo	10.2 ± 1.01[b]	15.7 ± 1.44	22.2 ± 2.02	5.1 ± 0.33	36.3 ± 3.45
Reward	8.7 ± 1.15	12.5 ± 1.63	17.6 ± 2.29	4.8 ± 0.37	30.4 ± 3.92
Nigra	0.0 ± 1.07	0.6 ± 1.53	1.1 ± 2.14	1 ± 0.35	0.02 ± 3.63
MBX	14.3 ± 1.24	19.1 ± 1.77	24.4 ± 2.47	5.4 ± 0.41	46.9 ± 5.61
Westar	8.3 ± 1.15	13.9 ± 1.63	19.2 ± 2.29	5. 1 ± 0.37	29.1 ± 3.92
Juncea	0.4 ± 1.15	0.7 ± 1.63	1.2 ± 2.29	1.2 ± 0.37	1.8 ± 3.92
JO65	1.8 ± 1.36	3.7 ± 1.94	4.5 ± 2.71	1.2 ± 0.44	4.7 ± 5.73
O70	0.1 ± 1.15	0.1 ± 1.63	0.1 ± 2.29	0.9 ± 0.37	0.08 ± 3.92
98-17-009	0.1 ± 1.15	0.1 ± 1.63	0.1 ± 2.29	0.9 ± 0.37	0.08 ± 3.92
DH21	3.2 ± 1.07	6.7 ± 1.53	9.7 ± 2.14	3.2 ± 0.35	11.3 ± 3.63
DH45	0.2 ± 1.15	2.4 ± 1.63	4.6 ± 2.29	1.8 ± 0.37	0.5 ± 3.92
DH51	0.0 ± 1.07	2.8 ± 1.53	5.7 ± 2.14	2.3 ± 0.35	0.01 ± 3.63

[a] "cm" refers to lesion length.
[b] mean ± standard error

3.3.3. Reaction of DH lines

A significant differences were found between the 58 DH lines for all the five disease severity parameters evaluated in this study (Table 3.2). Also, the "B+" group of the DH lines was significantly different from a subset of seven lines from the "B-" group at a single degree of freedom. Furthermore, individual

comparison of the two "B+" DH lines, DH45 and DH51,with the "B-" group, also revealed a significant differences for all five measurements (Table 2).

The "B+" lines DH45 and DH51, also showed a high level of resistance comparable to that of the species carrying the B-genome (*B. carinata*, *B. juncea* and *B. nigra*). The DH45 did not differ significantly from these resistant species for any of the five disease severity parameters, while DH51 differed significantly with respect to the severity score obtained on the 1-6 scale (Table 3). The AUDPC value for DH45 (0.5 ±3.92) was greater than that of DH51 (0.01 ±3.63); however, this difference was not significant. These two DH lines were significantly different from *B oleracea* var. *alboglabra*, *B. rapa* cv. Reward, and the two *B. napus* genotypes, 'MBX' and 'Westar' for all five disease severity parameters (Tables 3.1 and 3.3).

Table 3.3. Two tailed t-test results on differences between the individual parental lines and reference lines of different *Brassica* species for resistance to Sclerotinia stem rot.

Line	Parameter	Reward	Nigra	MBX	Westar	Juncea	JO65	O70	17-009	DH45	DH51
Albo	7 DAI	ns	**	*	ns	**	**	**	**	**	**
	15 DAI	ns	**	ns	ns	**	**	**	**	**	**
	21 DAI	ns	**	ns	ns	**	**	**	**	**	**
	score	ns	**	ns	ns	**	**	**	**	**	**
	AUDPC	ns	**	ns	ns	**	**	**	**	**	**
Reward	7 DAI		**	**	ns	**	**	**	**	**	**
	15 DAI		**	**	ns	**	**	**	**	**	**
	21 DAI		**	*	ns	**	**	**	**	**	**
	score		**	ns	ns	**	**	**	**	**	**
	AUDPC		**	*	ns	**	**	**	**	**	**
Nigra	7 DAI			**	**	ns	ns	ns	ns	ns	ns
	15 DAI			**	**	ns	ns	ns	ns	ns	ns
	21 DAI			**	**	ns	ns	ns	ns	ns	ns
	score			**	**	ns	ns	ns	ns	ns	**
	AUDPC			**	**	ns	ns	ns	ns	ns	ns
MBX	7 DAI				**	**	**	**	**	**	**
	15 DAI				*	**	**	**	**	**	**
	21 DAI				ns	**	**	**	**	**	**
	score				ns	**	**	**	**	**	**
	AUDPC				**	**	**	**	**	**	**
Westar	7 DAI					**	**	**	**	**	**
	15 DAI					**	**	**	**	**	**
	21 DAI					**	**	**	**	**	**
	score					**	**	**	**	**	**
	AUDPC					**	**	**	**	**	**
Juncea	7 DAI						ns	ns	ns	ns	ns
	15 DAI						ns	ns	ns	ns	ns
	21 DAI						ns	ns	ns	ns	ns
	score						ns	ns	ns	ns	*
	AUDPC						ns	ns	ns	ns	ns
JO65	7 DAI							ns	ns	ns	ns
	15 DAI							ns	ns	ns	ns
	21 DAI							ns	ns	ns	ns
	score							ns	ns	ns	ns
	AUDPC							ns	ns	ns	ns
O70	7 DAI								ns	ns	ns
	15 DAI								ns	ns	ns
	21 DAI								ns	ns	ns
	score								ns	ns	**
	AUDPC								ns	ns	ns
98-17-009	7 DAI									ns	ns
	15 DAI									ns	ns
	21 DAI									ns	ns
	score									ns	**
	AUDPC									ns	ns
DH45	7 DAI										ns
	15 DAI										ns
	21 DAI										ns
	score										ns
	AUDPC										ns

* is the significant level of p<0.001 and ** is the significant level of p<0.005. ns means not significant

Components of variance estimated for all parameters indicated a relatively large contribution of genotypic variance to total variance. Estimates of heritability were about 40% for disease symptom at 7, 15 and 21 DAI and AUDPC, while slightly higher heritability (54%) was estimated for the 1-6 disease severity score (Table 3.4).

Table 3.4. Components of variance and the estimates of heritability for Sclerotinia stem rot resistance in the DH lines of *B. napus* x *B. carinata* as measured by five parameters.

	7 DAI	**15 DAI**	**21 DAI**	**Score (1-6)**	**AUDPC**
Heritability	40%	37%	38%	54%	40%
Var (line) [a]	7.4134	14.4776	26.3912	1.3752	90.8393
Var (test)	8.6646	18.0141	36.6033	1.021	106.34
Var (line*test)	2.4072	6.507	6.5402	0.148	30.4476

[a] variance component estimates, form a mixed model in which all effect are random.

3.4. Discussion

The B-genome has been of interest to researchers as a source of traits for introgression into the AC-genome of *B. napus* (Schelfhout et al. 2006), particularly in cases when where these trait(s) are not otherwise available, e.g. blackleg resistance (Chevre et al. 1996; Plieske et al. 1998), shattering resistance (Prakash and Chopra 1988), etc. Several problems are generally associated with research on the interspecific introgression of traits, including hybrid sterility, chromosomal instability, stable introgression, and optimal expression of the trait in an alien genetic background. In the case of a trait such as resistance to *S. sclerotiorum*, which is often difficult to assess due to complex nature of this trait and pathogen (Toojinda et al. 2000), repetition of the experiment with the same genotype is essentially needed to generate reliable data.

In this study, a DH population derived from a *B. napus* x *B. carinata* interspecific cross was used. This allowed me to the repeat the inoculation experiments, taking advantage of the DH nature of the genetic material. This would not have been possible with a conventional F_2 or backcross population. To the best of my knowledge, this is the first study on the possible use of Sclerotinia resistance of *B. carinata* for the improvement of *B. napus*.

In the DH population, we found high levels of resistance in the "B+" lines, suggesting that the introgressed resistance was derived from the B-genome. Of the 58 DH lines used in this study, DH51 carried J13 and DH45 carried J13 and contained large segments of J18 of the B-genome chromosomes (Chapter 2). These two lines also showed resistance to Sclerotinia stem rot disease. However, based on the present data set, it is not possible to conclusively determine which B-genome chromosome may be conferring resistance to these two lines. Introgression of small segments of other B-genome chromosomes may have also occurred in these lines, which may not have been detectable with the chosen methods.

In addition to these two DH lines, some other DH lines possessed high levels of Sclerotinia stem rot resistance. In these DH lines, it was not possible to detect any B-genome introgression by use of the molecular marker set; therfore, introgression of Sclerotinia resistance from the C-genome of *B. carinata* cannot be ruled out. Regions of the C-genome chromosome(s) conferring resistance to this disease have also been reported in *B. napus* (Zhao and Meng 2003a; Zhao et al. 2004; Zhao and Meng 2003b; Zhao et al. 2006; Zhao et al. 2007). Further investigation on these resistant "B+" and "B-" DH lines is needed to determine which of the B and/or C-genome chromosomal regions of *B. carinata* may be conferring resistance to Sclerotinia stem rot. Based on the results of this study, it is clear from the study of various diploid and amphidiploid *Brassica* species that the species carrying the B-genome show resistance to Sclerotinia stem rot disease. However, present knowledge of the genetics of the resistance genes involved in expression of *Scletotinia* resistance is, still limited.

The frequency distribution of the DH lines for their response to *S. sclerotiorum* at 7, 15 and 21 DAI is presented in Fig. 3.2. The DH population was skewed towards the lower end for disease symptoms at 7 DAI; while at 15 DAI, the distribution of the population was almost normal. In contrast, at 21 DAI, lesion length was more pronounced than at 7 and 15 DAI, and the population

tended to fall into two distinct groups, one group with lesion length of 3-8 cm and the other group with a lesion length of 12-24 cm (Appendix 3.1).

Variation for resistance to Sclerotinia stem rot was continuous among the DH lines, reflecting polygenic control of this trait in this population (Fig 3.2). This is in accordance with the work of Zhao and Meng (2003b) and Zhao et al. (2006), who identified QTLs for this trait on 11 of the 19 *B. napus* chromosomes. Similarly, the polygenic nature of resistance to Sclerotinia stem rot has also been reported in the C-genome of *B. oleracea* (Baswana et al. 1991). A second key point illustrated in Figure 3.2 is that the optimum time for scoring for *Scletotinia* stem rot would be the third week after inoculation, when most susceptible plants have developed disease symptoms. Li et al (2006) also performed their scoring under field conditions in the third week.

The estimated broad sense heritability (H) for Sclerotinia stem rot resistance, based on the1-6 scores, was 54% (Table 4), which is quite similar to heritability of this disease estimated based on lesion length (57%) , in soybean recombinant inbred line population (Vuong et al. 2008). However, Sedun et al. (1989) reported low heritability for Sclerotinia stem lesion expression in *B. rapa* (23.9%) and *B. juncea* (27.1%). On the other hand, Baswana et al. (1991) found low to moderate heritability (35 to 57%) for *Sclerotinia* stem rot resistance in *B. oleracea*.

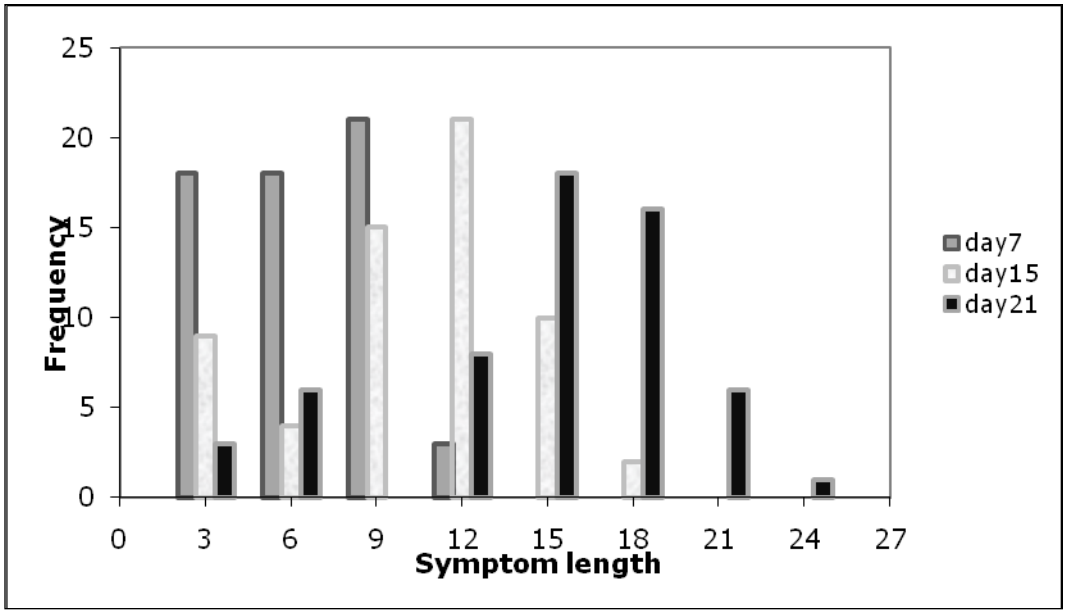

Fig. 3.2. Frequency distribution of the DH lines for Sclerotinia stem lesion length measured at 7th, 15th and 21st days after inoculation (DAI). (Note: Mean lesion length for *B. napus* cv.Westar is 8.3, 13.9 and 19.2 cm at 7, 15, 21 DAI, respectively, and 0.1 cm at all three dates for the *B. carinata* line 98-17-009).

