Transferring blackleg resistance from *Brassica carinata* and synthetic hexaploid *Brassica* accessions into *Brassica napus*

By

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ABSTRACT

Duoduo Wang. M.Sc., the University of Manitoba, 2016. Transferring blackleg resistance from *Brassica carinata* and synthetic hexaploid *Brassica* accessions into *Brassica napus*.

Blackleg caused by *Leptosphaeria maculans* (Desm.) Ces. & De Not. is one of the most serious diseases in canola production. A high level of blackleg resistance has been shown in *Brassica carinata* A. Braun (BBCC) and new synthetic hexaploid *Brassica* species (AABBCC) developed from the crosses of *B. rapa* L. and *B. carinata.* Blackleg resistance from *B. carinata* and hexaploid *Brassica* accessions was transferred into *B. napus* L. using interspecific hybridization followed by backcrossing to a susceptible *B. napus* cultivar 'Westar' three or four times and selfing one or two times to produce pure lines. *Leptosphaeria maculans* isolate 03-15-03 was used to select the resistant plants in each generation using cotyledon inoculation, and four *L. maculans* isolates (03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M) were utilized in advanced generations. In the cross of *B. napus* 'Westar' and *B. carinata*, all plants in the F_1 showed a high level of resistance to *L. maculans* isolate 03-15-03. According to the chi-square testing for goodness of fit, the segregation of resistant and susceptible plants fit a 1:1 ratio in the BC_1, BC_3, and BC_4. In the BC_3F_2, two families followed a 3:1 segregation ratio of resistant and susceptible plants. The results suggest that the resistance to *L. maculans* transferred from *B. carinata* into canola 'Westar' was controlled by a single locus.

Embryo rescue tissue culture was used to obtain F_1 plants of the crosses of 'Westar' and synthetic hexaploid *Brassica* accessions. In the BC_1 and BC_2, most families did not fit a

1:1 segregation ratio of resistant and susceptible plants. The segregation of resistant and susceptible plants fit a 3:1 ratio when inoculated with *L. maculans* isolates in the BC_1F_2-3.1.1s and BC_1F_3-3.1.1.1ss families. Meanwhile, the BC_2-3.1.1 family also followed a 1:1 segregation of resistant and susceptible plants inoculated with *L. maculans* isolate 03-15-03. The results suggest that the resistance to *L. maculans* introgressed from synthetic hexaploid *Brassica* species into *B. napus* is most likely controlled by a single locus.

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FORWORD

This thesis is written in manuscript style. A general introduction and review of literature precedes manuscripts that comprised the main part of the thesis. Each manuscript consists of an abstract, introduction, materials and methods, results, and discussion. A general discussion, a future research and a list of references cited follow the manuscripts.

TABLE OF CONTENTS

ABSTRACT.....ii

ACKNOWLEDGMENTS.....iv

FORWARD.....v

TABLE OF CONTENTS.....vi

LIST OF TABLES.....x

LIST OF FIGURES.....xiii

Chapter 1. General introduction.....1

Chapter 2. Literature review.....6

2.1 *Brassica* species.....6

2.1.1 Description.....6

2.1.2 History of *Brassica* species.....7

2.2 *Leptosphaeria maculans*.....11

2.2.1 Description.....11

2.2.2 Classification and variability.....12

2.3 Blackleg disease.....15

2.3.1 Blackleg disease symptoms.....15

2.3.2 Blackleg disease cycle.....15

2.3.3 History of blackleg disease.....17

2.3.4 Blackleg disease management.....18

2.4 Blackleg resistance.....21

2.4.1 Qualitative resistance in *Brassica napus*.....24

2.4.2 Qualitative resistance in *Brassica rapa*.....25

2.4.3 Qualitative resistance in the B genome containing *Brassica* species.....27

2.4.4 Quantitative resistance.....30

2.5 Durability of blackleg resistance.....32

2.6 Objectives of this project.....34

Chapter 3. Transferring blackleg resistance from *Brassica carinata* into *Brassica napus*.....36

3.1 Abstract.....36

3.2 Introduction.....36

3.3 Materials and methods.....38

3.3.1 Plant materials.....38

3.3.2 Crossing procedure.....39

3.3.3 Confirmation of the F_1 hybrids.....39

3.3.4 Production of BC_1 plants with tissue culture.....40

3.3.5 Production of backcrossing generations.....41

3.3.6 *Leptosphaeria maculans* isolates.....42

3.3.7 Inoculation tests.....44

3.3.8 Disease score.....45

3.3.9 Statistical analysis.....47

3.4 Results.....48

3.4.1 Evaluation of *Leptosphaeria maculans* isolates for virulence.....48

3.4.2 Resistance to *Leptosphaeria maculans* in the F_1 of the cross of *Brassica napus* cultivar

‘Westar’ and *Brassica carinata* ‘T4001’.....49

3.4.3 Resistance to *Leptosphaeria maculans* in the BC_1, BC_2, BC_3, BC_4, and BC_3F_2.....50

3.5 Discussion.....59

Chapter 4. Transferring blackleg resistance from ‘Meng’ (AABBCC) into *Brassica napus* (AACC) 64

4.1 Abstract.....64

4.2 Introduction.....64

4.3 Materials and methods.....66

4.3.1 Plant materials.....66

4.3.2 Crossing procedure.....68

4.3.3 Production of F_1 plants with tissue culture.....68

4.3.4 Production of backcrossing and selfing generations.....69

4.3.5 *Leptosphaeria maculans* isolates.....70

4.3.6 Inoculation tests.....72

4.3.7 Disease score.....72

4.3.8 Statistical analysis.....73

4.4 Results.....74

4.4.1 Resistance to *Leptosphaeria maculans* in the F_1 of the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’.....74

4.4.2 Resistance to *Leptosphaeria maculans* in the BC_1, BC_2, BC_1F_2, BC_2F_2, and BC_1F_3....74

4.5 Discussion.....85

Chapter 5. General discussion.....90

Chapter 6. Future research.....95

References.....96

LIST OF TABLES

Table 2.1 Blackleg resistance genes in *Brassica* species 23

Table 3.1 Blackleg disease scores for the evaluation of *Leptosphaeria maculans* isolates 47

Table 3.2 Blackleg disease scores for the evaluation of cotyledon resistance (Williams et al 1979) 48

Table 3.3 Blackleg disease severity (DS) caused by five *Leptosphaeria maculans* isolates in five *Brassica napus* accessions at 14 days after cotyledon inoculation 49

Table 3.4 Cotyledon and true leaf resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_1 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2014 52

Table 3.5 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_2 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2014 53

Table 3.6 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_3 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2014 55

Table 3.7 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03 and PG4-1-M in BC_4 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2015 56

Table 3.8 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in BC_3F_2 plants following the cross of *Brassica*

napus cultivar ‘Westar’ and *Brassica carinata* cultivar ‘T4001’ in a greenhouse in Winnipeg, MB in 2015 59

Table 4.1 Name, seed type, generation, and parents of synthetic hexaploid *Brassica* species ‘Meng’ 68

Table 4.2 True leaf resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in F_1 plants of the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’ in a greenhouse in Winnipeg, MB in 2014 75

Table 4.3 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_1 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’ in a greenhouse in Winnipeg, MB in 2014 77

Table 4.4 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_2 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’ in a greenhouse in Winnipeg, MB in 2014 79

Table 4.5 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_1F_2 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’ in a greenhouse in Winnipeg, MB in 2014 81

Table 4.6 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in the BC_2F_2 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’ in a greenhouse in Winnipeg, MB in 2015 82

Table 4.7 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M in BC_1F_3 plants following the cross of

Brassica napus cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’ in a greenhouse in Winnipeg, MB in 2015 86

LIST OF FIGURES

Figure 1.1 Production of canola/rapeseed in Canada from 1961 to 2013 (FAOSTAT data) 2

Figure 1.2 Yield of canola/rapeseed in Canada from 1993 to 2013 (FAOSTAT data) 3

Figure 1.3 Top five canola/rapeseed producing countries in 2013 (FAOSTAT data) 3

Figure 1.4 Global consumption of vegetable oils from 1995/1996 to 2014/2015, by oil type (in million metric tons) (Statista 2015) 4

Figure 2.1 Genetic relationships of oilseed *Brassica* species (Kimber et al 1995) 7

Figure 2.2 Blackleg disease cycle (Diagram courtesy of Department of Biology, University of Saskatchewan) 16

Figure 3.1 The acetocarmine staining of viable pollen grains (A) and non-viable pollen grains (B) (Photo credit: Minkyung Kang-Choi) 40

Figure 3.2 The process of the interspecific crossing of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' following by backcrossing with 'Westar' and selfing 41

Figure 3.3 Blackleg symptoms on *Brassica napus* cotyledons evaluated as 0, 1, 3, 5, 7, and 9 14 days after cotyledon inoculation with *Leptosphaeria maculans* isolate 03-15-03.....46

Figure 3.4 Blackleg symptoms on *Brassica napus* true leaves evaluated as resistant, intermediate, and susceptible plants 14 days after true leaf inoculation with *Leptosphaeria maculans* isolate 03-15-03 46

Figure 3.5 Blackleg disease scores for cotyledon inoculation of F_1 plants of the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2013 50

Figure 3.6 Blackleg disease scores for cotyledon inoculation of BC_1 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014 51

Figure 3.7 Blackleg disease scores for cotyledon inoculation of BC_2 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014 53

Figure 3.8 Blackleg disease scores for cotyledon inoculation of BC_3 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014 54

Figure 3.9 Blackleg disease scores for cotyledon inoculation of BC_4 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03 and PG4-1-M in a greenhouse in Winnipeg, MB in 2015 56

Figure 3.10 Blackleg disease scores for cotyledon inoculation of BC_3F_2-1.1.1s plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015 58

Figure 3.11 Blackleg disease scores for cotyledon inoculation of BC_3F_2-1.1.2s plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar

‘T4001’. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015 58

Figure 4.1 The process of the interspecific crossing of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’ following by backcrossing with ‘Westar’ and selfing 69

Figure 4.2 Blackleg disease scores for cotyledon inoculation of BC_1 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014 76

Figure 4.3 Blackleg disease scores for cotyledon inoculation of BC_2 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014 78

Figure 4.4 Blackleg disease scores for cotyledon inoculation of BC_1F_2 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014 80

Figure 4.5 Blackleg disease scores for cotyledon inoculation of BC_2F_2 plants following the cross of *Brassica napus* cultivar ‘Westar’ and synthetic hexaploid *Brassica* species ‘Meng’. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015 82

Figure 4.6 Blackleg disease scores for cotyledon inoculation of BC_1F_3-3.1.1.1ss plants

following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015 84

Figure 4.7 Blackleg disease scores for cotyledon inoculation of BC_1F_3-3.1.1.2ss plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015 84

Figure 4.8 Blackleg disease scores for cotyledon inoculation of BC_1F_3-7.3.1.1ss plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015 85

Chapter 1. General introduction

Rape is an English word applied to the oilseed forms of *Brassica napus* L. and *B. rapa* L., which have been cultivated for centuries (Downey 1966). Rape (*B. napus*) and turnip rape (*B. rapa*) have been cultivated in Europe since the thirteenth century (Appelqvist et al 1972). At the end of the middle Ages, rapeseed oil was mainly used for lighting and making soap, and has been noted to have been used in ancient civilizations of Europe, Asia and the Mediterranean (Appelqvist et al 1972; Gugel et al 1992). Later, rapeseed oil was used as a lubricant for steam powered engines (Canola Council of Canada 1988). In 1942, rapeseed was introduced in Canada due to the demand for rapeseed oil as lubricants for ships (Downey et al 1975). Subsequently, rapeseed was commercially grown and has become the most significant oilseed crop in Canada (Appelqvist 1972; Runciman et al 1975).