While t-test comparisons for symptom length at 7 DAI to 21 DAI are very similar, our reference lines had different and variable AUDPC values, which might suggest that these lines have different mechanisms of defense against the disease.

In conclusion, the results of this study provide evidence of the utility of the Sclerotinia stem rot resistance from *B. carinata* for the improvement of canola *B. napus*. The resistant DH lines identified in this study would be excellent material for understanding the *Brassica* B- and C-genome resistance and for efficient exploitation of these resistance genes in development of resistant canola cultivars. In this context, further research on the identification and mapping of B-genome-derived resistance genes in the *B. napus* background would be of particular interest.

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4. CHARACTERIZATION OF B-GENOME CHROMOSOMES IN AN INTERSPECIFIC CROSS BETWEEN *Brassica napus* × *B. carinata* AND THEIR ASSOCIATION WITH MORPHOLOGICAL TRAITS

4.1. Introduction

Interspecific crosses have been widely used to introgress desired traits into specific genetic backgrounds and to increase genetic diversity. However, they are not always successful. Factors such as limited chromosome alignment, low rates of recombination, and linkage drag may make it difficult to introgress the trait and may also result in the introgression of undesirable traits along with the trait of interest (Brown et al. 2003; Desloire et al. 2003).

Several tools are available to assess interspecific hybrids and to detect the introgression of traits from different species into the Brassica. The morphology of leaves, flowers, pods, etc. has been successfully used to distinguish the species of *Brassicaceae* (Gomez-Campo 1980), but may be ambiguous for the F_1 hybrids, which are often similar to either of the parents or intermediate between them, depending on the trait. In a backcross generation population derived from a wide cross, morphological traits can be used as markers to follow the introgression of gene(s) from one species to another. Cytogenetic markers, e.g. chromosome banding in wheat (Gill and Kimber 1977) and techniques of fluorescent *in situ* hybridization (Wang et al. 2006) may be used to visualize the genomic constitution of the hybrids and meiotic behavior of the chromosomes (Attia and Röbbelen 1986; Heneen and Jorgensen 2001). These markers can also be used to identify genomic regions in common between closely related species and to detect introgressed segments from wild relatives (Chevre et al. 2004). A third way to detect introgressed segments is to use molecular markers.

Today, a large number of DNA-based molecular markers are available for the Brassica genomes (A, B, and C), which can be used for detecting interspecific

hybrids and genetic introgressions. DNA-based molecular markers have been used in recent years for tagging simple Mendelian traits, as well as for mapping quantitative trait loci (Mahmood et al. 2006; Mahmood et al. 2007). The steps involved in QTL mapping include identifying the significant QTLs, positioning them in the genome, and exploring the effect of different QTL combinations (Darvasi 1998). Mapping of quantitative traits in populations derived from interspecific crosses can be very complex due to segregation distortion (Lorieux et al. 2000). In this chapter, QTL analysis was performed on a number of interspecific families derived from a BC_3 population using marker regression for a number of agronomic traits.

4.1.1 Background history of the plant material

The material used for this study was originally developed by Kiersten Stead in the laboratory of Dr. Allen Good, and is described in more detail by Stead (2009). In brief, an interspecific cross was made between *B. napus* line MBX and *B. carinata* line BCA-070 with the initial objective to transfer blackleg resistance from *B. carinata* into *B. napus*. The line MBX is an artificially synthesized *B. napus* derived from the cross between *B. rapa* × *B. oleracea*. This line was chosen based on the observation that it undergoes reduced chromosome pairing and, therefore, it might undergo more frequent non-homologous pairing in interspecific hybrids than a natural *B. napus* (Derek Lydiate; pers. comm.). The *B. carinata* line BCA-070 was received from Dr. Phil Salisbury (University of Melbourne, Australia), which is known to possess several desirable agronomic and disease resistant traits e.g. blackleg resistance (Purwantara et al. 1998). Ten F_1 plants of a *B. napus* × *B. carinata* cross were backcrossed to *B. napus* as the recurrent parent. The hybrid nature of these F_1 plants was confirmed by Southern hybridization and RFLP analysis (Stead 2009). The resultant BC_3 families and their self-pollinated generations were used in this study to generate BC_3S_1 Introgressed Families (IF) (Fig.4.1).

4.1.2 Objectives

The objective of this study was to analyze the BC_3S_1 introgressed families (IF) derived from the above mentioned interspecific cross, and to study the B-genome chromosomes and their association with morphological traits. The following investigations were made:

a) Characterize the introgression and inheritance of the B-genome chromosomes using B-genome molecular markers.
b) Visualize the introgressed B-genome using fluorescent *in situ* hybridization.
c) Determine the effect of the introgressed B-genome chromosomes on different morphological traits.

4.2 Materials and Methods

4.2.1 Plant material

Seeds from seventeen BC_3 S_1 families of the MBX × BCA-O70 cross were selected and used in this study. These BC_3S_1 families were selected based on the B-genome chromosome content of their corresponding BC_3 plant, as had been characterized by 400-500 microsatellite markers (Stead 2009). It should be noted that the B genome chromosomes will segregate in these interspecific families, since the B chromosome would only occur as a single copy, due to the fact that this is an interspecific backcross.

Twelve plants of each of the seventeen BC_3S_1 families and their parents were planted in Metro Mix® 290 (Grace Horticultural Products, Ajax, Ontario, Canada) and grown in a growth cabinet at 21°/18°C (day/night) with a 16 hour photoperiod. Fertilization was done every second week with 200 ppm Peres 20-20-20 (N-P-K) (Plant Products Inc. Brampton, Ontario, Canada). Plants were used for the evaluation of morphological traits as described below. The experiment was repeated once in the growth cabinet under the same environmental conditions.

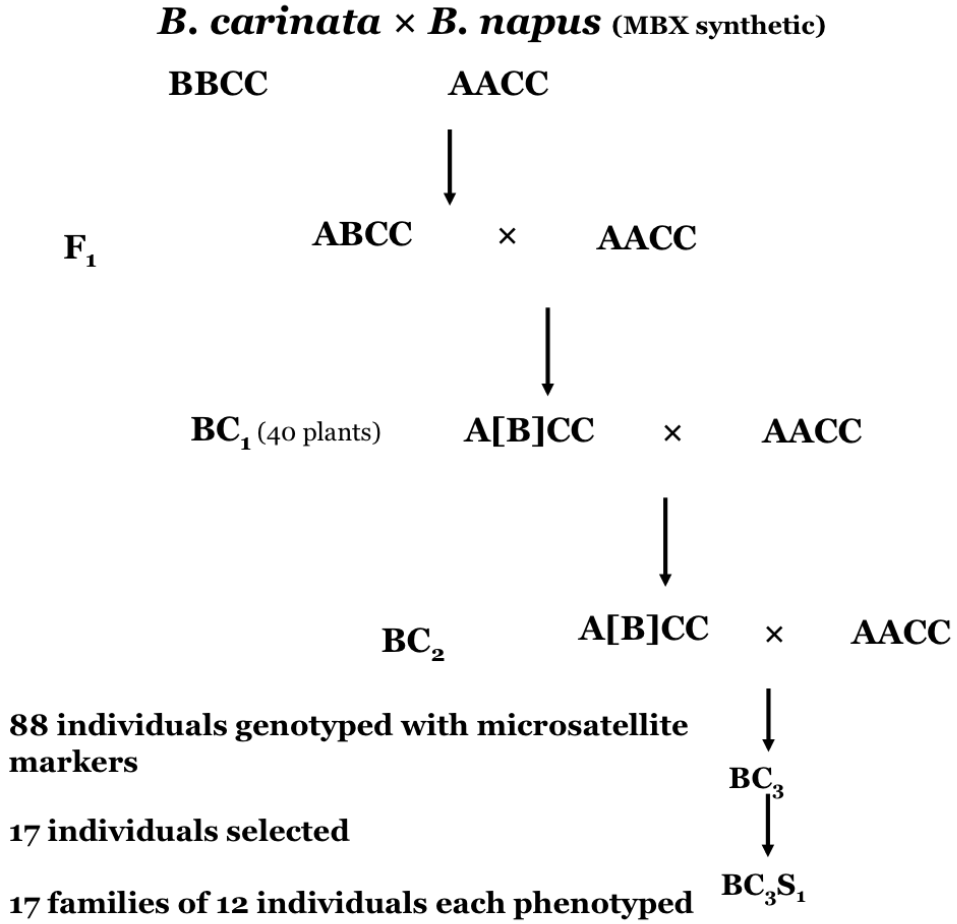


Fig. 4.1. Pedigree of the plant material used in this study. The crossings and development of the BC_3 families was done by Stead (2009).

4.2.2 Data recording

The following morphological traits were measured on 12 plants of each of the 17 BC_3S_1 families using the descriptors for *Brassica* (IBPGR 1990) with minor modifications.

At the three-leaf stage:

- Cotyledon retention (CR). A score of 3 indicates that the seedling dropped the cotyledon early, a score of 5 was assigned when the senesced cotyledon was not dropped, and a score of 7 indicates that the cotyledon was green and attached.

- Incision or leaf division (ILD) was scored 1 when the leaf had no division, 3 when the leaf divided moderately, and 5 when the leaf was deeply divided.
- Margin leaf division (MLD) was scored 0 when the margin had no divisions, 1 when the divisions were round, and 2 when divisions were sharp.

At flowering:

- Number of days to flowering (DTF) was measured when the main branch bloomed.
- Flower color (FC) was scored 1 as yellow, 2 as yellowish-white, and 3 as white.
- Stem color (STC) scored 1 when green, 3 when purplish-green, and 7 when it was purple.
- Stem length (STL) was measured when plant started to flower.
- Number of primary branches (NB) was measured at the flowering stage.

At ripening:

- Silique length (SL), silique width (SW), and beak length (BL) were measured at full maturity stage.
- Number of days to maturity (DTM) was measured when three quarters of the plant was dried; and counted as number of days from flowering to maturity.
- Seed color (SC) was scored 1 when yellow to brown, 3 when brown, 5 when dark brown to black, 7 when black.
- Number of seeds per silique (NSS) was measured at the maturity stage.

4.2.3 Marker analysis

Leaf tissues were collected from 11 plants from each of the 17 BC_3S_1 families at the 4-5 leaf stage, and DNA was extracted using Sigma's GenElute^{TM} Plant Genomic DNA Miniprep Kit (Sigma Chemical Co., St. Louis, MO).

Six BC_3S_1 families (IF) were selected based on their segregation of different morphological traits and used for molecular marker analysis using the microsatellite (SSR) marker set developed at Agriculture and Agri-Food Canada (AAFC), Saskatoon Research Station. From a total of 1242 B-genome microsatellites, 103 markers for the linkage groups J13, J15, J16 and J18 were selected for use in this study. The rationale for choosing these markers was that Kiersten Stead found that these four B-genome chromosomes were present in the parental BC_3 plants of these six BC_3S_1 families (Stead, 2009) . The other two key criteria for the selection of markers were their ability to amplify strong bands as evident from earlier mapping projects, and the fact that these markers had been used for mapping in other populations and therefore would provide the opportunity to cross reference the data set from this research with other map data. To prepare the PCR reaction, forward and reverse primers along with the genomic DNA were added to the Amplitaq Mastermix. The PCR products, labeled with three different fluorescent dyes, were loaded on to an ABI 3700 (Applied Biosystems Inc.) DNA Analyzer, and were analyzed using Fragment Profiler® (MegaBACE^{TM}) software. The details of the molecular marker analysis, including the PCR assay and sequencing protocol, is described in Chapter 2.

4.2.4. GISH assay

Flower buds from the six selected IF families that were used for marker analysis, were used for GISH/FISH analysis using *B. nigra* genomic DNA and a 45S DNA probe. Immature flower buds collected from two to three plants of each family were used for mitotic and meiotic chromosome spreads. Slides were prepared following enzyme maceration (Kato et al. 2004). *Brassica nigra* and *B. oleracea* genomic DNA and repeated sequences were labeled fluorescently (Chapter 2). A 45S DNA clone was also used as a probe to detect Nuclear Organizing Regions (NORs). Fluorescent *in situ* hybridization was performed following the method of Kato *et al*. (2004) with slight modifications, as described in Chapter 2.

4.2.5. Statistical analysis

Morphological data collected from the two tests were used to perform the statistical analysis. A marker regression approach (Kearsey and Hyne 1994) was used to detect the association of the B-genome chromosome(s) with each trait. Analysis of variance was performed using the PROC MIXED command of SAS® software, followed by "LSmean" and "boxplot" statements to calculate the statistics of the population (SAS Institute Inc. 1989). The null hypothesis of no effect of the B-genome chromosome on the trait was tested. In the simplest linear model of regression, the phenotypic value of individual *j* (Z_j) is a function of mean value (μ), effects (b_i) of different chromosomes (x_{ij}) on the phenotype and residual error (e_j) following the model:

$$Z_j = \mu + \sum b_i x_{ij} + e_j$$

When two or more markers are considered, the effect (b_i) corresponds to the multi locus marker genotype; the evidence of a linked QTL is provided by a significant R^2, which is the fraction of phenotypic variance accounted for by the marker genotype (Lynch and Walsh 1998). In addition, the segregation of the B-genome chromosomes for fit to a 3:1 ratio was tested by the χ^2 test, using the following formula:

$$\chi^2 = \sum (O_i - E_i)^2 / E_i$$

O_i is the observed number of the chromosome of either B+ or B- class in each family and E_i is the expected value under a segregation ratio of 3:1 assuming that the chromosomes are segregating in a Mendelian manner.

4.3 Results

4.3.1 Distribution of morphological traits

Significant differences ($P < 0.01$) as revealed by the ANOVA test were observed between families (IF) for the following traits: cotyledon retention, leaf incision, blade blistering, days to flowering, stem color, stem length, number of branches, silique length, silique width, days to maturity, and stem color (Table

4.1). For days to flowering (DTF), the family IF-72 with no B-genome chromosome content had the earliest flowering, with a mean value of 28.9; while the family IF-29, carrying three B-genome chromosomes, had the greatest number of days to flowering with a mean of 41.1. For days to maturity, the family IF-1, in which no B-genome chromosome could be detected, was the latest to mature with a mean value of 79.4 days to maturity; while the family IF-30, carrying J16, was the earliest maturing one, requiring an average 65.0 days to mature (Table 4.1). For stem color, the family IF-1, with no B-genome chromosomes, had the greenest color; while the families IF-29 and IF-30 had the highest value (3.3) with green purple stem color (Table 4.1). The family IF-81, carrying J13, was the tallest, with a mean height of 82.2 cm, while the family IF-72 had the lowest height (58.8 cm) among the introgressed families (Table 4.1).

The distributions of the BC_3S_1 plants in each of the 17 families for different morphological traits are presented as boxplots in Figure 4.2. Boxplots are useful for comparing distribution between several sets of data because they show the variation for the trait in each family (IF) and also show the mean, median, maximum and minimum values of the population. For qualitative traits such as leaf division, flower color, and seed color, there was little diversity within the families, and therefore this data is not presented.

Among the two ripening related traits, the number of days to flowering showed much less variability, with 50% of the data points within the families being closer to the median, compared to that of days to maturity, where significantly greater variation was observed (Fig. 4.2). Similar comparisons apply for silique width, which generally had less variability within the families compared to silique length (Fig. 4.2). The families IF29 and IF30 were the two main outliers for stem color, while IF115 and IF7 were the outliers for the number of primary branches.

Table 4.1. Analysis of variance and least square mean values for different morphological traits in the BC_3S_1Introgressed Families (IF). The B-genome chromosome content of these families is also given**.**

	B chromosome content	CR[1]	MLD[2]	ILD[3]	DTF[4]	STC[5]	FC[6]	STL[7]	NB[8]	SL[9]	SW[10]	BL[11]	DTM[12]	SC[13]	NSS[14]
Family		**	ns	**	**	**	ns	**	**	*	**	ns	**	**	ns
Test		**	.	**	**	**	.	**	**	**	.	**	**	.	.
Family*test		**	.	**	**	**	.	**	**	ns	.	ns	**	.	.
IF-1	**None**	5.5	0.9	2.2	32.7	1.5	1.4	67.5	4.6	24.8	0.4	10.6	79.4	6.5	2.7
IF-7	**Non**	5.0	1.0	2.2	38.2	2.3	1.4	63.6	5.5	17.4	0.3	7.8	72.4	6.0	1.1
IF-61	**None**	4.8	1.2	2.4	33.3	2.8	1.2	79.7	4.8	19.9	0.3	8.8	71.6	6.6	0.4
IF-72	**None**	3.4	1.3	2.2	28.9	2.3	1.0	58.8	3.5	23.3	0.3	8.0	67.8	4.9	1.7
IF-80	**None**	6.0	1.1	2.2	39.6	2.6	1.2	78.5	3.6	24.5	0.4	10.2	71.6	6.7	1.0
IF-13	**J13**	5.2	0.9	2.2	40.0	1.9	1.7	65.5	4.5	17.4	0.3	9.1	72.5	5.0	1.6
IF-42	**J13**	4.3	0.9	2.3	32.4	2.4	1.5	64.6	4.6	16.6	0.3	8.4	69.3	5.8	1.0
IF-81	**J13**	5.9	1.3	2.1	33.9	3.0	1.2	82.2	4.1	21.6	0.3	8.4	73.0	7.0	0.5
IF-30	**J16**	5.8	1.3	2.4	31.6	3.3	1.8	80.0	4.3	23.4	0.4	12.8	65.0	6.8	1.2
IF-38	**J16**	4.1	0.8	2.1	35.8	2.5	1.4	71.1	4.4	20.9	0.4	10.5	67.7	5.8	0.6
IF-52	**J16**	4.5	1.3	2.3	35.1	2.2	1.3	71.0	4.6	21.4	0.3	8.2	69.8	6.1	1.4
IF-20	**J16**	4.5	1.3	2.1	36.9	2.4	1.4	67.5	4.3	21.4	0.4	9.9	73.8	7.1	0.1
IF-103	**J17**	5.0	1.0	2.1	35.5	2.6	1.2	66.1	4.0	20.1	0.3	10.3	72.7	5.9	1.3
IF-115	**J17**	3.8	0.8	2.1	30.8	2.0	1.7	69.8	5.8	18.3	0.3	7.4	70.1	6.8	1.3
IF-174	**J18**	4.5	0.8	2.2	37.4	2.2	1.7	67.8	4.8	21.9	0.4	10.2	70.5	6.3	1.5
IF-15	**J13/J18**	4.4	0.7	2.0	35.4	2.0	1.3	72.0	4.1	17.7	0.5	9.1	73.5	7.1	0.0
IF-29	**J13/J17/J18**	4.3	1.3	2.3	41.1	3.3	1.2	78.7	4.0	18.6	0.3	10.2	74.8	6.4	0.4
MBX *B. napus*	**None**	7.0	.	1.7	35.3	1.0	2.3	87.2	4.5	3.6	0.4	1.1	83.4	.	6.4
O-70 *B. carinata*	**All**	3.0	.	1.0	45.0	7.0	1.0	126.6	8.6	3.3	0.7	0.4	78	.	13.6

[1] cotyledon retention (CR), margin leaf division (MLD), [3] incision of leaf division (ILD), [4] days to flowering (DTF), [5] stem color (STC), [6] flower color (FC), [7] stem length (STL), [8] number of primary branches (NB), [9] silique length (SL), [10] silique width (SW), [11] beak length (BL), [12] days to maturity (DTM), [13] seed color (SC), and [14] number of seeds per silique (NSS).

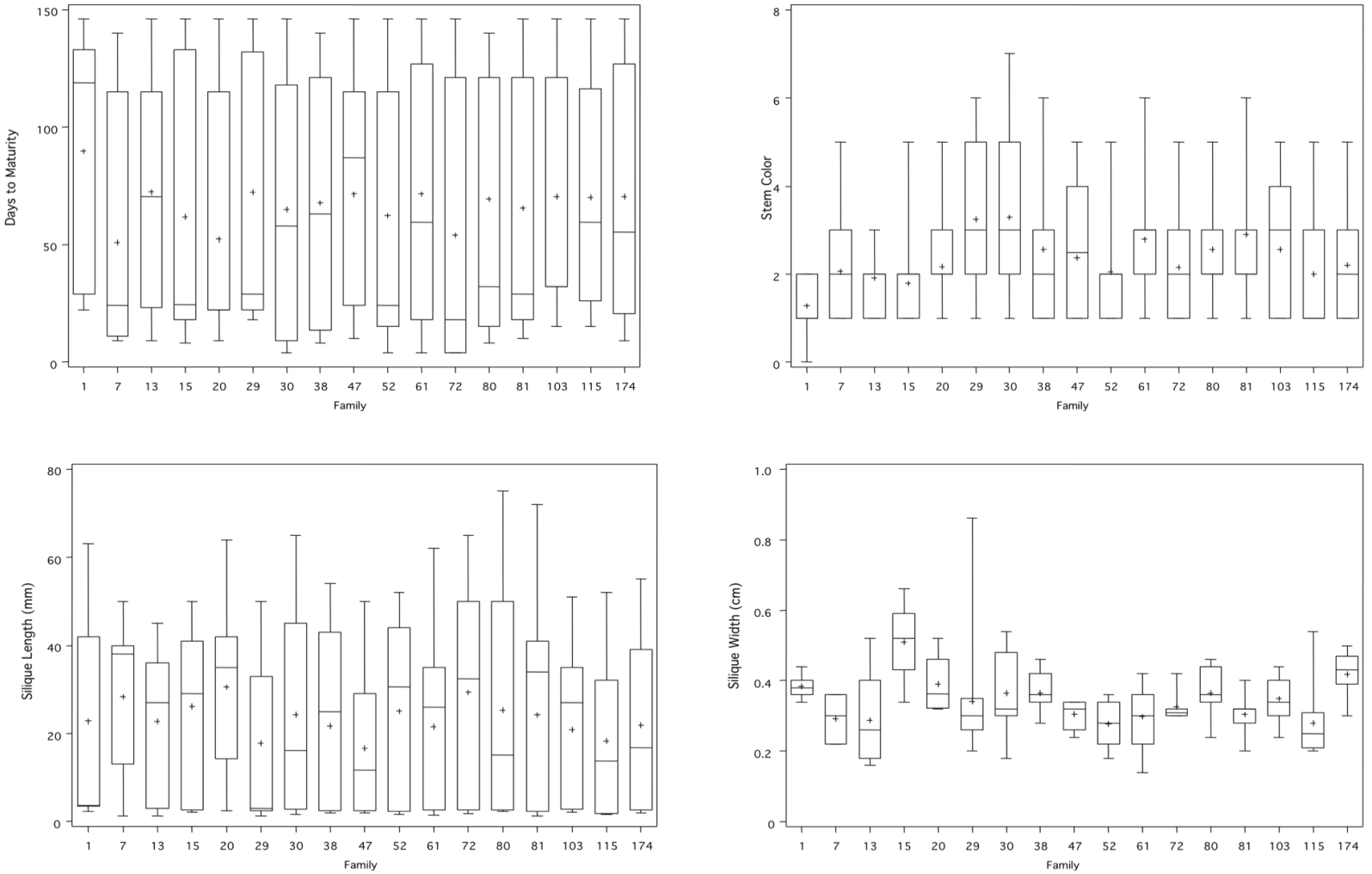
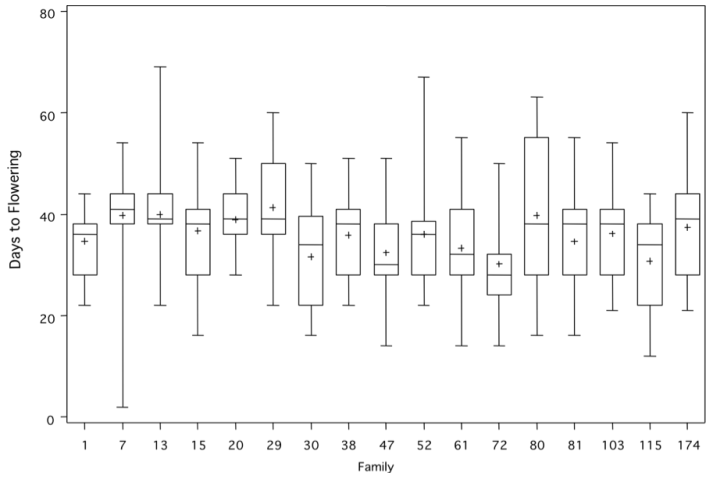
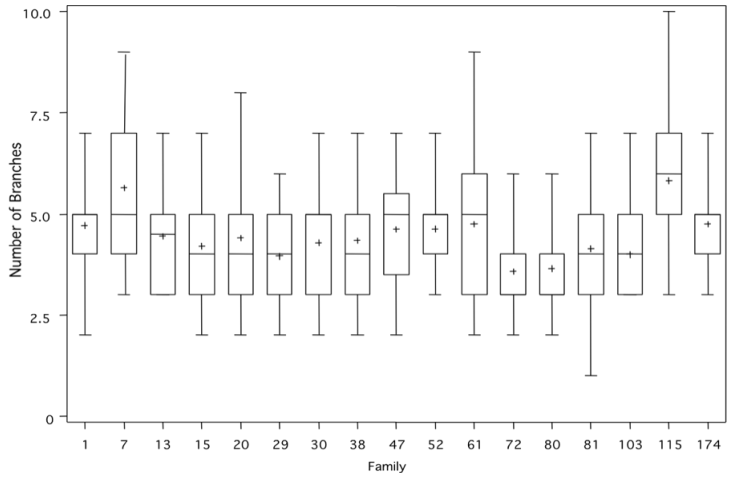
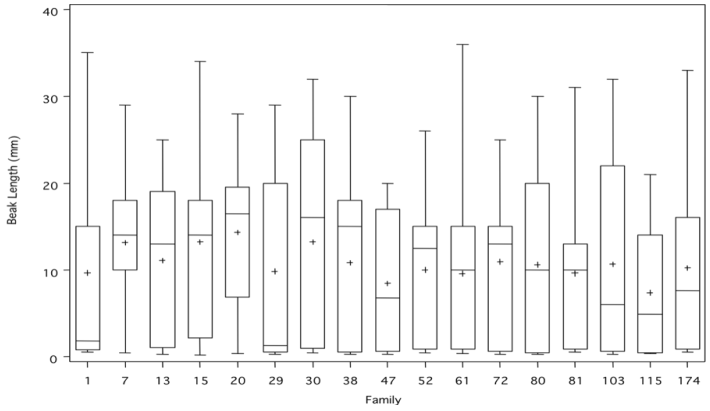