Actually, World War Ⅱ was a milestone for worldwide development of cultivation and utilization of rapeseed oil (Downey 1966; Snowdon et al 2007). In Canada, the first edible oil was extracted from rapeseed in 1956 (Canola Council of Canada 1988; Fereidoon 1990). Subsequently, rapeseed was introduced into Australia in 1968 (Gugel et al 1992). During the period of 1975-1985, because of the increased production and use, rapeseed oil became the third most important oil crop in the world following palm and soybean (Downey et al 1989).

It is known that erucic acid and glucosinolates in rapeseed have an adverse effect on taste and animal health when rapeseed meal is used as feed or food (Snowdon et al 2007). Accordingly, two components have been significantly reduced by breeders for rapeseed oil use in food and cooking (Snowdon et al 2007). The first *B. napus* variety ‘Oro’ with low erucic acid was licensed in 1968 in Canada, and it contained less than 5% erucic acid (Eskin

2013; Roman 2011). Low glucosinolates content was found in *B. napus* variety 'Bronowski' in 1967 (Kondra et al 1970). The first *B. napus* variety with both low erucic acid and low glucosinolates called 'Tower' was developed and licensed in 1974 (Stefansson et al 1975; Snowdon et al 2007). Canola is the name applied to low erucic acid and glucosinolates rapeseed varieties (Canola Council of Canada 1988; Mendham et al 2016).

Canola/rapeseed's tolerance to low temperature explains why it is grown in temperate and high altitude agricultural areas (Downey et al 1989). The major regions of rapeseed production in the world include Canada, China, India and Northern Europe. Since 1975, the production and yield of canola/rapeseed in Canada has substantially increased (Figure 1.1 and Figure 1.2). As a result, Canada has become a major canola/rapeseed production and export country in the world. In fact, Canada was the top canola/rapeseed producing country in 2013 (Figure 1.3), and the oil occupied 86 % of Canadian vegetable oil supply in 2013. Meanwhile, it was the third in global vegetable oil consumption (Canola Council of Canada 2011, 2013; Figure 1.4). The canola/rapeseed production in Canada is mainly centered in Alberta, Saskatchewan and Manitoba, where temperature and moisture are desirable for canola/rapeseed production (Fereidoon 1999).

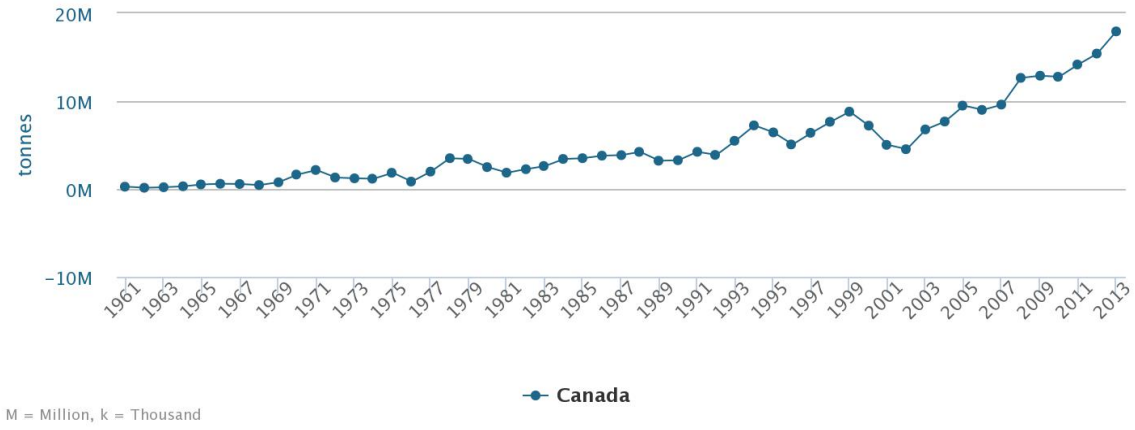


Figure 1.1 Production of canola/rapeseed in Canada from 1961 to 2013 (FAOSTAT data).

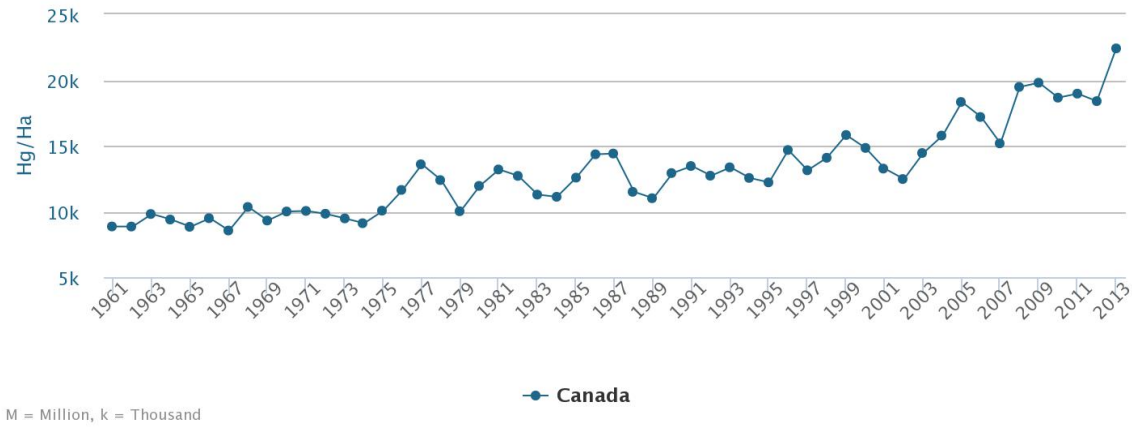


Figure 1.2 Yield of canola/rapeseed in Canada from 1993 to 2013 (FAOSTAT data).

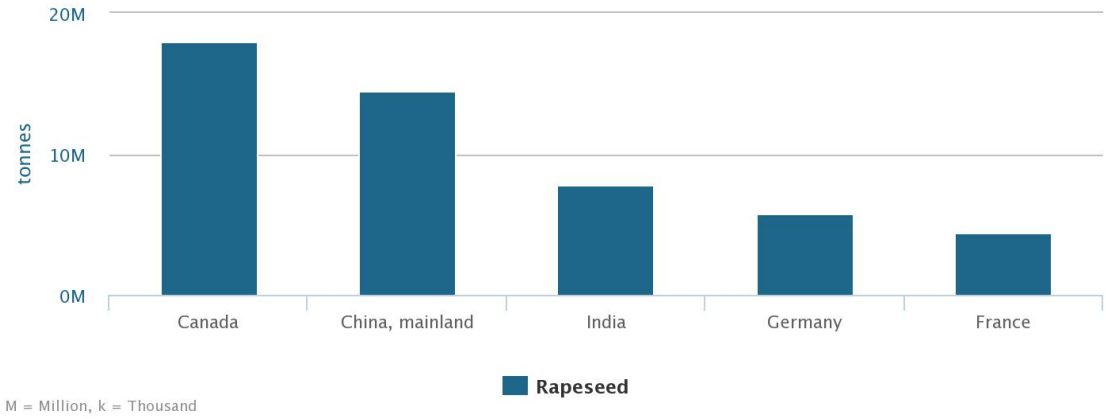


Figure 1.3 Top five canola/rapeseed producing countries in 2013 (FAOSTAT data).

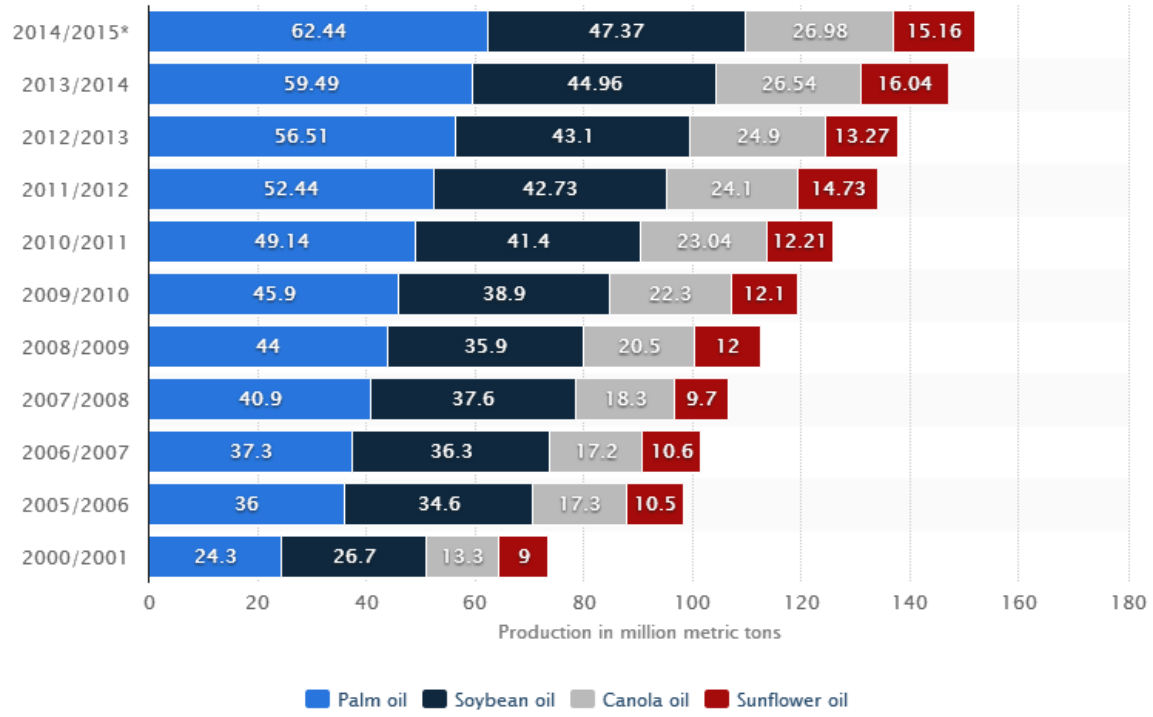


Figure 1.4 Global consumption of vegetable oils from 1995/1996 to 2014/2015, by oil type (in million metric tons) (Statista 2015).

Several pathogens are considered to cause serious yield losses in canola/rapeseed and are widespread in production fields in western Canada. White rust and other diseases of rapeseed were already detected in Manitoba and other provinces when rapeseed was introduced in Canada (Conners 1967). In 1961, blackleg disease was identified, but did not seem to result in a severe damage (Vanterpool 1961). Since 1966, blackleg disease has been prevalent in the worldwide regions of rapeseed growth (Gugel et al 1992). In 1975, blackleg disease was severely epidemic in Australia, Europe, and Canada. Blackleg disease caused significant damage in some canola/rapeseed fields due to the lack of a proper rotation. In 1977, some canola/rapeseed fields were 100 % infested by blackleg disease with a 50 % yield loss (Gugel et al 1992).

To date, blackleg (*Leptosphaeria maculans* (Desm.) Ces. &De Not.), sclerotinia stem

rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), and clubroot (*Plasmodiophora brassicae* Woronin) consistently cause significant yield losses in the production of canola/rapeseed around the world (Canola Council of Canada 2014). In addition to the disease control approaches employed, such as rotation, weed control, clean seed use and application of fungicides, the development of resistant cultivars has become an effective control for blackleg disease in canola/rapeseed. The use of resistant cultivars has significantly reduced canola/rapeseed yield losses in Canadian production (Gugel et al 1992; Rimmer et al 1992).