Fig 4.2. Boxplots showing the variation for different morphological traits in 17 BC_3S_1 Introgressed Families (IF). Maximum, minimum and median values are marked. Each box represents the interquartile range, which contains 50% of the values. The whiskers are lines that extend from the box to the highest and lowest values, excluding outliers. The solid line across the box indicates the median. The mean value is indicated by '+' sign.







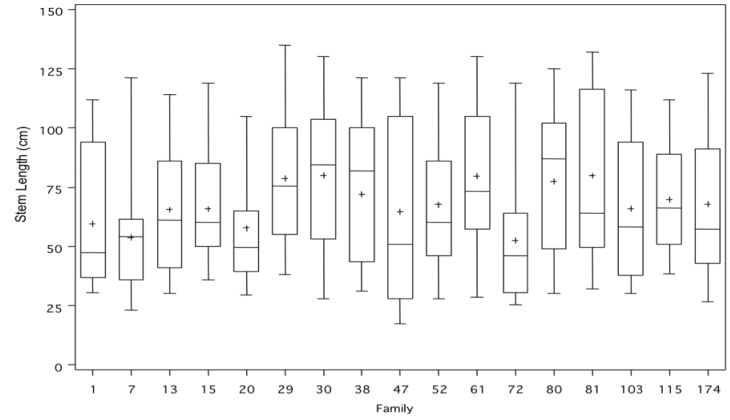


Fig 4.2. (continued)

4.3.2 Microsatellite analysis of BC_3S_1 families

The genomic location of the alleles amplified by the 103 B-genome specific SSR markers for the B-genome linkage groups in the present material was assigned based on a panel of DNA from the control plants. The details of this are explained in Chapter 2. It was found that most of the B-genome chromosomes in BC_3S_1 plants tended to be inherited as intact linkage groups (Fig. 4.3). However, loss of a terminal segment or translocation was detected in several cases (Figure 4.3). In this Figure, the different B-genome chromosomes (either intact or as specific segments) segregating in the families are illustrated. For example, the family IF-42 carried only the terminal segment of J17, while it was present as a whole chromosome in IF-29, IF-103, and IF-115 (Fig. 4.3). Similarly, the terminal segment of J13 was present in IF-103, while the full chromosome J13 was present in IF-29 and IF-42 (Fig. 4.3). The chromosome J18 was found to exist as a whole chromosome in IF-174; no deletions of this chromosome appeared to have occurred in this family (Fig. 4.3).

In case of the BC_3S_1 families, where B-genome chromosome(s) were detected, logically the BC_3 female plant would have been carrying one copy of these chromosomes. Assuming Mendelian segregation and no differential selection on the male or female gametes in these BC_3 plants, it is expected that one quarter of the BC_3S_1 plants would carry two copies of the extra chromosome, one half would carry one copy, while one quarter of them would be missing this extra chromosome. Based on SSR marker data it was not possible to differentiate between the BC_3S_1 plants carrying two or one copy of a given B-genome chromosome, therefore a 3:1 segregation ratio for the presence or absence of the B-genome chromosome was tested in the BC_3S_1 families.

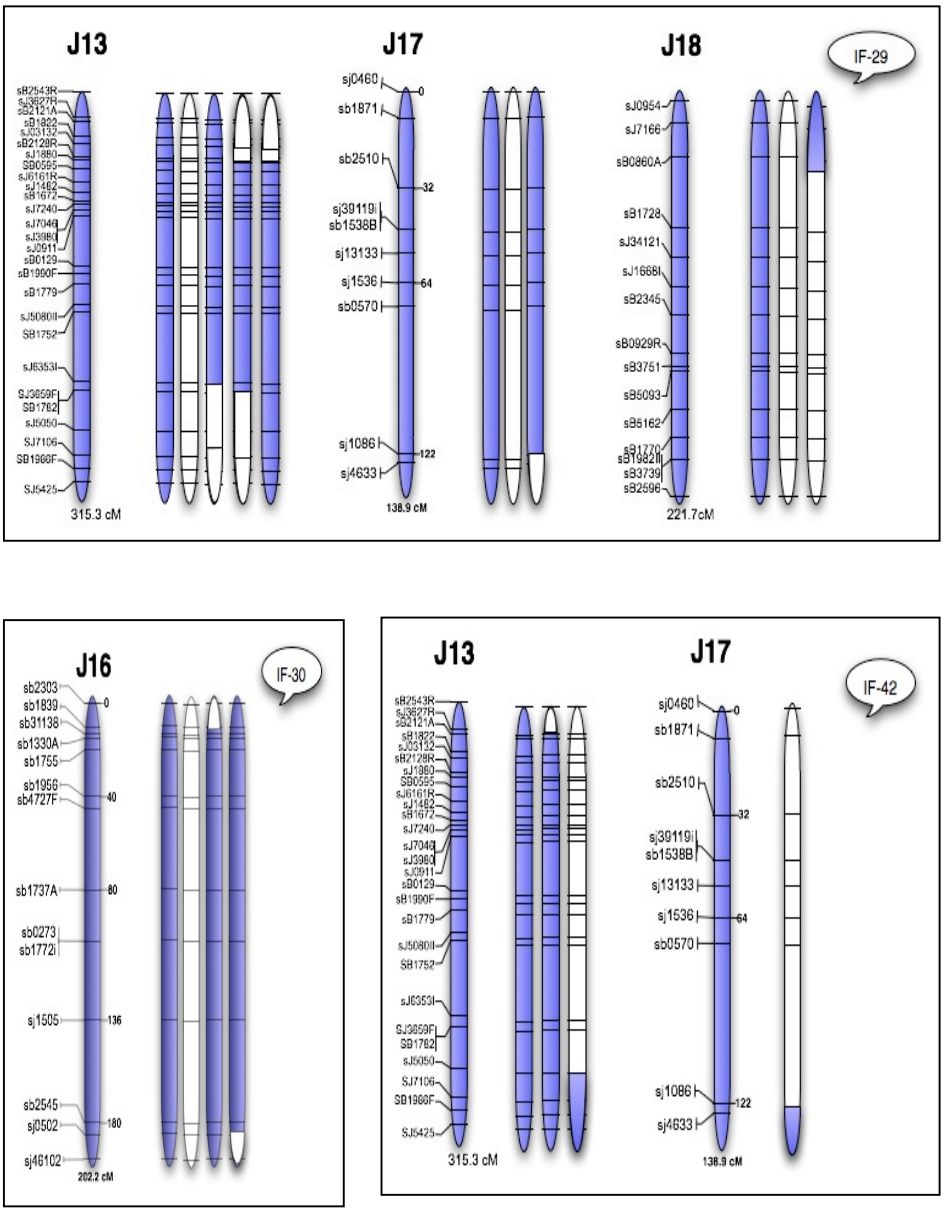


Fig 4.3. Physical representation of the different types of B-genome linkage groups observed in the 11 individuals of BC_3S_1 families. Location of the markers selected on the four B-genome linkage groups is based on the AAFC reference maps. Shaded parts represent the presence of the segment and white segments represent its absence.

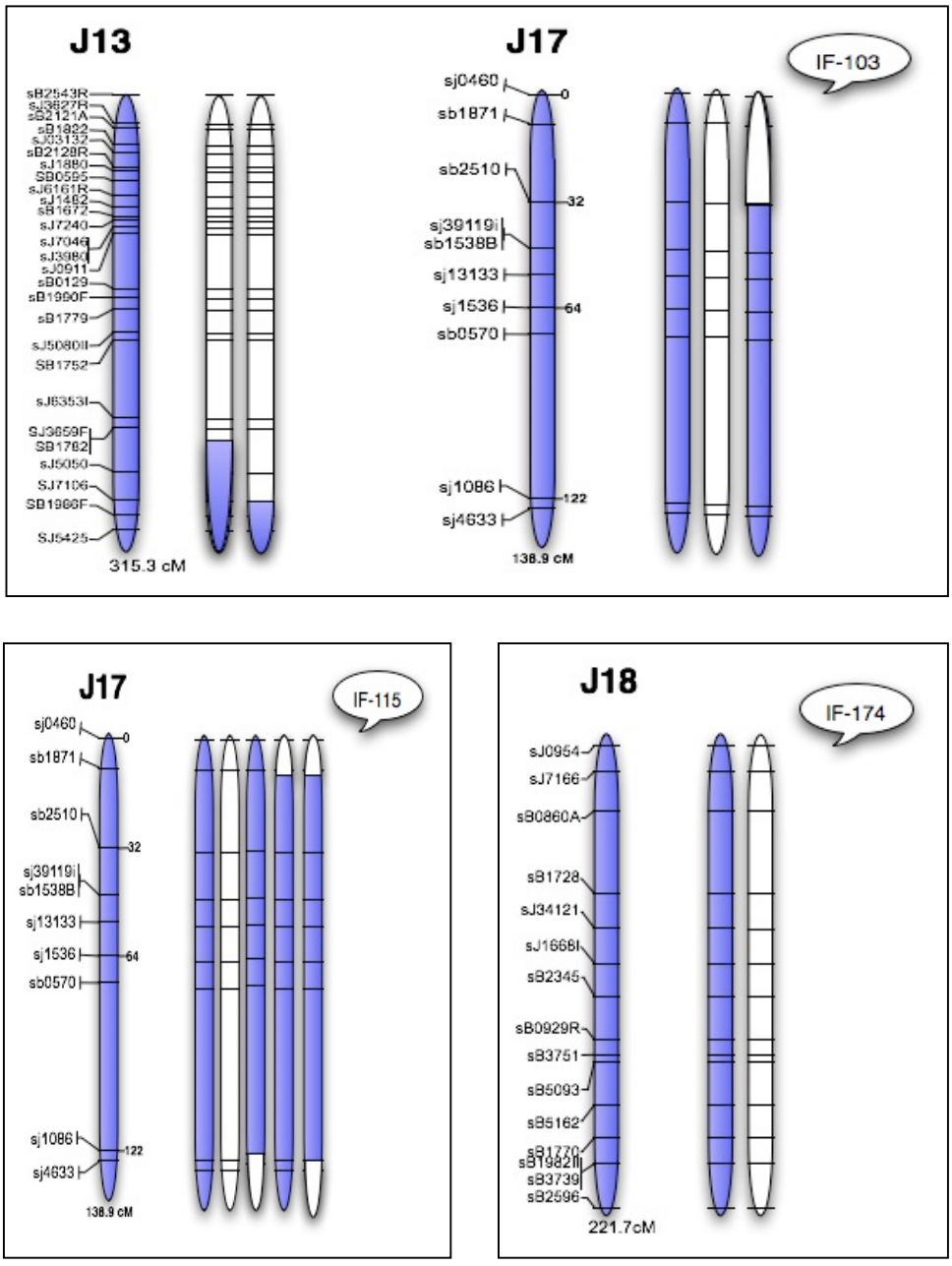


Fig 4. 3. (continued)