Effective blackleg control requires that resistant cultivars be integrated into an integrated management strategy (Kutcher et al 2011). Blackleg resistance imparted from the *B. napus* cultivar 'Jet Neuf' has been used in a wide range of Australian cultivars with a major reduction in disease impact (Rouxel et al 2003b). It was also observed that in fields with short rotation periods, resistance to blackleg broke down (Brun et al 2000). Genetic variation among *L. maculans* races presents a major problem in maintaining high levels of blackleg resistance in *B. napus* cultivars (Liban et al 2015; Zhang et al 2015). Combining the diversity of blackleg resistance resources (referred to as pyramiding) appears to be an effective strategy to achieve somewhat sustainable resistance (Brun et al 2001; Rimmer et al 2006; Sprague et al 2006; Stachowiak et al 2006).

Chapter 2. Literature review

2.1 *Brassica* species

2.1.1 Description

The genomic relationships among *Brassica* species are illustrated as the so-called "Triangle of U" (Figure 2.1), which consists of three diploids *Brassica rapa* L. (AA genome, 2n=20), *B. nigra* (L.) Koch (BB, 2n=16), and *B. oleracea* L. (CC, 2n=18) and three amphidiploids *B. juncea* (L.) Czem. & Coss. (AABB, 2n=4x=36), *B. napus* L. (AACC, 2n=4x=38), and *B. carinata* A. Braun (BBCC, 2n=4x=34) (Kimber et al 1995). The amphidiploid species are the result of the crosses of two corresponding diploid *Brassica* species (Cheng et al 2013; Morinage 1934). The amphidiploid *Brassica* species have enormously complicated genomes (Lagercrantz et al 1996; Chalhoub et al 2014). Duplications frequently occur within and among chromosomes of the A, B and C genomes. Such duplications are called intragenomic duplication and intergenomic duplication, respectively (Panjabi et al 2008). It is rather common to observe homeologous recombination between the A and C genomes, while the homeologous crossovers between the B genome and the A or C genomes are rare (Attia et al 1986, 1987; Mason et al 2010). In the A, B and C genomes, chromosomal rearrangements including inversions, translocations, insertions and deletions can also occur with duplications (Long et al 2011; Mayerhofer et al 2005; Parkin et al 2003). It has been observed that there is a translocation between chromosomes N7 and N16 in *B. napus* (Parkin et al 2005).

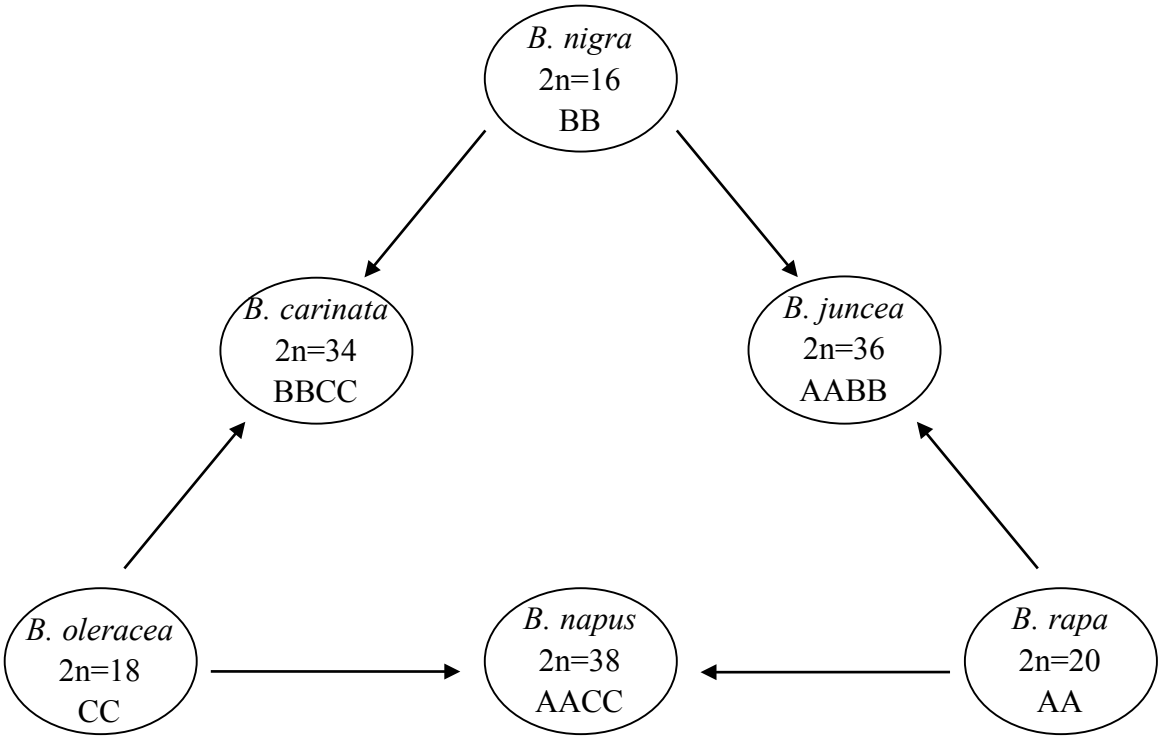


Figure 2.1 Genetic relationships of oilseed *Brassica* species (Kimber et al 1995).

2.1.2 History of *Brassica* species

2.1.2.1 *Brassica napus*

Brassica napus is the most widely cultivated and commercialized species among the six *Brassica* species (Snowdon et al 2007). It is also an amphidiploid species derived from the cross of *B. rapa* and *B. oleracea* (Dixon 2007; Downey et al 1989; Snowdon et al 2007). This species includes two subspecies, *B. napus ssp. napobrassica* and *B. napus ssp. napus*. Winter and spring types of the latter are cultivated for use as leaf vegetable, fodder, and oil. This species originated in the Mediterranean through natural interspecific hybridization between turnip (*B. rapa*) and cabbage (*B. oleracea*) (Snowdon et al 2007).

In the 16th century, rapeseed was used to produce oil as the main source of lamp fuel in Europe (Kimber et al 1995). Throughout history, oil produced by rapeseed was generally considered to be inedible oil and poisonous and it was not until the development of zero

erucic acid and low glucosinolate cultivars that it came into use as cooking oil and foodstuff (Downey et al 1989). Development of zero erucic acid and low glucosinolate cultivars was a major turning point in expanded human use of canola/rapeseed oil as it was previously proven that the toxic byproducts released during the digestion of glucosinolate impair the function of liver, kidney, and lymphnodes (Fenwick et al 1983; Snowdon et al 2007). Additionally, erucic acid imparted a bitter taste to the oil and had been proven to have a range of adverse health effects (Snowdon et al 2007).

The first double low (zero erucic acid and low glucosinolates) cultivar of spring *B. napus*, labelled 'Tower', was developed from a Polish spring *B. napus* cultivar 'Bronowski', and released in 1974 (Stefansson et al 1975; Snowdon et al 2007). With agricultural adoption of the cultivar the emergence of double low *B. napus* production commenced and led to *B. napus* becoming one of the most significant oil crops in the world (Snowdon et al 2007). The high yield of *B. napus*, possibly due to high chloroplast numbers and a large chloroplast volume, also contributed to the development of *B. napus* as the most productive *Brassica* oilseed species (Rakow 2004). It is noteworthy that canola oil contains less than 2 % erucic acid and 30 umol aliphatic glucosinolates per gram of residual meal (Rimmer et al 1992).

2.1.2.2 *Brassica carinata*

Brassica carinata, derived from an interspecific hybridization between *B. nigra* and *B. oleracea*, is utilized in the Ethiopian region of eastern Africa (Kimber et al 1995; Rakow 2004). In Ethiopia, people grow it as a leafy vegetable and extract mustard oil from the seed. The wild form of *B. carinata* has not been identified (Dixon 2007; Rakow 2004*). Brassica carinata* contains several desirable agronomic traits, including drought tolerance, pod

shattering resistance, and disease resistance (Alonso et al 1991; Rimmer et al 1992). *Brassica carinata* cultivar 'Braun' has been introduced into many parts of the world and is known to have originated in eastern Africa (Rimmer et al 1992). This species generally contains high levels of erucic acid and grows slowly compared with other *Brassica* species which contribute to its low level of agricultural adoption around the world (Kimber et al 1995; Rimmer et al 1992).

2.1.2.3 *Brassica juncea*

Brassica juncea is an amphidiploid species obtained from the cross of *B. nigra* and *B. rapa* (Fransden 1943). Its wild type was first found in the Near East and Iran (Rakow 2004). In India, *B. juncea* is known as brown mustard or Indian mustard. It produces brown seeds and has been cultivated as an oilseed in various regions of the Near and Middle East (Edwards et al 2007; Rakow 2004). In China, *B. juncea,* a yellow seeded variety is cultivated as a leaf vegetable (Dixon 2007; Edwards et al 2007; Rakow 2004). This species is grown as a condiment crop in western Canada (Rakow 2004).

Brassica juncea exhibits tolerance to heat and drought, and it also has a number of important agronomic characters such as disease resistance and oil content (Edwards et al 2007). Therefore, it has been considered as a potential *Brassica* oilseed to be introduced into western Canada and Australia (Oram et al 1999; Woods et al 1991). It displays a high level of resistance to *L. maculans* (Gugel et al 1990; Helms et al 1979; Keri 1991; Roy 1978b). However, some *B. juncea* cultivars are susceptible to *L. maculans* isolates (Ballinger et al 1991; Keri 1991; Sjodin et al 1988). Keri (1991) reported that three of 296 Canadian lines of *B. juncea* are susceptible to *L. maculans* isolates growing in fields in Manitoba, Canada. Most

of the infections appeared on the roots following cotyledon inoculation (Keri 1991).

2.1.2.4 *Brassica rapa*

Brassica rapa originated in the highlands near the Mediterranean Sea as a vegetable species, and was introduced to Scandinavia and eastern Europe (Mizushima et al 1967; Nishi 1980). *Brassica rapa* has a higher level of cold tolerance than other Brassica species (Kimber et al 1995; Mendham et al 1995). In China, several varieties of *B. rapa* are grown in the low temperature northern regions as a leafy vegetable crop, and these varieties are considered to be introduced from western Asia or Mongolia (Dixon 2007; Rakow 2004). *B. rapa* has been cultivated as an oilseed crop in India, Sweden, Finland, and Canada (Rakow 2004). Spring *B. rapa* cultivars accounted for 75% of the rapeseed area in Canada in the 1970s, falling to 50% in the 1990s (Quijada et al 2007). The first low erucic acid *B. rapa* cultivar 'Span' was released in 1971 (Downey et al 1975; Roman 2011). *Brassica rapa* cultivar 'Candle' with low erucic acid and low glucosinolates was later released in 1977 by Keith Downey (the National Research Council of Canada in Saskatoon) (Roman 2011). *Leptosphaeria maculans* resistant cultivars or breeding lines of *B. rapa* have not been identified (Helms et al 1979; Kuther 1990; Sjodin et al 1988).

2.1.2.5 *Brassica nigra*

Brassica nigra grows in the Mediterranean as a wild weed (Rakow 2004; Tsunoda 1980). This species contains the B genome and has been used as the donor of the B genome to other *Brassica* species (Dsa et al 2007). It has often been considered a genetic source for imparting high resistance to *L. maculans* in other *Brassica* species (Dixon 2007; Rakow 2004).

2.1.2.6 *Brassica oleracea*

Brassica oleracea was first found in the Europe as a vegetable species (Rakow 2004). It has been cultivated worldwide as kale, cabbages, kohlrabi, inflorescence kales, branching bush kales, and Chinese kale (Rakow 2004; Snogerup 1980). This species contains the C genome and blackleg resistance has not been identified in the accessions of *B. oleracea*, suggesting that the C genome may not contain dominant blackleg resistance genes (Mithen et al 1987; Monteiro et al 1989; Sjodin et al 1988).

2.2 *Leptosphaeria maculans*

2.2.1 Description

Leptosphaeria maculans (Desm.) Ces. & De Not., the teleomorph of *Phoma lingam* (Tode ex Fr.) Desm., causes blackleg or phoma stem canker on *Brassica* species (Anonymous 1957; Koch et al 1991; Punithalingam et al 1972). Previously, all isolates recovered from plants with blackleg disease were assigned to *L. maculans*, however, isolates of *L. maculans* were subsequently divided into groups with contrasting pathogenicity affects characterized as virulent and avirulent (McGee et al 1978), aggressive and nonaggressive (Koch et al 1989), pathotype A and pathotype NA (Badawy et al 1991), the A group and the B group (Johnson et al 1994), Tox^0 and Tox^+ (Balesdent et al 1992), or highly virulent and weakly virulent (Sippell et al 1995; Williams et al 1999). Furthermore, these contrasting groups can be distinguished by several other diagnostic characteristics. For example, the isolates that belong to the aggressive group were observed to grow slowly on agar media in comparison with nonaggressive ones, which grew more rapidly (Koch et al 1989; McGee et al 1978).

Aggressive isolates did not produce a yellow-brown pigment in liquid culture (Bonman et al 1981; Koch et al 1989, 1991) and were differentiated from the nonaggressive isolates by the presence of various compounds during metabolism. For example, the aggressive isolates synthesized a phytotoxin sirodesmin (Balesdent et al 1992; Gall et al 1995; Koch et al 1989). Additionally, the aggressive isolates were observed to produce short germ tubes of conidia when they were cultivated on water agar media (Petrie 1988).

2.2.2 Classification and variability

Koch et al (1991) divided the non-aggressive group into three subgroups, NA1, NA2, and NA3, based on restriction fragment length polymorphism (RFLP) studies. This classification of the non-aggressive group was confirmed by soluble protein analysis and isozyme analysis (Gall et al 1995). Subsequently, Shoemaker et al (2001) termed the NA1 subgroup as *Leptosphaeria biglobosa* sp. nov. based on isolates from *B. juncea*. It was observed that in Canadian collections *L. biglobosa* did not cause as severe damage on host organs and tissues as did *L. maculans* (Gugel et al 1992). *Leptosphaeria maculans* appeared as lesions on cotyledons and stems, and *L. biglobosa* generally caused lesions on cotyledons and was located in pith without external lesions on stems (Johnson et al 1994; West et al 2002). Also, *L. maculans* was generally located in the cortex, which caused stem base cankers in the field (West et al 2002).

Based on the phenotypes of *L. maculans* isolates tested on *B. napus* cultivars ‘Westar’, ‘Quinta’, and ‘Glacier’, aggressive isolates were distinguished into three pathogenicity groups (PG) including PG2, PG3, and PG4 (Koch et al 1991; Mengistu et al 1991). Non-aggressive isolates were categorized into PG1, which were not virulent on all of the

‘Westar’, ‘Quinta’, and ‘Glacier’ cultivars (Koch et al 1991; Mengistu et al 1991).

Pathogenicity group 2 isolates caused sporulating lesions on ‘Westar’ but not on ‘Glacier’, and were dominant among *L. maculans* isolates from western Canada 1988-2000 (Chen et al 2006; Kutcher et al 2007). PG3 isolates were virulent on both ‘Westar’ and ‘Glacier’, and both PG2 and PG3 isolates produced a few non-sporulating lesions on cotyledons of ‘Quinta’ (Keri 1999; Koch et al 1991; Mengistu et al 1991). Infestation of PG4 isolates showed sporulating lesions on cotyledons of ‘Westar’, ‘Glacier’ and ‘Quinta’ (Koch et al 1991; Mengistu et al 1991). Subsequently, a new pathotype pathogenicity group T (PGT) was found to be virulent on ‘Glacier’ but not on ‘Quinta’, and was observed frequently in western Canada (Rimmer et al 2006).

Applying the A group and the B group designations of pathogenicity affects (Johnson et al 1994), *L. maculans* was classified into six A groups, A1 to A6, according to cotyledon inoculation on *B. napus* cultivars ‘Lirabon’, ‘Glacier’, ‘Quinta’, and ‘Jet Neuf’ (Badawy et al 1991; Kuswinanti et al 1995). In this classification, response in the winter type *B. napus* cultivar ‘Lirabon’ actually replaced the spring cultivar ‘Westar’ in the PG classification. Also, *B. napus* cultivar ‘Jet Neuf’ was added to split each PG2, PG3, and PG4 into two groups, respectively, totaling, six groups assigned to “A” of the A/B pathogenicity designation (Balesdent et al 2002; Li et al 2013).

Regarding virulence as an expression of pathogenicity, various combinations of avirulence genes in *L. maculans* were found to induce resistance in cultivar ‘Quinta’, ‘Glacier’, and ‘Jet Neuf’, and these avirulence genes were used to divide *L. maculans* (Balesdent et al 2005; Rouxel et al 2005). The gene *AvrLm1* induces blackleg resistance in

'Quinta' (Ansan-Melayah et al 1995); *AvrLm2* and *AvrLm3* trigger blackleg resistance in 'Glacier' (Balesdent et al 2002), and *AvrLm4* triggers blackleg resistance in 'Jet Neuf' (Balesdent et al 2001). With the discovery of numerous combinations of avirulence genes, previous terminology was found inadequate to describe the diversity of *L. maculans* at a race level. Accordingly, this terminology was replaced with a new terminology for races of *L. maculans* using avirulence genes. Races of *L. maculans* are described as the avirulence gene composition (Balesdent et al 2005; Rouxel et al 2005).

Previously, nine *L. maculans* avirulence genes (*AvrLm1-9*) had been mapped, and seven were grouped into two genetic clusters, *AvrLm1-2-6* and *AvrLm3-4-7-9* (Balesdent et al 2002; Gout et al 2006). Currently, seven of the nine *L. maculans* avirulence genes (*AvrLm1, AvrLm2, AvrLm3, AvrLm4-7, AvrLm6, AvrLm11*, and *AvrLmJ1*) have been cloned (Balesdent et al 2013; Fudal et al 2007; Gout et al 2006; Ghanbarnia et al 2015; Plissonneau et al 2015; Parlange et al 2009; Van de Wouw et al 2014).

With collection and survey of 96 *L. maculans* isolates in Canada over the period of 1997 to 2005 it was observed that *AvrLm2, AvrLm6, AvrLm10*, and *AvrLepR3* were found in over 90 % of the isolates. Avirulence genes *AvrLm3, AvrLm4, AvrLm5*, and *AvrLm7* were found in 10.4 to 29.2 % of the 96 isolates surveyed (Kutcher et al 2010a). A recent analysis of 674 isolates collected and surveyed in Canada in 2010 and 2011 showed that *AvrLm2, AvrLm4, AvrLm6*, and *AvrLm7* were detected in more than half of the isolates. And Liban et al (2015) observed that the frequency of *AvrLm3* had reduced to 8 % in Canadian *L. maculans* populations.

2.3 Blackleg disease

2.3.1 Blackleg disease symptoms

Two types of symptoms, including canola/rapeseed leaf lesions and stem cankers, are caused by *L. maculans* in its different developmental stages (Canola Council of Canada 2014). Leaf lesions on both cotyledons and true leaves at the vegetative growth stage are usually grey-green water stains or yellow-brown eruptions and include small black pycnidia (Rimmer et al 1995). Stem cankers, seen as pale necrotic lesions with a dark margin, appear at the base of the stems of adult plants, at and around flowering time and after, while infected roots display dark discoloration (Hammond et al 1985; Rimmer et al 1995).

2.3.2 Blackleg disease cycle

The life cycle of *L. maculans* (Figure 2.2) includes a symptomless biotropic period with the infection of cotyledons, leaves and stems and necrotropic phases (Delourme 2012; Hall 1992; Williams 1992). The primary inoculum of the disease is mainly from pycnidia (conidia or pycnidiospores) and pseudothecia (ascospores) in crop residue and on seeds (Hall 1992). Conidia are released from pycnidia and scattered by rain (Travadon et al 2007; Vanniasingham et al 1989). Ascospores are produced from pseudothecia and are wind disseminated as far as 1.5 to 8 km from the source (Bokor et al 1975; Gladders et al 1980). Conidia and ascospores germinate to produce hyphae which penetrate into cotyledons and leaves through stoma or wounds (Hammond et al 1985; Rimmer et al 1995).

Subsequent to infestation, the fungus interacts with the plant by killing cells and tissue to result in symptoms of green or green-gray leaf lesions (Rimmer et al 1995). At the same time, nutrients from the necrotized plant lesions are utilized to produce pycnidia (Williams

1992). Conidia and ascospores produce hyphae that grow to the stem through the petiole. Within the plant tissue, the fungus mainly colonizes the xylem cells, intercellular spaces and cells of the cortex (Hammond et al 1985; Sexton et al 2001). *Leptosphaeria maculans* infection of the stem cortex often results in cankers at the base of the stem, and canker girdled plants tend to lodge in the field (Hammond et al 1985, 1987). Stem cankers are usually caused by pycnidiospores that originate from a large number of pycnidia growing on leaf or cotyledon lesions. However, stems can also be infected by ascospores released from pycnidia in crop residue of the same or neighboring fields (Gugel et al 1992; Hammond et al 1985). Hammond et al (1985) reported that undamaged petioles and stems can not be infected by pycnidiospores or ascospores directly. Therefore, the main pathway for stem cankers is through leaves and petiole into stems (Hammond et al 1985).

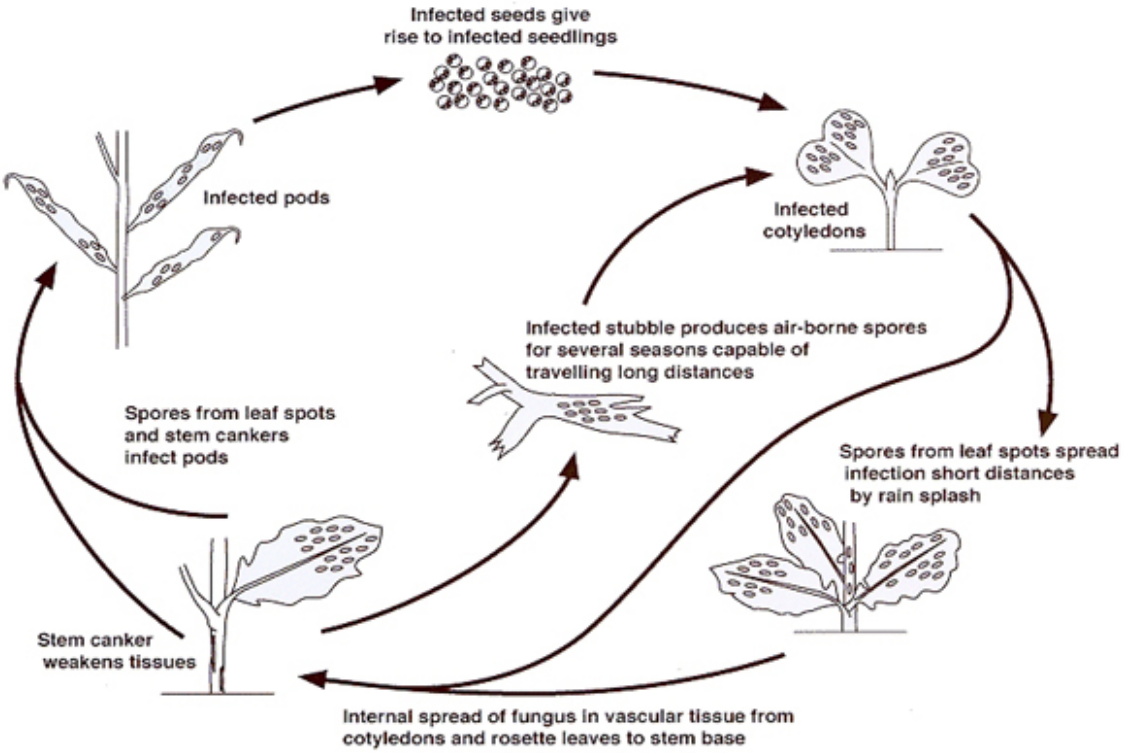


Figure 2.2 Blackleg disease cycle (Diagram courtesy of Department of Biology, University of Saskatchewan).