The transmission rate of B-genome chromosomes from the BC_3 to the BC_3S_1 generation was found to vary depending on the chromosome (Table 4.2). For example, eight of the 11 plants of the family IF-30 inherited J16, displaying a 3:1 segregation ratio. In contrast, six of the 11 plants of the family IF-29 inherited the chromosome J18, which clearly deviated from a 3:1 segregation (Table 4.2). From this Table, it can be observed that J13 and J16 segregate in a Mendelian fashion, whereas J17 and J18 are clearly selected against. Moreover, the segregation of the B genome linkage groups is not determined by the specific line (at least based on IF-29), although clearly this is too small a sample to provide any more general conclusions.

Table 4.2. Chi-square (χ^2) test of the segregation for the B-genome chromosomes in Introgressed BC_3F_2 Families (IF).

	B-genome linkage group present	**B-genome linkage group absent**	**N**	$\chi^2_{3:1}$	**p-value df=1**
IF-29					
J13	7	4	11	0.76	0.39
J17	3	8	11	13.36	<0.005
J18	5	6	11	5.12	0.025
IF-30					
J16	8	3	11	0.03	0.86
IF-42					
J13	10	1	11	1.48	0.25
IF-103					
J17	3	8	11	13.36	<0.005
IF-115					
J17	3	8	11	13.36	<0.005
IF-174					
J18	2	9	11	18.94	<0.005

4.3.3 GISH assay

Genomic DNA from *B. nigra* was used to detect the B-genome chromosome content in the BC_3S_1 families (Fig. 4.4). Based on the molecular marker data, it was possible to predict the number of B-genome chromosomes introgressed into the BC_3S_1 segregating families. The GISH technique allowed visualization of the B chromosomes in these materials. Based on the presence of B-genome specific alleles using molecular markers, it was determined that the BC_3S_1 family IF-29, as well as its corresponding BC_3 parent, carried the chromosomes J13, J17 and J18. GISH analysis on three plants of this family also showed one or two signals from the *B. nigra* chromosomes (Fig 4.4a, b and c), with chromosome counts of 2n=37+1B, 2n=38+2B or 2n=38+1B, (Table 4.3). The plant IF-29-2 of this family was found to carry 37 AC chromosomes (2n=37+1B), apparently due to loss of an A- or C-genome chromosome. However, this plant gained a B-genome chromosome, which makes the total number of its chromosomes 38. In the case of the IF-30, signals from J16 were expected to be observed, as this family was segregating for this chromosome. GISH assay supported this with a chromosome count of 2n=38+1B (Fig 4.4d) in one of the three plants, while the other two plants had 2n=40 chromosomes. However, none of these chromosomes displayed the red signal from the B-genome chromosome, indicating that these plants might have gained an A- and/or C-genome chromosome due to abnormal chromosome segregation (Table 4.3 and Fig 4.4e and h). The family IF-42 segregates for both J13 and J17 (Table 4.3). One of the two plants of this family produced three signals specific to the B-genome chromosomes (2n=39+3B). This is probably due to the presence of a broken B-genome fragment (from J13 or J17) in this plant, which I was able to detect by SSR markers (Fig 4.4g). The other plant of this family carried 2n=36 chromosomes and produced only faint signals from small segments of the B-genome chromosomes (Fig 4h and Table 4). The two plants of IF-103 carried J17, as only one red signal was observed using the *B. nigra* genomic DNA, and this plant had 37 AC-genome chromosomes. This family apparently lost two AC-

genome chromosomes and gained a B-genome chromosome (Fig 4.4i and Table 4.3).

In case of the family IF-115, two B-genome chromosomes, J13 and J17, were expected to be observed. The GISH assay displayed two signals in one of the three plants, presumably from two B-genome chromosomes, and had a chromosome count of 2n=38+2B (Fig 4.4j). The second plant carried 37 chromosomes (Fig 4.4k) with signals from either J13 or J17. This plant was missing two A- or C- chromosomes with the addition of one B chromosome. The third plant of this family had 2n=36 chromosomes and did not display any red signal, indicating that this plant was missing two A or C chromosomes (Table 4.3). Based on molecular marker data, it was expected that the chromosome J18 would be present in some of the plants of IF-174. However, no B-genome chromosome signal was detected in the three plants studied from this family (Fig. 4.4m, n and o). These plants had chromosome numbers of 2n=36, 37 and 38 (Table 4.3), indicating that two of these plants have lost an A or C chromosome.

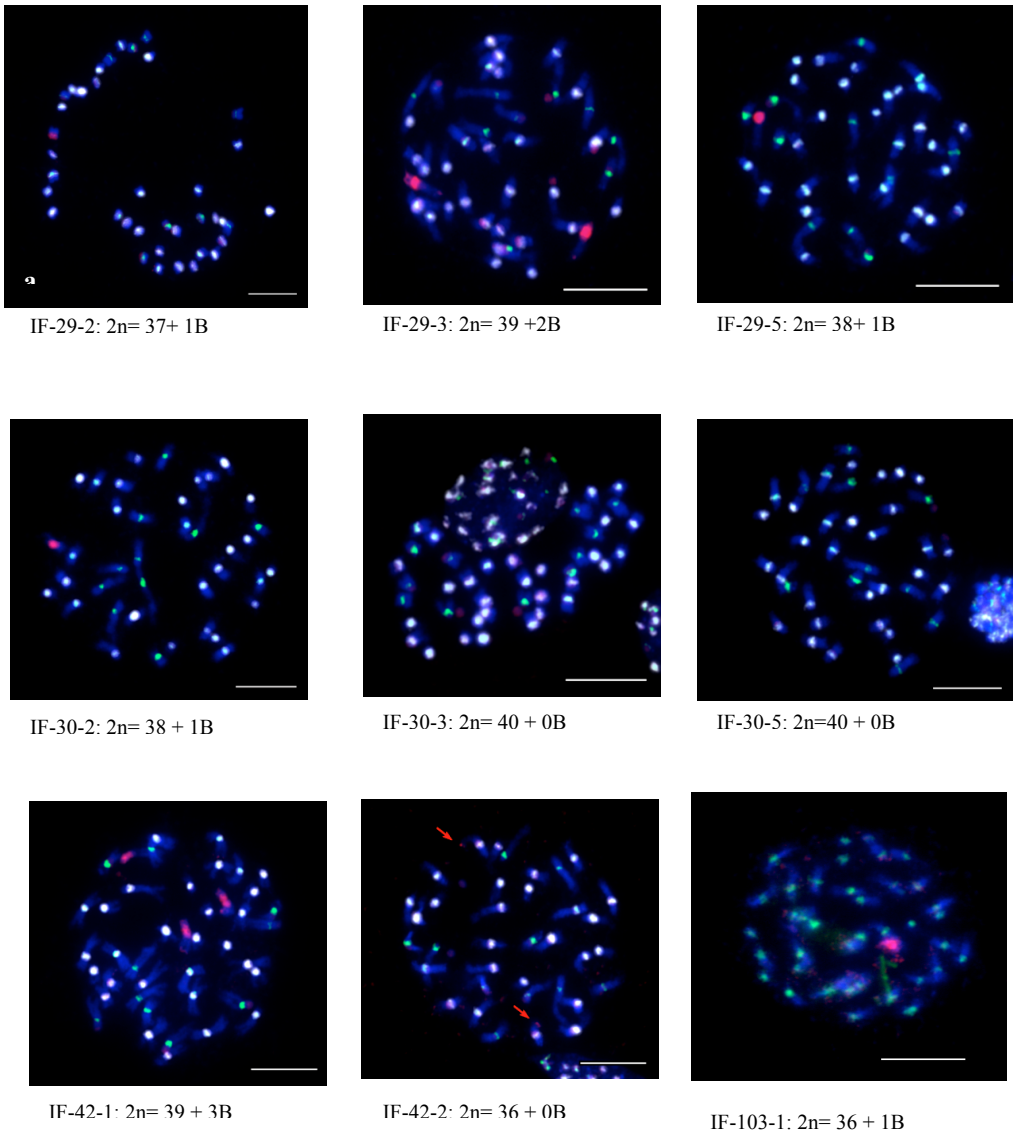
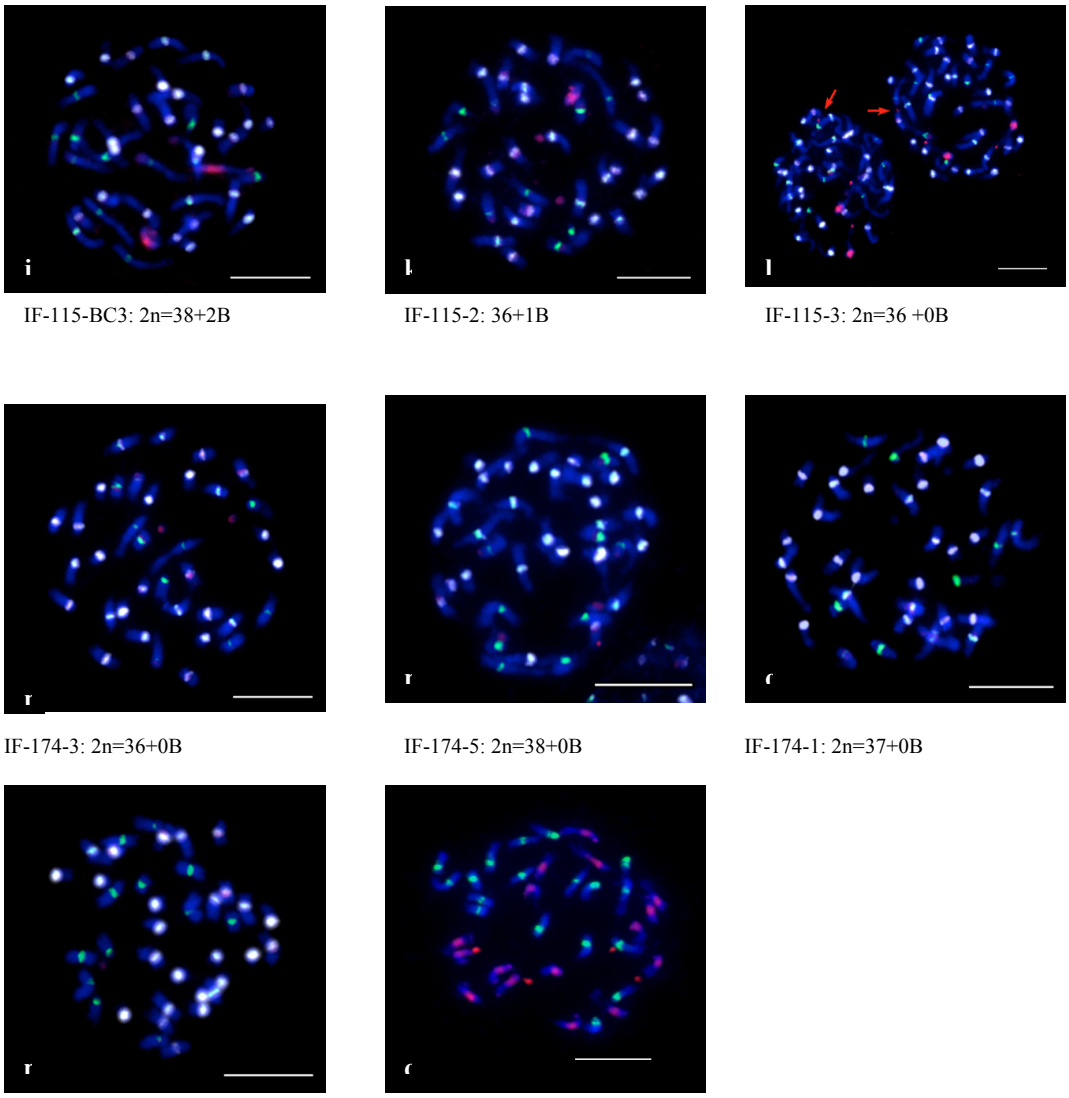


Fig 4.4. Chromosome painting at late prophase II with 60X magnification, using the GISH technique. *B. nigra* genomic DNA is fluorescently labeled in red, *B. oleracea* genomic DNA is labeled green and the 45S DNA is labeled white. Arrows show chromosomal fragments. Each bar equals to 10 µm.