2.3.3 History of blackleg disease

Blackleg disease was identified in the early eighteen century in Europe and America (Henderson 1918). The yearly incidence of blackleg disease in France has been reported to be up as high as 50 % to 80 % (Gugel et al 1992). Actually, the production of rapeseed in Canada was not significantly limited by blackleg disease until 1961 (Vanterpool 1961). However, since 1966 blackleg disease has increased in Canada, and even more so in Europe as well as in some other growing areas of the world (Gugel et al 1992).

In 1968, rapeseed was introduced into Australia as an alternative crop to wheat. As early as 1972, blackleg disease caused significant reduction in Australian rapeseed production (Gugel et al 1992; McGee et al 1977). To the contrary, blackleg disease was not serious in China and India where rapeseed production had occurred for centuries (Rimmer et al 1995). In China, the stems were always used as fuel, and rapeseed lands were subsequently flooded to produce rice. This practice led to the rapid decomposition of infected residue in warm and wet conditions, reducing the source of inoculum and the risk of blackleg (Fitt et al 2006; Rimmer et al 1995). In India, the low incidence of blackleg was probably attributed to the use of *B. juncea* with the high blackleg resistance (Rimmer et al 1995).

In Canada, after rapeseed production surged from 1965 to 1971, production losses caused from blackleg spread across the provinces of Saskatchewan and Manitoba, particularly since 1975 (McGee et al 1978; Peter 1978; Runciman et al 1975). Continual rapeseed yield losses stirred establishment of breeding programs aimed at developing blackleg resistant cultivars. In 1978, a cultivar ‘Wesreo’ that contained blackleg resistance from a French cultivar ‘Major’ was developed and released (Roy 1978a). In the next year,

more blackleg resistant cultivars were developed, i.e. ‘Jet Neuf’ and ‘Rafal’ (Gugel et al 1992). However, blackleg resistance was reported to have broken down in Saskatchewan by 1984 and 1985 (Petrie 1986).

2.3.4 Blackleg disease management

Blackleg disease is caused by the interaction of three factors, host, pathogen, and environment (Agrios 1988). Resistant cultivars, crop rotation, fungicide application, seed treatment, and control of volunteers are common strategies used in the management of blackleg disease. The sustainable and economic strategies to manage this disease cannot employ only one management strategy (Kutcher et al 2013). For example, the utilization of blackleg resistant cultivars does not necessarily mean a short rotation can be adopted. A completely integrated management strategy is needed for effective management of blackleg disease in most canola/rapeseed growing regions of the world (Gugel et al 1992; Rimmer et al 1995).

Three general blackleg disease management strategies, i.e. resistant cultivars, crop rotation, and fungicide application, will now be discussed.

2.3.4.1 Resistant cultivars

The development of resistant cultivars is the most important approach to manage blackleg disease. Starting with the eighteen century resistant cultivars for blackleg were available in Australia, Canada, and Europe (Gugel et al 1992). In Europe, the original winter *B. napus* cultivars were derived from a French cultivar ‘Jet Neuf’, while the primary spring *B. napus* cultivars included a French cultivar ‘Cresor’ and Australian cultivars ‘Maluka’ and ‘Taparoo’ (Roy et al 1983; Delourme et al 2006). Blackleg resistance in many Australian

cultivars was obtained from Japanese cultivars, like ‘Chisaya’, ‘Chikuzen’, and ‘Mutu natane’ (Rimmer et al 1995; Salisbury et al 1999). Japanese spring cultivars and French winter cultivars are still considered as the principal resource of blackleg resistance in Australian breeding programs (Salisbury et al 1999).

Some blackleg resistant *Brassica* cultivars are used to effectively restrict blackleg disease causing devastating yield losses. For example, the introduction of *B. napus* cultivar ‘Jet Neuf’ in Europe was successful at managing the prevalent blackleg disease at the time (Rimmer et al 1992). However, an increasing number of reports showed that the blackleg resistance from the primary resistant cultivars had broken down due to the appearance of more virulent strains of *L. maculans* (Brun et al 2000; Li et al 2003b; Sprague et al 2006). In Canada, over one hundred canola cultivars with blackleg resistance were registered (Rimmer 2006). However, Zhang et al (2015) reported that of 104 registered Canadian canola cultivars, 56.7 % carry the same blackleg resistance gene *Rlm3* and 30.8 % carry only the single *Rlm3* blackleg resistance gene.

2.3.4.2 Crop rotation

Crop rotation is considered a single effective natural practice for reduction of the risk of incidence blackleg disease. Crop rotation is primarily aimed at limitation of blackleg inoculum, i.e. ascospores from pseudothecia and conidia (pycnidiospores) from pycnidia (Gugel et al 1992). The control of cruciferous weeds (volunteers) significantly reduces inoculum of blackleg ascospores infesting young canola plants, as pseudothecia produced on these volunteers, and the source of these spores, are limited (Petrie 1975).

Crop rotation addresses blackleg inoculum arising from pycnidia and conidia by

reducing crop residues, the source of the infective agents, through natural decomposition processes. Such decomposition requires time to effectively limit infected residue, thereby reducing the primary inoculum of conidia (Gugel et al 1992). The period of recommended crop rotation is distinct in different countries according to climatic conditions in each growing region. For example, three or four year crop rotations are recommended in western Canada due to the more limited decomposition of residue in the frozen soil during the long winter (Kutcher et al 2013). In Australia, the effective period of crop rotation is similar to that of western Canada, since the hot and dry environment limits the rate of residue decomposition (Marcroft et al 2011). However, in Europe, the temperate climate and deep plowing practices increase the decomposition rate of infected residue (Rimmer et al 1995).

2.3.4.3 Fungicide application

Fungicide applications practices for blackleg disease control include foliar spray and seed treatment. Fungicide applications are usually a last resort due to cost limitations and the potential negative impact on applicator health and the environment (Gugel et al 1992; Kutcher et al 2011a). Furthermore, it is difficult to obtain effective and economically viable results using a foliar fungicide application, especially on winter-type production because ascospores are discharged over the entire growing season (Downey et al 1989). Therefore, timing of fungicide application is critical for efficient control of disease (Gladders et al 1998; West et al 1999). An effective foliar fungicide application can be achieved only if it coincides with the climax of ascospores release (West et al 1999).

In both Australia and Canada, fungicide applications showed varied levels of success (Elliott et al 2011; Kutcher et al 2011a; Marcroft et al 2008). Effective control of blackleg

disease has been demonstrated in England through the combination of two or more types of fungicides, but the results were inconsistent (Rawlinson et al 1979). Peng et al (2015) reported that application of three registered fungicides (Headline, Quadris, and Quilt Xcel) on the susceptible cultivar 'Westar' at the 2 to 4 leaf stage reduced blackleg across Alberta, Saskatoon, and Manitoba.

Elliott et al (2011) reported that seed treatment was more effective in reducing the severity of blackleg than a foliar fungicide application. In Canada, seed treatment was initiated in 1978 (Petrie 1979). Currently, Syngenta canola seeds are treated with Helix Vibrance in Canada, which prevents early-season diseases, like seed-borne blackleg. Seed treatment was also applied in Australia, where it reduced severe blackleg disease infection as well as mortality of susceptible canola cultivars in the field (Marcroft et al 2008). However, seed treatment had no effect on controlling blackleg in the areas where the disease had prevailed (Gugel et al 1992).

2.4 Blackleg resistance

The resistance to *L. maculans* in canola can be classified into two types: qualitative resistance and quantitative resistance (Balesdent et al 2001; Rimmer et al 1992). Qualitative resistance is assigned to cases of race-specific resistance controlled by a few resistance genes and is called vertical (Rimmer 2006). It is temporally displayed from the cotyledon to the adult stages (Delourme et al 2006; Rimmer 2006). Such resistance is suggested to be consistent with a gene-for-gene interaction (Ansan-Melayah et al 1995; Delourme et al 2006; Rimmer 2006). Quantitative resistance is assigned to cases in which there is regulation by

many genes with minor effects (Hayward et al 2012; Rimmer 2006). This type of resistance is considered to be horizontal and race non-specific, which affects the adult stage (Delourme et al 2006; Hayward et al 2012; Rimmer 2006).

In actuality, there have been studies which have established that adult resistance to blackleg is not only controlled by polygenes but may also be controlled by single major genes (Dion et al 1995; Ferreira et al 1995; Mayerhofer et al 2005; Rimmer et al 1999). For example, Dion et al (1995) detected a single major resistance gene, *LmFr1*, in *B. napus* cultivar 'Cresor', which controlled blackleg disease at the adult stage.

In previous studies, more than a dozen race-specific resistance genes, *Rlm1* to *Rlm11*, *LepR1* to *LepR4*, *BLMR1*, and *BLMR2*, have been inferred in *Brassica* species (Table 2.1) (Raman et al 2013). Some of these resistance genes have been mapped in *B. napus* or introgressed from the related species to *B. napu*s (Delourme et al 2006; Raman et al 2013). To date, some race-specific resistance genes, like *Rlm1* and *LepR3* in France and Australia respectively, have been introduced into commercial cultivars, and have been reported to no longer impart resistance to blackleg due to the evolution of *L. maculans* populations (Yu et al 2013). *Rlm4* is the most common resistance gene in Australian *Brassica* cultivars especially prior to 2002 (Marcroft et al 2012a; Rouxel et al 2003b). A recent paper reported that *Rlm3* is a dominant gene in Canadian *B. napus* accessions and germplasm (Zhang et al 2015). *Rlm1, Rlm2, Rlm4, Rlm9, RlmS, LepR1*, and *LepR2* showed low presence in 104 Canadian *B. napus* accessions, and *Rlm6* and *Rlm7* were not detected (Zhang et al 2015).

Table 2.1 Blackleg resistance genes in *Brassica* species.

Species	R gene	Map position	Cultivar	Reference
B. napus	*Rlm1*	N7	Quinta	Ansan-Melayah et al (1998)
			Maxol	Delourme et al (2004)
B. napus	*Rlm2*	N10	Glacier	Ansan-Melayah et al (1998)
			Darmor	Delourme et al (2004)
B. napus	*Rlm3*	N7	Maxol	Delourme et al (2004)
B. napus	*Rlm4*	N7	Quinta	Balesdent et al (2001)
				Delourme et al (2004)
B. juncea	*Rlm5*		Aurea, Picra	Balesdent et al (2002)
B. juncea	*Rlm6*		Breeding line	Chevre et al (1997)
B. napus	*Rlm7*	N7	23.1.1	Delourme et al (2004)
B. rapa	*Rlm8*		156-2-1	Balesdent et al (2002)
B. napus	*Rlm9*	N7	Darmor-bzh	Delourme et al (2004)
B. nigra	*Rlm10*	B4	Addition line	Delourme et al (2008)
				Eber et al (2011)
B. rapa	*Rlm11*		02-159-4-1	Balesdent et al (2013)
B. rapa	*RlmS*		Surpass 400	Van de Wouw et al (2009)
B. rapa	*LepR1*	N2	Breeding line	Yu et al (2005)
B. rapa	*LepR2*	N10	Breeding line	Yu et al (2005)
B. rapa	*LepR3*	N10	Surpass 400	Yu et al (2008)
B. rapa	*BLMR1*	N10	Surpass 400	Long et al (2011)
B. rapa	*BLMR2*	N10	Surpass 400	Long et al (2011)
B. rapa	*LepR4*	N6	Breeding line	Yu et al (2013)
B. juncea	*LMJR1*	J13(B3)	AC Vulcan	Christianson et al (2006)
B. juncea	*LMJR2*	J18(B8)	AC Vulcan	Christianson et al (2006)
B. juncea	*Rjlm2*			Saal et al (2005)

2.4.1 Qualitative resistance in *Brassica napus*

Several major resistance genes to *L. maculans* have been mapped by using doubled haploid (DH) populations derived from single F_1 plants between resistant and susceptible cultivars (Ferreira et al 1995; Mayerhofer et al 1997; Rimmer et al 1999). A race-specific blackleg resistance gene *LEM1* was mapped to linkage group 6 using a DH population derived from the cross between resistant 'Major' and susceptible 'Stellar', using a PG2 blackleg isolate PHW1245 (Ferreira et al 1995). In another report, Mayerhofer et al (1997) used DH populations derived from the cross of a resistant cultivar 'Shiralee' and susceptible breeding lines of *B. napus* to map a major blackleg resistance locus *LmR1* and explored the resistance to *L. maculans* isolates in western Canada. However, it is clear that *LEM1* and *LmR1* are located in distinct map positions on the same chromosome (Mayerhofer et al 1997). Rimmer et al (1999) mapped cotyledon and adult blackleg resistance genes *cRLMc*, *cRLMrb*, *aRLMrb*, and *aRLMm* in populations derived from the crosses of a susceptible cultivar 'Westar' and resistant cultivars 'Cresor', 'Maluka' and 'RB87-62'. The resistance gene *cRLMc* in 'Maluka' was possibly linked to the resistance gene *cRLMrb* in 'RB87-62' (Rimmer et al 1999). Meanwhile, the resistance gene *cRLMc* was also linked to a major adult resistance locus *LmFr1* in 'Cresor' (Dion et al 1995; Rimmer et al1999). The location of RFLP makers proved that *LEM1* was located at the bottom of the *cRLMm* locus region in linkage group 6 (Rimmer et al 1999). Even though different makers were used to map *LmR1* and *cRLMm*, it is likely that they are identical due to the similar pedigree of 'Maluka' and 'Shiralee' (Delourme et al 2006; Mayerhofer et al 2005; Rimmer et al 1999).

Several race-specific blackleg resistance genes have been mapped in *B. napus* by using

the gene-for-gene interaction between avirulence (*AvrLm*) genes in pathogen and resistance (*Rlm*) genes in plants (Delourme et al 2004). *Rlm1*, *Rlm3*, *Rlm4*, *Rlm7*, and *Rlm9* were mapped in linkage group N7, while *Rlm2* was mapped in linkage group N10 (Delourme et al 2004). *Rlm1* was found in 'Quinta' originally and was also found in the *B. napus* cultivar 'Maxol', and corresponded to avirulence gene, *AvrLm1*. *Rlm2* was observed in 'Glacier', and later found in 'Samourai', and corresponds to *AvrLm2* in *L. maculans* (Ansan-Melayah et al 1998; Delourme et al 2004). Balesdent et al (2001) detected the blackleg resistance gene *Rlm4* in 'Jet Neuf' using the interaction with *AvrLm4*. Subsequently, Delourme et al (2004) mapped *Rlm4* in 'Quinta', which also harbored *Rlm1*. It was suggested that *Rlm1* and *Rlm4* are linked in linkage group N7. A single gene, labeled *Rlm3* in 'Maxol', was found to be resistant to *AvrLm3* blackleg isolates. *Rlm1* was also suggested to be linked to *Rlm3*, but not allelic. Therefore, it was concluded that *Rlm1* was distinct from *Rlm3* and *Rlm4* (Delourme et al 2004). In a *B. napus* accession '23-1-1' *Rlm7* was postulated to impart resistance to avirulence gene *AvrLm7* (Balesdent et al 2002; Delourme et al 2004). *Rlm9* was observed in *B. napus* cultivar 'Darmour' as a major blackleg resistance gene distinguished from a minor one in linkage group N10 (Delourme et al 2004). However, the relationship among *Rlm3*, *Rlm4*, *Rlm7*, and *Rlm9* was not clear. These R genes could be tightly linked to form a cluster or they might be different alleles of the same gene (Delourme et al 2004, 2006; Leflon et al 2007; Long et al 2011).

2.4.2 Qualitative resistance in *Brassica rapa*

Crouch et al (1994) used three wild accessions of *B. rapa* subsp. *sylvestris* to cross with *B. oleracea* subsp. *alboglabra*. It was the first time the synthetic amphidiploid *B. napus* with

blackleg resistance from wild *B. rapa* was obtained (Crouch et al 1994). After Pacific seeds Pty Ltd. improvement of seed quality using crosses of canola varieties and synthesis of *B. napus* with blackleg resistance from *B. rapa* subsp. *sylvestris*, canola varieties 'Surpass 400' and others with this blackleg resistance were released in Australia in 2001-2002 (Buzza et al 2002). 'Surpass 400' resistance to *L. maculans* was reported to be controlled by a single dominant gene at both the seedling and adult stages inoculated by Australian isolates (Li et al 2003a).

Subsequently, several race-specific resistance genes *LepR1*, *LepR2*, *LepR3*, *LepR4, BLMR1,* and *BLMR2,* which were introgressed from wild *B. rapa* subsp. *sylvestris* into *B. napus,* have been mapped (Long et al 2011; Yu et al 2005, 2008, 2013). *LepR1* was mapped in linkage group N2, while *LepR2* was in linkage group N10 in DH95 and DH96 populations. These two DH lines were derived from the backcross of the original F_1 population developed by Crouch et al (1994). Here, *LepR2* showed an intermediate level of resistance to most *L. maculans* isolates from Canada, Australia, Europe, and Mexico. *LepR1* conferred a high level of blackleg resistance preventing fungal penetration (Yu et al 2005). *LepR3* was mapped in linkage group N10 in 'Surpass 400' and has been recently cloned (Larkan et al 2013; Yu et al 2008). It has also been identified to encode a receptor-like protein and causes an incompatible reaction with *AvrLm1*, which corresponds to the blackleg resistance gene *Rlm1* (Ansan-Melayah et al 1998; Larkan et al 2013). However, the isolate 3R5 that was used to test the blackleg resistance in 'Surpass 400' has been found to harbor both *AvrLm1* and *AvrLmS*. Therefore, *RlmS* was hypothesized as another blackleg resistance gene in 'Surpass 400' (Larkan et al 2013; Van de Wouw et al 2009). A recent report showed that *LepR3* was

co-localized with another blackleg resistance gene, *Rlm2*, in the same linkage group N10 (Larkan et al 2014). *BLMR1* and *BLMR2* were mapped in linkage group N10 in 'Surpass 400'. *BLMR1* was fine mapped and showed complete resistance to a blackleg isolate 87-41, and *BLMR2* provided intermediate resistance to this isolate (Long et al 2011). Based on its location in linkage group N10, *LepR3* was considered to be identical to *BLMR1* (Larkan et al 2013; Long et al 2011). Two alleles at the *LepR4* locus, *LepR4a* and *LepR4b*, were mapped in linkage group N6 and reported to be recessive in nature. *LepR4a* showed a high level of both cotyledon and adult resistance to *L. maculans*. Unlike *LepR4a*, *LepR4b* was only resistant to some blackleg isolates and conferred weak adult resistance to blackleg in the field (Yu et al 2013).

In addition to the above blackleg resistance genes, a blackleg resistance gene in *B. rapa* termed *Rlm8* was recognized by the interaction with a *AvrLm8 L. maculans* isolate (Balesdent et al 2002). *Rlm11* was another single major resistance gene corresponding to *AvrLm11* in *B. rapa* accession '02-159-4-1' (Balesdent et al 2013). All the resistance genes described above are located in the A genome of *Brassica* species. To date, no resistance gene has been mapped in the C genome of *B. oleracea* and *B. napus* (Rimmer et al 1992, 2006).

2.4.3 Qualitative resistance in the B genome containing *Brassica* species

Brassica species with the B genome, *B. nigra*, *B. juncea*, and *B. carinata*, have been described as exhibiting a high level of blackleg resistance (Gugel et al 1990; Helms et al 1979; Keri 1991; Roy 1978b). Cotyledon resistance to blackleg observed in *B. nigra, B. juncea, and B. carinata* were considered to be controlled by genes most probably located in the B genome (Roy 1984; Rimmer et al 1992; Sacristan & Gerdemann 1986; Sjodin et al 1988). Since

genome triplication in each of the A, B, and C genomes was suggested before their divergence (Mason et al 2010), the conserved chromosomal regions and sequence similarity are shared among the A, B, and C genomes (Mason et al 2010; Raman et al 2013). The B genome has less polymorphism than the A genome, but the B genome shares some common segments with the A and C genomes (Panjabi et al 2008). The homeologous crossovers between the A, B and C subgenomes could occur to some degree (Panjabi et al 2008). Therefore, it should be possible to transfer blackleg resistance from the B genome of *B. nigra*, *B. juncea*, and *B. carinata* into the A or C genomes in *B. napus* (Navabi et al 2011).

2.4.3.1 Qualitative resistance in *Brassica nigra* and *Brassica juncea*

Due to a high level of blackleg resistance in the *Brassica* species containing the B genome, numerous attempts have been made at introgressing blackleg resistance from *B. nigra* and *B. juncea* into *B. napus* (Chevre et al 1996, 1997; Gerdemann-Knorch et al 1995; Plieske et al 1998; Roy 1978b, 1984; Sacristan et al 1986; Struss et al 1996; Zhu et al 1993). Early studies achieved the introgression of blackleg resistance genes from *B. juncea* into *B. napus* through homeologous recombination between *B. napus* and *B. juncea* (Barret et al 1998; Chevre et al 1997; Pang et al 1996; Plieske et al 1998; Roy 1984). It proved difficult to obtain stable introgression of blackleg resistance from *B. nigra* and *B. carinata* into *B. napus* through interspecific hybridization (Chevre et al 1996; Roy 1984; Sacristan et al 1986). This difficulty was probably due to the low level of recombination between the A or C subgenomes of *B. napus* as well as the fact that the B genome harbors the resistance gene in *B. nigra* or *B. carinata* (Barret et al 1998; Leflon et al 2007; Saal et al 2004).