IF-115-BC3: 2n=38+2B

IF-115-2: 36+1B

IF-115-3: 2n=36 +0B

IF-174-3: 2n=36+0B

IF-174-5: 2n=38+0B

IF-174-1: 2n=37+0B

B. napus (AACC)2n=38

B. carinata (BBCC) 2n=34

Fig. 4.4. (continued).

Table 4.3. Summary of GISH assay for individual plants of the five selected BC_3S_1families. Expected B-genome chromosome based on SSR marker analysis, total chromosome number and observed number of *B. nigra* genomic signals are presented.

BC_3S_1 plant	**Expected B-genome chromosome**	**Number of red GISH signals**	**Total number of chromosomes**
IF-29-2	J13/J17/J18	1	38
IF-29-3	J13/J17/J18	2	40
IF-29-5	J13/J17/J18	1	39
IF-30-2	J16	1	39
IF-30-3	J16	0	40
IF-30-5	J16	0	40
IF-42-1	J13/tip of J17	3	42
IF-42-2	J13/ tip of J17	Fragments	36
IF-103-1	J17	1	37
IF-103-2	J17	1	37
IF-115-1	J17	2	40
IF-115-2	J17	1	37
IF-115-3	J17	0	36
IF-174-1	J18	0	37
IF-174-3	J18	0	36
IF-174-5	J18	0	38
Westar	None	0	38
Carinata	All	8	34

4.3.4 QTL analysis

The alien B-genome chromosome(s) in the AC-genome background of the present material were found to have significant effects on cotyledon retention, leaf margin, leaf incision, days to flowering, stem color, flower color, stem length, beak length and days to maturity. The R^2 values showing the amount of variation explained by these B-genome chromosomes are presented in Table 4.4.

The B-genome chromosome J13 was found to have a significant effect on leaf margin, explaining 16% of the phenotypic variation. It also had a significant effect on stem color and stem length, explaining 5% of the total phenotypic variation for either of these traits (Table 4.4). The chromosome J16 explained 22% of the total phenotypic variation for cotyledon retention and also had a

significant effect on stem color and flower color, explaining 6% and 8% of the phenotypic variation, respectively (Table 4.4). While J17 explained 23% of the variation for leaf margin, this chromosome also had significant effect on leaf incision, flower color and beak length, explaining 8%, 6% and 5% of the variation, respectively (Table 4.4). The only chromosome having significant effect on days to flowering was J18, which explained 8% of the total phenotypic variation. This chromosome also explained 6% and 8% variation for flower color and days to maturity, respectively (Table 4.4).

Table 4.4. Effect of the B-genome chromosomes on different morphological trait. R^2 values explain the amount of phenotypic variation due to the B-genome chromosome**.**

		CR[1]	MLD[2]	ILD[3]	DTF[4]	STC[5]	FC[6]	STL[7]	NB[8]	SW[9]	BL[10]	DTM[11]	SC[12]
J13	**P value**	ns	*	ns	ns	*	Ns	*	ns	ns	ns	ns	.
	R2	0.04	0.16	0.01	0	0.05	0	0.05	0	0	0.03	0.02	0
J16	**P value**	**	ns	ns	ns	*	*	ns	ns	ns	ns	ns	ns
	R2	0.22	0	0.02	0.03	0.06	0.08	0.01	0	0.01	0.01	0.01	0
J17	**P value**	ns	*	*	ns	ns	*	ns	ns	ns	*	ns	ns
	R2	0.03	0.23	0.08	0	0.03	0.06	0.02	0.02	0.02	0.05	0.02	0.03
J18	**P value**	ns	.	ns	*	ns	*	ns	ns	ns	ns	*	.
	R2	0	0	0.02	0.07	0.01	0.06	0	0.03	0.02	0.03	0.05	0

[1] cotyledon retention (CR), margin leaf division (MLD), [3] incision of leaf division (ILD), [4] days to flowering (DTF), [5] stem color (STC), [6] flower color (FC), [7] stem length (STL), [8] number of primary branches (NB), [9] silique width (SW), [10] beak length (BL), [11] days to maturity (DTM), and [12] seed color (SC).

4.4 Discussion

Stead (2009) reported that the B-genome linkage groups were inherited as intact segments in the BC_3 plants that were used to generate the BC_3S_1 families used in this study. She also found that there appeared to be no intergenomic recombination occurring between the B-genome chromosomes and A- or C-genome chromosomes. This was one of the rationales for using the BC_3S_1 segregating families to study the inheritance of the B-genome chromosomes. Different B-genome chromosomes and combinations of them were detected in these families, along with significant differences for some of the morphological

traits within and between families. High levels of segregation distortion for the B-genome chromosomes were observed in this study. This is due to the aneuploid nature of the BC_3 plants, which can be explained by the interspecific origin of the material and the fact that homeologous chromosomes from the B- and A-genomes do not pair (Ky et al. 2000; Lorieux et al. 2000; Parkin and Lydiate 1997). This was previously reported in interspecific *Brassica* hybrids that contained distantly related genomes (Chevre et al. 2007; Chevre et al. 1998). Heneen and Jorgensen (2001) also reported the effect of aneuploidy on the behavior of *B. oleracea (alboglabra)* alien chromosomes in a *B. rapa* background.

In this chapter, the marker regression method was used for QTL mapping. This method detects the association of the trait value and the genotype for a single locus (in this case a full chromosome). Interval mapping, which estimates the position of a QTL (Lynch and Walsh 1998), could not be applied to this data set, as recombination between the B-genome chromosomes with AC-genome chromosomes was highly restricted in meiosis. Therefore, with the available marker data, construction of a reasonable linkage map or mapping of QTLs was not feasible.

With the GISH assay, it was possible to distinguish the B-genome chromosomes from those of A and C, but not possible to differentiate the linkage groups or to detect translocations. Conversely, SSRs do not provide the copy number of chromosomes or indicate whether they are addition or substitution lines. However, a combination of these two approaches does allow for linkage group differentiation and chromosomal copy number., It is not possible to identify specific B-genome linkage groups using GISH with *B. nigra* genomic DNA, since the probe paints the centromere area of any B-genome chromosome. To distinguish and visualize different B-genome chromosomes, one needs to use probes of BAC clones specific for a B-genome chromosome (Hasterok et al. 2005; Howell et al. 2008). However, in this case, GISH did not detect B-genome chromosomes in all of the lines where it was expected to be present based on

marker data. This was due to the small sample size and the segregating nature of the BC_3S_1 generation.

Due to the nature of the cross, I focused on B-genome SSR markers and, therefore, could only recognize the lines with B chromosomal segments and lines without them. Lines with no B chromosomes have either lost or gained an A or C chromosome. This is not surprising, considering the number of backcrosses these lines have gone through and the fact that the A and C chromosomes can pair readily (Attia et al. 1987; Parkin and Lydiate 1997). Future studies would be needed to explain this further. Hence for future work, I would suggest that the development and analysis of lines with: B-genome additions and B-genome introgression to fully characterize the A and C component of *Brassica* genome in these lines. This is the approach taken by Howell et al. (2008) to detect the A7/C6 translocation in 'Westar'.

From a plant breeding perspective, the material characterized and generated in this study is an excellent source of genetically diverse genotypes, which possess valuable traits such as early flowering, early maturity, number of seed per silique, plus other traits. It would be desirable to introgress these traits into one line, such as members of the IF-72 family, which are relatively early flowering, and early in maturity. However, this material also carries some negative traits such as high glucosinolate content in the seed (data not shown). Such linkage drag, if associated with the desired traits, will need to be addressed in future research on this germplasm. Recurrent crossing with selection for the desired trait(s) often leads to breaking of the large chromosomal segments and can be used to generate desirable lines.

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5. GENERAL DISCUSSION

5.1. Introduction

Brassica napus is an important oilseed crop that is grown in North America, Europe, Australia and China and provides $13 billion annually to Canada's agricultural industry. In Canada, *B. napus* is attacked by several pathogens; therefore, any improvement in disease resistance would also improve the yield for the producer and reduce the environmental impact of pesticide applications. There are many genes carried by the *Brassica* B-genome, which can provide some degree of resistance or adaptation to some diseases and stresses. However, to date, researchers have yet to resolve the genetics of many of these traits. This thesis describes my research to identify and exploit novel sources of morphological and disease resistance genes and alleles found in the Brassica B-genome and transferring them into A- or C-genomes of *B. napus*.

Genome homology in the Crucifers has been most thoroughly characterized by analyzing the detailed genetic and physical maps of *Arabidopsis* and aligning these with the genetic maps of different *Brassica* species (Lagercrantz and Lydiate 1996; Panjabi et al. 2008). These studies indicate that there were multiple events of genome polyploidization and subsequent chromosome rearrangements (Lagercrantz 1998; Parkin et al. 2002) which occurred in the Brassicaceae lineage after the divergence of *Arabidopsis* and *Brassica*, between 14.5 and 20.4 Million years ago (Mya). This was followed by the separation of the *B. nigra* (B) and the *rapa/oleracea* (AC) lineages about 7.9 Mya (Lysak et al. 2005; Yang et al. 1999). It has been demonstrated that there are high levels of colinearity between the A- and C-genomes of *B. napus*, *B. oleracea* and *B. rapa* (Lydiate et al. 1993; Panjabi et al. 2008; Parkin et al. 2005). As a result, there have been a number of reports of homeologous recombination events within *Brassica napus* (Leflon et al. 2006; Udall et al. 2005). However the B-genome is significantly diverged from the A- and C-genomes and there is some limited data suggesting that there are regions of the B-genome which are

homologous with the Brassica A- and C-genomes (Lagercrantz et al. 1996; Panjabi et al. 2008; Parkin et al. 2005). As explained earlier in this thesis, the *Brassica* B-genome containing species (*B. nigra, B. carinata and B. juncea*) contain many valuable agronomic traits. Introgression of certain traits (i.e. disease resistance) from allied species can increase the frequency of desired traits within a breeding population; but the problems with unstable introgressions and the incorporation of agronomically undesirable traits (linkage drag) is often a problem and this needs to be resolved to maximize the benefits of interspecific hybridization.

To characterize the genetic changes that occur during the B-genome introgression process and to identify traits associated with the B-genome chromosomes, I used two segregating populations derived from interspecific crosses between *B. carinata* and *B. napus* (Chapters 2 and 4), and addressed the following objectives:

a) Determine the behavior of the B-genome chromosomes in the progenies of the interspecific crosses by using SSR markers.
b) Visualize the B-genome chromosomes using GISH in order to karyotype the specific genotypes.
c) Identify the amount of B chromosome introgression in interspecific hybrid progenies.
d) Determine the effect of B-genome introgression on morphological traits.
e) Determine the association of the resistance to Scletotinia and Blackleg with the B-genome linkage groups.