At least three different chromosomes in *B. nigra* may contain blackleg resistance (Zhu

et al 1993). Blackleg resistance at the seedling stage was observed in *B. nigra* on chromosome B4 (Chevre et al 1996). However, chromosome B8 was characterized to carry blackleg resistance in *B. juncea* (Chevre et al 1997). Genetic analysis of inheritance of blackleg resistance in the DH line and RIL populations suggested that blackleg resistance in *B. juncea* was under the control of two single genes (Chevre et al 1997; Keri et al 1997; Rimmer et al 1992). By using independent crosses between one susceptible and three resistant *B. juncea* accessions, Keri et al (1997) reported that one dominant gene and one recessive gene controlled blackleg resistance in *B. juncea.*

To date, blackleg resistance genes, *Rlm5*, *Rlm6*, *rjlm2*, *LMJR1* and *LMJR2*, have been detected in *B. juncea* and presence of *Rlm10* has been deduced for *B. nigra* (Balesdent et al 2002; Chevre et al 1997; Christianson et al 2006; Delourme et al 2008; Eber et al 2011; Saal et al 2005; Eber et al 2011 cited by Raman et al 2013). *Rlm5* was detected in two *B. juncea* cultivars, 'Aurea' and 'Picra', and shown to interact with *AvrLm5* (Balesdent et al 2002). *Rlm6,* which interacts with *AvrLm6* (Balesdent et al 2002), is most likely located on chromosome B8 of the two *B. juncea* cultivars and is identical to the locus identified by Chevre et al (1997). *Rlm10* was reported to be in linkage group B4 of *B. nigra* (Delourme et al 2008; Eber et al 2011). Saal et al (2004, 2005) reported a recessive blackleg resistance gene *rjlm2* that was derived from the B genome of *B. juncea*. Subsequently, *LMJR1,* a dominant gene, and *LMJR2*, a recessive gene, were been mapped on J13 (B3) and J18 (B8) of *B. juncea* (Christianson et al 2006; Raman et al 2013).

2.4.3.2 Qualitative resistance in *Brassica carinata*

Sacristan and Gerdemann (1986) compared *B. juncea* and *B. carinata* as possible sources for transferal of blackleg resistance genes into *B. napus*. They reported that blackleg resistant plants were almost lost in the first backcross between *B. carinata* and *B. napus*. Navabi *et al.* (2010) successfully transferred fragments of the B genome blackleg resistance genes from *B. carinata* into *B. napus* through an advanced backcross method. The authors backcrossed twice and selfed three times to produce doubled haploid lines that carried stable B genome chromosomal fragments. Moreover, the B genome chromosomes were tracked by genomic in situ hybridization (GISH) analysis. The results showed that the chromosomes from the B genome appeared to be lost while some introgressed fragments remained over several generations. These results were not consistent with previous studies where the B genome chromosomes were eliminated in the early generations of interspecific hybrids (Li et al 2004; Navabi et al 2010, 2011). By using simple sequence repeat (SSR) markers to analyze the genotype of the B chromosomes, chromosome B3 from *B. carinata* was shown to exhibit the blackleg resistance in the cotyledon stage (Fredua-Agyeman et al 2014). However, none of the B chromosomes present in the DH lines contained blackleg resistance. One SSR markers, SN9756, was associated with cotyledon resistance to *L. maculans* in the C genome (Rahman et al 2007). By the identification of SSR markers, Rahman et al (2007) demonstrated that blackleg resistance genes from *B. carinata* were introgressed into the A or C genomes of *B. napus.*

2.4.4 Quantitative resistance

Quantitative resistance to *L. maculans* is controlled by many genetic factors that have

been mapped in quantitative trait loci (QTL) studies (Yu et al 2013). Even though quantitative resistance is partial and provides only moderate resistance to *L. maculans*, it is more durable than the blackleg resistance controlled by race-specific genes (Brun et al 2000). Several studies have explored quantitative resistance to blackleg in the *B. napus* cultivar 'Jet Neuf', which shows a high level of blackleg resistance in the field during the adult stage. With wide use in Europe, blackleg resistance of 'Jet Neuf' persisted throughout the 1970s and 1980s (Delourme et al 2006). A French *B. napus* cultivar 'Darmor' with quantitative resistance to *L. maculans* was derived from 'Jet Neuf' through backcrosses (Delourme et al 2008; Pilet et al 1998).

In a number of studies, quantitative resistance to blackleg derived from *B. napu*s has been reported as QTLs that were associated with blackleg resistance in the field in a number of studies (Rimmer et al 2006). Two QTLs on LG12 and LG21 were associated with field blackleg resistance. They were detected in DH lines that were derived from a cross between the resistant 'Major' and the susceptible 'Stellar' cultivars (Ferreira et al 1995). Subsequently, several studies identified QTLs that were associated with quantitative blackleg resistance originally derived from 'Jet Neuf'. Pilet et al (1998) detected ten QTLs for blackleg resistance from the cross of 'Darmor-bzh' × 'Yudal'. Four out of these ten QTLs contributed to the reduction of blackleg disease severity in field surveys done in 1995 and 1996 (Pilet et al 1998). Furthermore, in a cross between the resistant cultivar 'Darmor' and the moderately resistant cultivar 'Samourai', Pilet et al (2001) detected ten QTLs for quantitative resistance to *L. maculans*. Among the ten QTLs, six were identified in a DH line population while others were in an $F_{2:3}$ population derived from the same parents (Pilet et al 2001). In another

report, 16 QTLs were detected in a DH line population and $F_{2:3}$ families, while only four QTLs were commonly identified in both crosses 'Darmor-bzh' × 'Yudal' and 'Darmor' × 'Samourai' (Delourme et al 2006; Rimmer et al 2006). Also, Pilet et al (1998, 2001) observed that three QTLs were detected in two DH line populations from 'Darmor-bzh' × 'Yudal' and 'Darmor' × 'Samourai', and one QTL was in the DH population from 'Darmor-bzh' × 'Yudal'. The four QTLs from 'Darmor-bzh' × 'Yudal' were further analyzed through near isogenic lines with 'Darmor-bzh' (Delourme et al 2008). Furthermore, Jestin et al (2011) used association mapping to validate the previous QTLs detected in the cultivar 'Darmor'. In addition, QTLs associated with blackleg resistance were detected in populations derived from four crosses among five Australian *B. napus* cultivars (Kaur et al 2009).

2.5 Durability of blackleg resistance

The development of durable blackleg resistance is becoming a significant objective for *Brassica* breeding programs (Brun et al 2010; Rimmer et al 2006). Sudden breakdown of blackleg resistance happened in some canola production regions, including a 90% yield loss in Australia (Sprague et al 2006). As a result of breakdown of several blackleg resistance genes in different cultivars, pathologists and breeders have realized the importance and priority of the development of durable blackleg resistance in oilseed rape (Rouxel et al 2003a).

Several cultivars with major resistance genes have been reported to be infected by *L. maculans* in experimental fields or on commercial farms (Brun et al 2000, 2001; Li et al 2003b; Sprague et al 2006). Blackleg field evaluation has demonstrated that the resistance

gene *Jlm1* introduced from *B. juncea* to *B. napus* was overcome by *L. maculans* isolates from their own residues in the third year (Brun et al 2000). Li et al (2003a) initially reported that the *B. napu*s cultivar 'Surpass 400', having a single dominant blackleg resistance gene and grown in a western Australian experimental field, was overcome by *L. maculans*. The resistance gene was derived from *B. rapa* subsp. *sylvestris* (Li et al 2003a). Subsequently, the breakdown of the blackleg resistance introgressed from *B. rapa* subsp. *sylvestris* was reported in South Australia after this commercial cultivar had been released for only three years (Sprague et al 2006). Blackleg resistance decrease from breakdown of the resistance gene *Rlm1* has been observed in three independent commercial farms, which could reflect the situation in France from 1994 to 2000 (Rouxel et al 2003a). Resistance gene *Rlm1* effectively controlled blackleg disease in France when the frequency of *AvrLm1* in pathogen was over 80 %. However, the frequency of *AvrLm1* reduced to 19.6 % after introduction of commercial cultivars with *Rlm1* was widely used in France (Balesdent et al 2006; Rouxel et al 2003a).

Race-specific resistance genes were suggested to cause an additional selection pressure on *L. maculans*, which resulted in genetic change of *L. maculans* isolates in the same field (Sprague et al 2006). Race-nonspecific resistance to *L. maculans* should be more durable than race-specific resistance because of lower selection pressure on the virulence of *L. maculans* (Brun et al 2000). Therefore, using race non-specific genes in commercial cultivars may increase the durability of race-specific resistance genes (Brun et al 2000, 2010; Sprague et al 2006). One strategy of increasing the durability of blackleg resistance is to combine single major race-specific resistance genes to *L. maculans* with minor race-nonspecific resistance genes or pyramid several race-specific resistance genes from different resistant sources into

one cultivar (Brun et al 2001; Rimmer et al 2006; Sprague et al 2006; Stachowiak et al 2006). However, pyramiding of multiple race-specific genes leads to the selection pressure towards *L. maculans* with the corresponding avirulence genes (Marcroft et al 2012b). In western Canada, blackleg management has achieved success in the past 20 years using mainly resistant cultivars and crop rotation (Kutcher et al 2011b; Peng et al 2015). However, more than half of Canadian *B. napus* cultivars currently carry the same resistance gene *Rlm3* (Zhang et al 2015). The limited blackleg resistance gene in the commercial cultivars will be a challenge in managing blackleg disease by rotation of race-specific resistance genes (Zhang et al 2015). Liban et al (2015) have reported that the frequency of *AvrLm3* is only 8.0 % in Canadian *L. maculans* populations. Rotation of *B. napus* cultivars with blackleg resistance genes that have a high frequency of the corresponding avirulence genes will slow the breakdown of *Rlm3* in Canada (Zhang et al 2015). Therefore, the knowledge of dominant blackleg resistance genes in commercial *Brassica* cultivars and frequency of avirulence genes in the pathogen population is useful in developing effective strategies for maintaining durable resistance (Kutcher et al 2010a, 2010b; Zhang et al 2015).

2.6 Objectives of this project

Blackleg is the most damaging disease in the production of canola in Canada, Australia, and Europe (Fitt et al 2006; Howlett 2004). Since canola has an important contribution to Canadian economy, blackleg disease is a threat to the economy of Canada (Canola Council of Canada 2013). Currently, the change of *L. maculans* race structure is a challenge for blackleg management using genetic resistance and a four year rotation (Zhang et al 2015). It is

necessary to know blackleg resistance genes in current commercial cultivars and the frequency of avirulence genes in *L. maculans* populations (Marcroft et al 2012a, 2012b; Zhang et al 2015). Novel blackleg resistance derived from *Brassica* relatives is useful in commercial cultivars, which enhances blackleg resistance in canola (Zhang et al 2015). *Brassica carinata* and synthetic hexaploid *Brassica* species 'Meng' carry a high level of blackleg resistance (Gugel et al 1990; Helms et al 1979; Keri 1991; Roy 1978b). The knowledge of blackleg resistance transferred from *B. carinata* and synthetic hexaploid *Brassica* species 'Meng' is beneficial for breeding blackleg resistant cultivars.

The objectives of this research are:

1. To transfer blackleg resistance from *B. carinata* cultivar 'T4001' and synthetic hexaploid *Brassica* species 'Meng' into *B. napus*.
2. To investigate the interaction between the blackleg resistance genes transferred from *B. carinata* cultivar 'T4001' and synthetic hexaploid *Brassica* species 'Meng' into *B. napus* and four *L. maculans* isolates (03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M).
3. To study the inheritance of blackleg resistance genes transferred from *B. carinata* cultivar 'T4001' and synthetic hexaploid *Brassica* species 'Meng' into *B. napus* in different backcross and selfing generations.