5.2. Conclusions

Using microsatellite molecular markers and the cytological GISH technique, I was able to identify interspecific progeny lines carrying B-genome chromosomes, including addition lines (family IF-29, IF-30, IF-115), deletion lines (IF-42, IF-115, IF174, DH21) and substitution lines (DH45, DH51). This

knowledge and the characterized germplasm can be used for identifying the genes of agronomic importance and for the development of genetically diverse introgressed germplasm. My novel findings in this thesis were the following:

- This is the first report of interspecific cross-derived DH lines, which carry "stable" B-genome chromosomes or chromosomal segments.
- Detection of B genome introgressed segments in an interspecific population using genome specific microsatellite markers.
- Visualizing the B-genome chromosomes and identification of the type of lines as substitution, deletion or addition lines.
- Identifying the morphological traits associated with the B-genome chromosomes.
- Introducing J13 as a candidate for blackleg resistant genes.
- Introducing B-genome as a novel source of resistant genes for Sclerotinia stem rot.

Microsatellite markers can be successfully used to identify specific linkage groups of the B-genome; however one limitation to these markers is that they cannot identify the number of copies of the chromosome or identify translocations. I combined the marker data with cytogenetics, to overcome this limitation by having the ability to visualize and count the chromosomes and hence identify the nature of the lines as substitution, addition or deletion lines. I was not able to detect which A- or C-genome chromosomes were replaced, added or deleted in the B-genome introgressed lines. Future work with A, B, and C-genome chromosome specific markers would allow more precise cytogenetic mapping of the introgressions.

5.2.1. Microsatellite evidence of the introgression of B-genome chromosomes in the advanced backcross populations

I selected B-genome microsatellite markers from the *B. juncea* linkage maps and using the order and map distance of these markers from the AAFC

reference maps it was possible to identify the B-genome chromosomes in the advanced backcross populations. It was evident that although markers are designed to be B-genome specific, however, deviation can occur (Stead 2009). It is well established today that the *Brassica* A- and C- genome share many collinear regions (Parkin et al. 2005), and there are also regions between the A- and B-genome that share homoeology (Plieske and Struss 2001, Panjabi et al. 2008). This may explain why it is often difficult to identify the B-genome specific alleles. Therefore in this study a panel of control lines from different *Brassica* species was used to detect the possible B-genome specific alleles, as explained in Chapter 2 and Appendix 2.1.

In these backcrosses, I would not expect the B-genome chromosomes to pair with their A and C homoeologous chromosomes (Attia et al. 1987; Busso et al. 1987; Meng et al. 1998), and since these chromosomes would not have a homologue to pair with, they will either be lost or inherited as a complete chromosome. As a result of a lack of a homologue to pair with, the construction of linkage maps or mapping of QTLs was not possible. The difficulties in mapping due to suppressed recombination between introgressed regions and the corresponding region of the homologous/homeologous linkage group have been discussed by Mayerhofer et al. (2005).

However, the colinearity between the AC- and B-genome chromosomes might have an influence on the presence or loss of the B-genome chromosome(s) during the backcrossing of the ABCC hybrids with *B. napus* (AACC). Based on the present data it was not possible to deduce whether any specific B chromosomes are lost at a higher frequency. In addition, the population size of the "B+" genotypes (carrying B-genome chromosomes) was not large enough to apply comparisons between different lines with different B-genome linkage groups.

5.2.2. Cytogenetic evidence of introgression of B-genome chromosomes in the advanced backcross populations

I also used *B. nigra* genomic DNA in an *in situ* hybridization assay (GISH) to target B-genome chromosomes in the advanced interspecific backcross populations to find the copy number of the intact chromosomes in these lines. This technique could effectively visualize and distinguish the B-genome chromosomes from the AC chromosomes, as has been previously demonstrated by several researchers (Ge and Li 2007; Hasterok et al. 2005; Maluszynska and Hasterok 2005; Snowdon et al. 1997). However, this technique did not allow me to visualize homoeologus translocations involving only chromosome arms or to identify the different B-genome linkage groups, as it specifically paints the centromeres. In order to identify small translocations, BAC clone sequences need to be used as chromosome-specific DNA probes, and this can help to identify the individual B-genome chromosomes to allow a more precise cytogenetic mapping of introgressions (J.C. Pires, unpublished data).

Researchers have used *Brassica* species and have developed interspecific crosses between *B. napus* and *B. juncea* and successfully used FISH to determine the presence of B-genome linkage groups (Maluszynska and Hasterok 2005; Schelfhout et al. 2006). Although some of the researchers were able to identify additional B-genome linkage groups, they did not use locus specific molecular markers and therefore it was not possible to identify the specific linkage groups (Lagercrantz and Lydiate 1995; Panjabi et al. 2008; Schelfhout et al. 2006).

5.2.3. Disease resistance traits associated with the B-genome chromosomes in an interspecific population of *Brassica napus* x *B. carinata*

I analyzed the interspecific populations for several morphological and agronomically important traits. Furthermore, I evaluated different *Brassica* species for resistance to Sclerotinia and found that the species carrying the B-genome are resistant to *Sclerotinia sclerotiorum*. Although I can identify the B-genome as a new source for resistance to *S. sclerotiorum*, the population of "B+" lines was not large enough to make reliable associations between these traits and the specific B-genome introgression.

In this research, two of the DH lines were found to carry J13 and show resistance to blackleg. A blackleg resistant gene has been previously mapped on J13 of *B. juncea* (Christianson et al. 2006) and this genomic region aligns to the conserved C1E segment of the *Arabidopsis* genome, and also aligns with the segment of N7 where the blackleg resistance gene identified in the Australian cultivar Shiralee, was mapped (Mayerhofer et al. 2005; Panjabi et al. 2008). Therefore, I suggest that blackleg resistance observed in these DH lines is derived from the B-genome and located within the E segment of J13. The observation of J13 in common between DH lines can be explained as a consequence of selection for blackleg resistance during the development of the DH lines (Fig. 2.1).

5.3. My contribution to science as presented in this thesis

The majority of *Brassica* research has been focused on the A- and C-genome containing species (*B. rapa, B. oleracea* and *B. napus*), while the B-genome has remained an relatively unexplored source of novel traits. It could be used as a valuable source of diversity for developing improved cultivars, provided a more in depth understanding of the different B-genome chromosomes becomes available.

In my research I focused on two significant fungal diseases of Brassica species; Blackleg caused by *Leptosphaeria maculans,* which can cause yield losses of greater than 50% (Canola Council of Canada. 2006) and stem rot caused by *Sclerotinia sclerotiorum*, which cause yield losses up to 80% (Bolton et al. 2006). While the best method of control to both of them is through improvement of disease resistance in canola cultivars, here I introduce new sources of resistance genes for these diseases.

In this thesis research, two of the DH lines were found to carry J13 and show resistance to blackleg and I suggest that blackleg resistance derived from the B-genome can be located within the E segment of J13. These two lines also showed higher levels of resistant to *S. sclerotiorum*.

In the germplasm that I characterized, I found lines with specific B-genome linkage groups. This research helps in the understanding of traits located on the B-genome and the mapping of those traits. Hopefully, knowledge gained from my research can be used to develop a set of B chromosome addition lines, which would be valuable to the wider *Brassica* research community for understanding of the B-genome.

5.4. Future research

The material developed in my research is a valuable source of interesting traits, which can be used in developing improved cultivars. For breeding resistant lines and improved cultivars, the traits have to be associated and mapped to specific B-genome chromosomes with more in depth understanding.

Introgression and genetic mapping can be challenging because of the duplication events found in polyploids and complex genomes. In this regard, I would propose that any future research with this material includes making several additional crosses and backcrosses to induce recombination between B-genome chromosome(s) and the *B. napus* chromosomes to break the linkage groups, with the goal of reducing linkage drag. As a result, a QTL analysis of several traits including disease resistance, followed by saturation mapping of the regions of interest can be useful. From a genetics perspective the material characterized in this thesis is a valuable source for the development of a series of B-genome addition lines to help build a B-genome karyotype in which linkage groups can be clearly indentified. For this matter the availability of a library of chromosome-specific BAC clones is essential (Hasterok et al. 2005).

The results reported in this thesis will assist other researchers to exploit this genetic material to identify key agronomic genes from the B-genome that could be introgressed into *B. napus* breeding material. The current genetic material would facilitate Canadian canola breeding programs for the development of canola cultivars with multiple blackleg resistance genes for durable resistance to this disease and provide a platform for further targeted gene discovery.

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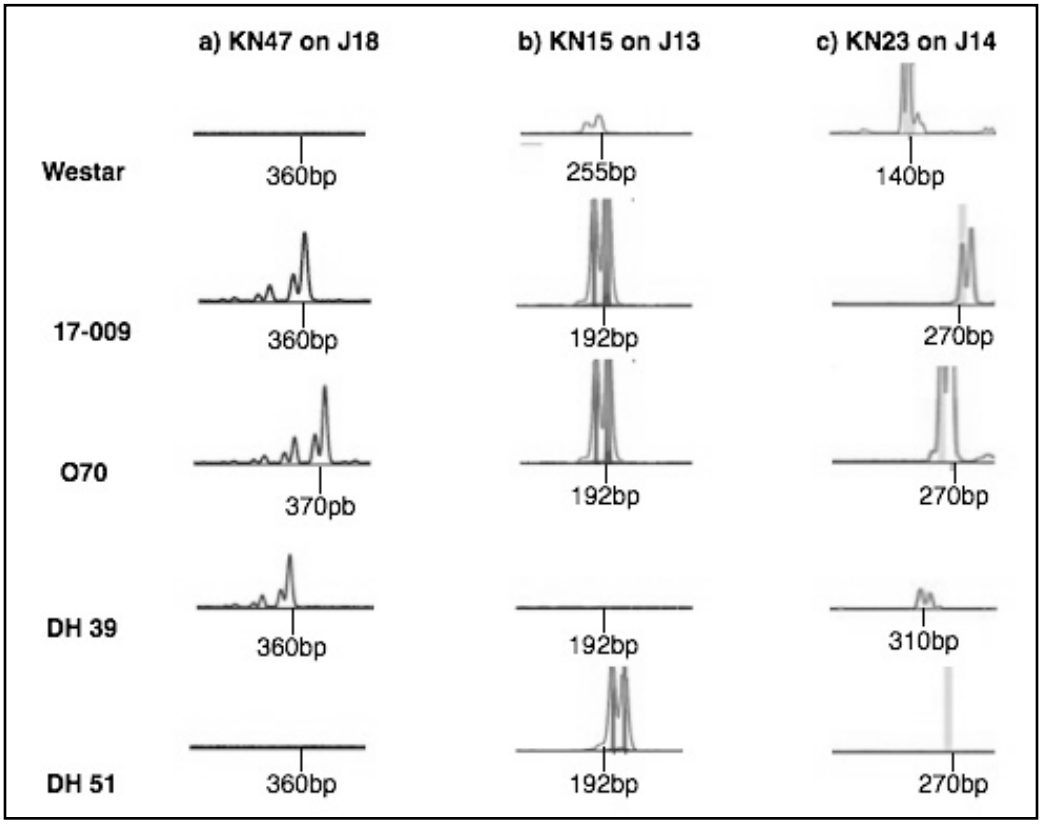
6. Appendices



Appendix 2.1 Typical marker characteristics of a number of primers used in this study to identify B-genome specific alleles. Westar is the *B. napus* control, 98-17009 and O70 are two of B. carinata controls, while DH39 and DH51 are two of the B-genome containing DH lines. (a) a marker that amplifies only the B-genome alleles, but the allele size varies depending on the material (b) a marker that amplifies preferentially the B-genome allele but also amplifies a distinctly different allele in the A-genome and (c) a marker that amplifies B-genome allele but also amplify B-genome allele of different size (usually ± 50bps).

Appendix 2.2. List of 48 publicly available B-genome SSR markers and their expected allele size from AAFC data and our observed allele size in *B. carinata*.

Primer Name	*B. juncea* Linkage Group[a]	*B. nigra* Linkage Group[b]	Distance from Top[c]	No. of alleles in *B. juncea*[d]	Expected Product Size	*B. carinata* allele size (bp)
sJ3838F	J11	G1	0	3	270-280	304
sJ4933	J11	G1	27.8	2	335-341	np
sJ84165	J11	G1	34.5	2	288-334	320
sJ0644	J11	G1	47.7	2	438-451	450
sJ3891	J11	G1	61.4	2	100-110	122
sB0563I	J11	G1	78.3	2	436-438	454
TOTAL			**88.8 cM**			
sJ6846	J12	G2	0	4	319-372	362
sB1870	J12	G2	0	3	481-489	319
sJ3302RI	J12	G2	10	4	392-409	430
sJ03104	J12	G2	45.7	2	380-390	np
sJ7079	J12	G2	59.1	2	211-214	F
sB4817R	J12	G2	65.8	2	250-364	309
TOTAL			**90.1 cM**			
sJ3627R	J13	G3	10.6	2	283-291	326
sB1822	J13	G3	17.5	2	250-267	278
sB1672	J13	G3	37.7	3	191-256	192
sJ7046	J13	G3	41.1	2	277-287	302
sB1990F	J13	G3	51.1	2	491-498	F
sB1752	J13	G3	61.5	3	406-422	392
TOTAL			**61.5 cM**			
sB2131	J14	G4	0	2	311-321	346
sA0306	J14	G4	16.8	2	362-396	np
sB0372	J14	G4	20.1	3	233-245	247
sB2141AI	J14	G4	26.8	2	381-389	F
sB1935A	J14	G4	40.4	2	257-260	270
sJ8033	J14	G4	47.7	4	150-225	243
TOTAL			**47.7 cM**			
sB0202I	J15	G5	0	2	108-187	160
sB3140	J15	G5	6.6	3	230-243	220
sJ3874I	J15	G5	9.9	4	164-185	190
sJ6842	J15	G5	19.9	3	331-358	345
sB2556	J15	G5	23.3	2	208-213	228
sB3872	J15	G5	60.9	2	276-183	184
TOTAL			**68.2 cM**			
sB31138	J16	G6	0	2	188-199	np
sJ7104	J16	G6	35.5	2	322-337	354
sJ0338	J16	G6	45.6	3	307-341	235
sJ1505	J16	G6	59.2	3	268-327	282
sJ3640I	J16	G6	82.9	2	327-346	344
sJ0502	J16	G6	104.1	2	249-253	264
TOTAL			**114.5 cM**			
sB1871	J17	G7	0	2	417-423	326
sJ39119I	J17	G7	25.8	3	329-388	362
sJ13133	J17	G7	25.8	3	294-300	318
sJ1536	J17	G7	29.5	2	210-213	222
sB1937	J17	G7	42.8	2	262-279	283
sJ4633	J17	G7	64.4	2	307-312	F
TOTAL			**64.4 cM**			
sB1728	J18	G8	24.8	2	445-490	484
sJ34121	J18	G8	24.8	3	320-340	344
sJ1668I	J18	G8	24.8	3	278-308	309
sB3751	J18	G8	52	2	165-198	179
sB5162	J18	G8	62.9	2	274-316	360
sB3739	J18	G8	76.1	3	373-377	325
TOTAL			**76.1 cM**			

Appendix 2.3. Statistics and comparison statements of the DH lines in two field trial seasons for fatty acid profile of oil.

Trait	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Population mean	0.008(0.0)[a]	0.03(0.0)	3.9(0.02)	0.23(0.002)	1.7(0.02)	53.7(0.75)	20.4(0.17)	11.7(0.13)
Mean of DH lines	0.008(0.0)	0.03(0.0)	3.9(0.02)	0.23(0.002)	1.8(0.02)	55.6(0.47)	20.7(0.15)	11.3(0.08)
98-17-009	0(0.0)	0.04(0.01)	2.6(0.19)	0.15(0.02)	0.7(0.23)	9.6(2.34)	11.2(1.21)	17.6(1.10)
Westar	0.009(0.0)	0.05(0.01)	3.7(0.19)	0.2(0.02)	1.8(0.23)	63.3(2.34)	17.6(1.21)	10.3(1.10)
DH39	0(0.0)	0.06(0.01)	5.3(0.2)	0.3(0.03)	2.6(0.25)	50.8(3.96)	25.2(1.58)	12.2(1.49)
DH45	0.005(0.0)	0.04(0.01)	3(0.2)	0.2(0.02)	1.6(0.23)	30.7(2.34)	15.7(1.21)	9.5(1.10)
DH 51	0.009(0.0)	0.04(0.01)	3(0.2)	0.2(0.02)	1.7(0.23)	33(2.34)	15.1(1.21)	9.4(1.10)
B-	0.009(0.0)	0.04(0.0)	3.9(0.1)	0.2(0.01)	1.8(0.07)	58.8(0.62)	21.0(0.38)	11.3(0.4)
B+[b] vs B-[c]	ns	**	ns	ns	**	**	**	ns
B- vs 39 d	*	**	**	**	**	*	**	ns
B- vs 45	ns	ns	**	ns	*	**	**	*
B- vs 51	ns	ns	**	ns	ns	**	**	*
DH45 vs DH51	ns	ns	ns	ns	ns	ns	ns	ns
98-17-009 vs Westar [e]	**	ns	**	**	**	**	**	**
98-17-009 vs DH39	ns	*	**	**	**	**	**	**
98-17-009 vs DH45	ns	ns	ns	ns	**	**	**	**
98-17-009 vs DH51	**	ns	ns	ns	**	**	**	**
Westar vs DH39	*	ns	**	*	**	**	**	ns
Westar vs DH45	ns	ns	**	ns	ns	**	*	ns
Westar vs DH51	ns	ns	**	ns	ns	**	**	ns
year	ns	ns	ns	ns	ns	ns	ns	ns
line	ns	*	**	*	**	**	**	**
line*year	*	**	**	**	**	ns	ns	**

Appendix 2.3. continued

Trait	C20:0	C20:1	C20:2	C22:0	C22:1	C24:0	C24:1	SATURATED
Population mean	0.6(0.006)	2.8(0.25)	0.2(0.02)	0.3(0.01)	3.5(0.58)	0.16(0.01)	0.4(0.04)	6.8(0.04)
Mean of DH lines	0.6(0.005)	2.8(0.27)	0.2(0.01)	0.3(0.01)	1.9(0.32)	0.14(0.00)	0.3(0.01)	6.8(0.04)
98-17-009	0.6(0.07)	6.2(0.65)	1.1(0.090	0.5(0.03)	45.9(1.07)	0.4(0.02)	2.8(0.09)	5.0(0.50)
Westar	0.6(0.07)	1.3(0.65)	0.1(0.09)	0.3(0.03)	0.1(1.07)	0.1(0.02)	0.1(0.09)	6.7(0.50)
DH39	0.9(0.08)	1.0(1.23)	0.1(0.15)	0.5(0.05)	0(2.1)	0.2(0.04)	0.4(0.19)	9.7(0.58)
DH45	0.7(0.07)	14.7(0.65)	0.7(0.09)	0.3(0.03)	21.2(1.07)	0.2(0.02)	1.0(0.09)	6.0(0.50)
DH 51	0.7(0.07)	15.2(0.65)	0.7(0.09)	0.3(0.03)	19.1(1.07)	0.1(0.02)	0.9(0.09)	6.0(0.50)
B-	0.6(0.02)	1.2(0.04)	0.1(0.01)	0.3(0.01)	0.2(0.04)	0.1(0.01)	0.2(0.01)	6.8(0.14)
B+[b] vs B-[c]	**	**	**	**	**	**	**	ns
B- vs 39 d	**	ns	ns	**	ns	**	ns	**
B- vs 45	**	**	**	ns	**	*	**	**
B- vs 51	**	**	**	ns	**	ns	**	**
DH45 vs DH51	ns	ns	ns	ns	ns	ns	ns	ns
98-17-009 vs Westar [e]	**	**	**	**	**	**	**	**
98-17-009 vs DH39	**	**	**	ns	**	**	**	**
98-17-009 vs DH45	*	**	**	**	**	**	**	**
98-17-009 vs DH51	**	**	**	**	**	**	**	**
Westar vs DH39	**	ns	ns	**	ns	**	ns	**
Westar vs DH45	**	**	**	ns	**	*	**	*
Westar vs DH51	**	**	**	ns	**	ns	**	*
year	ns	.	ns	ns	.	ns	.	ns
line	**	**	**	**	**	**	**	**
line*year	**	ns	**	ns	.	ns	.	**

Appendix 3.1. LsMeans of the reaction of 58 lines to Sclerotinia stem rot as estimated by five parameters (chapter 3).

Line		Group	7 DAI	15 DAI	21 DAI	MDS_{ij}	AUDPC
DH	1	B-	1.4	2.3	3.5	1.5	3.7
DH	3	B-	0.7	2.1	5.1	2.2	2.5
DH	6	B-	0.2	0.9	1.7	1.2	0.7
DH	12	B-	1.5	3.0	5.0	2.1	5.3
DH	13	B-	0.6	6.9	14.1	2.1	211.3
DH	15	B-	3.9	8.3	12.0	3.3	11.7
DH	17	B-	6.5	11.5	16.8	4.4	23.2
DH	18	B-	5.3	9.1	14.2	4.1	18.4
DH	19	B-	4.9	9.0	13.9	3.8	15.5
DH	21	B-	3.3	6.8	9.8	3.3	11.4
DH	22	B-	0.0	0.4	1.2	1.1	0.0
DH	23	B-	6.4	11.2	17.7	5.0	23.0
DH	26	B-	8.0	13.5	19.0	3.4	33.5
DH	30	B-	2.7	7.2	11.6	3.5	7.3
DH	31	B-	7.3	11.9	15.1	4.0	25.3
DH	32	B-	5.8	10.5	15.1	4.2	19.8
DH	33	B-	1.8	4.8	9.1	3.3	5.6
DH	36	B-	8.3	12.5	16.7	4.5	28.8
DH	42	B-	5.3	10.2	16.3	4.5	18.8
DH	45	B+	0.3	2.5	4.7	1.9	0.5
DH	46	B-	2.5	6.4	9.3	3.0	8.8
DH	50	B-	2.6	7.8	13.3	3.3	8.0
DH	51	B+	0.0	2.9	5.8	2.4	0.0
DH	52	B-	3.6	6.2	10.2	3.5	13.1
DH	53	B-	7.6	12.7	21.1	4.9	26.2
DH	55	B-	7.1	11.8	16.9	4.6	25.0
DH	58	B-	6.8	12.1	16.7	4.3	17.0
DH	59	B-	5.3	11.0	15.1	4.3	18.6
DH	60	B-	6.4	10.5	14.5	4.0	22.3
DH	62	B-	0.6	2.2	4.3	2.2	3.0
DH	63	B-	6.7	10.0	15.5	4.5	24.0
DH	66	B-	3.5	7.9	13.6	4.2	11.7
DH	68	B-	8.6	12.9	16.8	4.8	31.0
DH	69	B-	6.6	11.5	14.5	4.3	18.8
DH	73	B-	5.0	7.9	12.8	3.9	17.4
DH	75	B-	4.3	8.6	11.6	3.5	14.8
DH	79	B-	3.9	8.3	13.1	4.3	13.5
DH	81	B-	4.8	10.6	14.2	4.2	17.1
DH	82	B-	1.8	6.9	14.4	4.8	4.7
DH	83	B-	7.8	11.7	16.5	4.4	23.2
DH	86	B-	0.0	0.7	1.4	1.3	0.0
DH	88	B-	2.6	6.8	10.6	3.2	9.1
DH	91	B-	2.9	5.1	10.1	2.8	10.1
DH	92	B-	5.4	10.3	14.9	4.8	17.9
DH	93	B-	10.0	12.7	19.4	5.3	386.0
DH	94	B-	7.0	10.6	14.9	3.7	24.7
DH	95	B-	5.7	10.4	15.2	4.3	20.0
DH	96	B-	8.2	11.8	14.5	4.3	25.3
DH	97	B-	4.8	8.6	14.5	4.2	17.1
DH	98	B-	6.9	10.3	14.6	4.3	24.1
DH	99	B-	4.7	9.3	15.7	4.6	17.0
DH	100	B-	6.4	10.7	15.3	4.5	20.6
DH	102	B-	10.3	16.1	20.9	5.1	36.1
DH	104	B-	8.8	13.8	20.1	4.9	31.7
DH	105	B-	7.4	13.6	18.2	4.9	26.1
DH	106	B-	4.2	8.1	12.3	4.1	14.8
DH	107	B-	6.3	14.7	19.3	5.0	21.4
DH	108	B-	6.6	11.7	16.7	4.5	23.2