Chapter 3. Transferring blackleg resistance from *Brassica carinata* into *Brassica napus*

3.1 Abstract

Blackleg caused by *Leptosphaeria maculans* (Desm.) Ces. & De Not. is one of the most important diseases in canola production in the world, especially in Australia, western Canada, and Europe. Resistance to *L. maculans* was transferred from *Brassica carinata* A. Braun into *B. napus* L. through interspecific hybridization. To overcome low seed set, some individual plants in the BC_1 were subjected to tissue culture. One *L. maculans* isolate 03-15-03 was utilized to select resistant individual plants in each backcrossing and selfing generations. All plants in the F_1 showed complete resistance to *L. maculans*, which was similar to the *B. carinata* parent. According to the chi-square test for goodness of fit, the segregation of resistant and susceptible plants fit a 1:1 ratio in the BC_3 and BC_4 while the segregation of resistant and susceptible plants deviated from a 1:1 ratio in the BC_2. In the BC_3F_2, two tested families followed a 3:1 segregation ratio of resistant and susceptible plants. The results suggest that the resistance to *L. maculans* transferred from *B. carinata* into *B. napus* is controlled by a single locus.

3.2 Introduction

Canola developed from *Brassica napus* L. rapeseed contains low erucic acid (less than 2 %) and low glucosinolates (less than 30 umol g^{-1} of meal) (Rimmer et al 1992). Canola oil provides about 15% of the total vegetable oil in the world and contributes $19.3 billion to the Canadian economy annually (Canola Council of Canada 2013; Rahman et al 2013). However, blackleg caused by fungus *Leptosphaeria maculans* (Desm.) Ces. & De Not. is one of the

most serious diseases in canola, and causes serious yield losses especially in Europe, Australia and North America (Fitt et al 2006; Gugel et al 1992; Howlett, 2004; West et al 2001). Several blackleg resistance loci (*Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9*, *BLMR1*, *BLMR2*, *LepR1*, *LepR2*, *LepR3*, and *LepR4*) have been mapped on the A genome, and two of them, *LepR3* and *Rlm2*, have been cloned to date (Delourme et al 2004; Larkan et al 2013, 2015; Long et al 2011; Yu et al 2005, 2008, 2013). However, the breakdown of resistant cultivars with a single race-specific locus has been reported in canola (Sprague et al 2006). In order to extend the durability of blackleg resistance, it is necessary to identify more blackleg resistance genes from various resistance sources.

Several studies showed that the B genome containing *Brassica* species, *B. juncea* (L.) Czem. & Coss., *B. nigra* (L.) Koch, and *B. carinata* A. Braun, have a high level of resistance to *L. maculans*. Therefore, breeders attempted to introgress blackleg resistance from these species into *B. napus* (Chevre et al 1996, 1997; Gerdemann-Knorch et al 1995; Plieske et al 1998; Roy 1978b, 1984; Sacristan et al 1986; Struss et al 1996; Zhu et al 1993). Resistance to *L. maculans* in *B. carinata* was reported to be transferred into *B. napus* (Navabi et al 2010; Sacristan et al 1986). Rahman (2012) also reported the resistance in a *B. carinata* accession to various *L. maculans* isolates was transferred into canola at the University of Alberta. Researchers suggested that the complete resistance to *L. maculans* in *B. carinata* is from loci in the B genome (Roy 1984; Rimmer et al 1992; Sacristan et al 1986; Sjodin et al 1988).

Although the diploid genomes AA, BB, and CC were suggested to be derived from a common ancestor with a primary number of x=6 chromosomes (Attia et al 1986), the B genome is different from the A and C subgenomes, which makes it difficult to introgress *L.*

maculans resistance gene(s) to the B subgenome of *B. carinata* into the A or C subgenomes of *B. napus* (Lagercrantz et al 1996). The low homeology between the B and A or C subgenome is a limiting factor to transfer the high level of resistance to *L. maculans* from the B subgenome into the A or C subgenomes. However, it has been reported that homeologous recombinations between the B and AC subgenomes can still happen (Mason et al 2010; Parkin et al 2003, 2005). Therefore, it may be possible to introgress blackleg resistance genes between two different *Brassica* species.

The objective of this research was to introgress resistance to *L. maculans* from *B. carinata* into *B. napus*. The resistance to different *L. maculans* isolates and the inheritance of the transferred resistance was analyzed through cotyledon inoculation.

3.3 Materials and methods

3.3.1 Plant materials

Brassica napus cultivar 'Westar' and *B. carinata* 'T4001' were used for interspecific hybridization. 'Westar' is completely susceptible to *L. maculans* and used in blackleg research worldwide (Fredua-Agyeman et al 2014). 'T4001' shows a high level of resistance to *L. maculans* isolates. Seeds of 'T4001' and 'Westar' were obtained from the University of Manitoba. 'T4001' and 'Westar' were planted in a 54 × 27 × 6 cm (length × width × height) tray with 96 cells each with the dimensions of 4 × 3 × 5 cm (length × width × height) using LA4 professional growing mix (Sungro Horticulture, Agawam, Canada). The plants were kept in a growth chamber at 20 ℃ / 18 ℃ (day/night) with a 16 hour photoperiod and 10 % humidity. Following inoculation (see below), resistant plants were transferred into 15 × 15

cm (diameter × deep) plastic pots with the potting mixture of sand : peat : soil in a ratio of 2:2:1, along with 16-20-0-14 Milorganite fertilizer with 4 % iron. The plants were grown in a greenhouse at 20 - 25 ℃ with a 16 hour photoperiod. The plants were treated with pesticides imidacloptoprid and spinosyn (Bayer Corporation, Robinson Township, USA; Dow Agrosciences, Indianapolis, USA) to control aphids and thrips after they were transferred into pots. The plants were watered daily and fertilized every second week with 20 - 20 - 20 (N - P - K) water soluble fertilizer (Plant-Prod, Leamington, Canada).

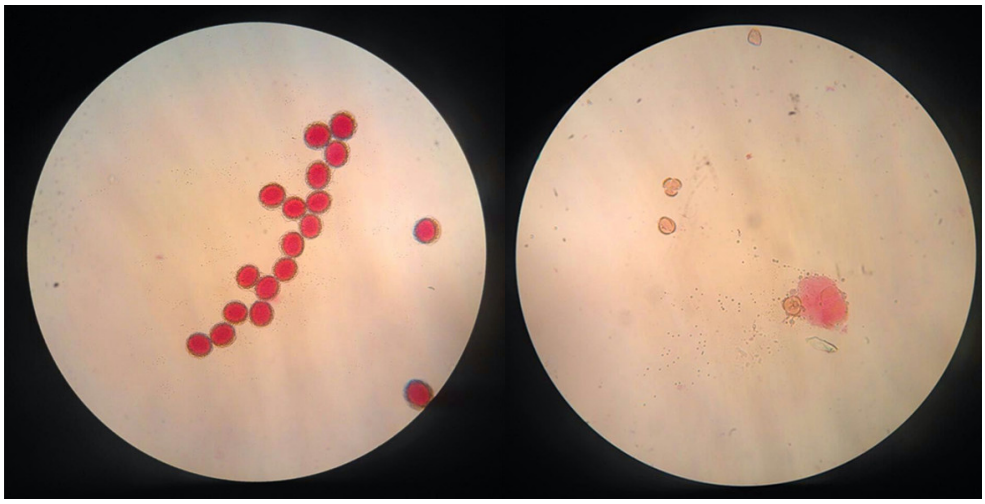
3.3.2 Crossing procedure

The flower buds of female plants were carefully emasculated using forceps without hurting the pistil (Branca 2008). In short, anthers were completely removed. Then stigmas of female plants were pollinated with fresh pollen grains from newly opened flowers of male plants. Female and male plants in different generations are shown in Figure 3.2. The crossing procedure was complete when numerous fresh mature pollen grains were exposed on the anthers. After pollination, the branches used for crossing were labeled and bagged to prevent pollen contamination from other branches. The bags were removed one or two weeks after pollination.

3.3.3 Confirmation of the F_1 hybrids

In order to confirm F_1 plants between *B. napus* cultivar 'Westar' and *B. carinata* 'T4001', the standard acetocarmine staining technique was used to evaluate the viability of pollen from F_1 plants (Heslop-Harrison 1992). The anthers with fresh pollen grains from opened flowers were touched on a microscope slide, and then one drop of acetocarmine (Aldon Crop, New York, USA) was added before adding a coverslide (Fisher Scientific,

Waltham, USA). After three to five minutes, pale pink and dark pink pollen grains were observed with a standard light microscope (Zeiss, Jena, Germany). The pale pink pollen grains were produced on interspecific hybrids with non-viable pollen. The dark pink pollen grains were viable in normal plants, which were used as the controls (Figure 3.1).



A B

Figure 3.1 The acetocarmine staining of viable pollen grains (A) and non-viable pollen grains (B) (Photo credit: Minkyung Kang-Choi).

3.3.4 Production of BC_1 plants with tissue culture

Siliques were collected two to three weeks after pollination and sterilized with 75 % ethanol for 10 min in a 50 mL conical tube (Fisher Scientific, Waltham, USA). The siliques were then rinsed twice with autoclaved distilled water. Subsequently, the siliques were split lengthwise from the dehiscence zone by using a surgical blade and forceps, which were sterilized with 95% ethanol. The developing ovules were excised and put on 1/2 Murashige & Skoog (1962) (MS) medium (2.2 g MS/L, 10 g sucrose/L, 8 g Agar/L, PH=5.8) sealed with parafilm (Vwr, Radnor, USA). All these steps were conducted in a laminar hood under sterile

conditions. When the developing seeds germinated and produced cotyledons, the seedlings were transferred into 8.5 × 8.5 × 8.5 cm (length × width × height) pots with growing mix (Sungro Horticulture, Agawam, Canada). One tray could accommodate 18 pots covered with a transparent plastic cover to keep moisture for two or three days.

3.3.5 Production of backcrossing generations

Brassica napus cultivar 'Westar' was crossed with *B. carinata* cultivar 'T4001' to obtain the F_1. The F_1 was backcrossed with 'Westar' to obtain the BC_1, BC_2, and BC_3 (Figure 3.2). During the production of the BC_3, the BC_2 was also self-pollinated to obtain the BC_2F_2. The BC_3 was also backcrossed with 'Westar' to produce the BC_4 and self-pollinated to be the BC_3F_2 (Figure 3.2).

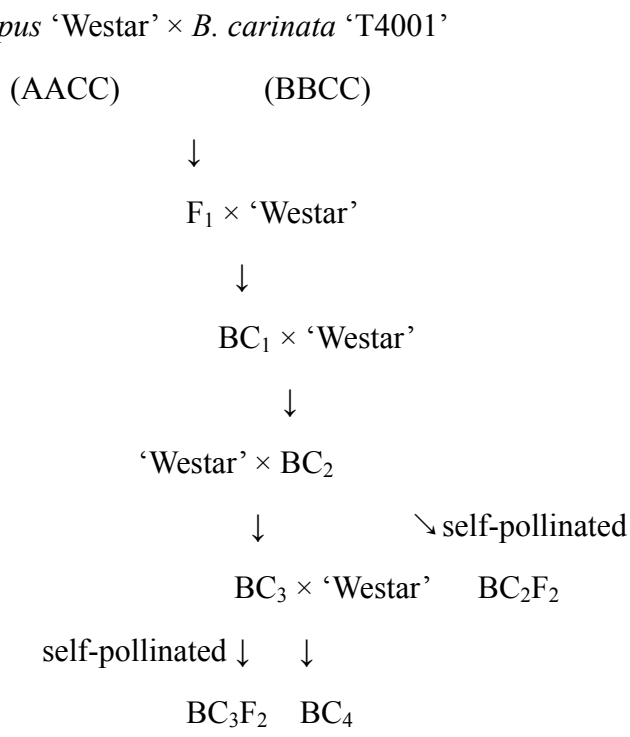


Figure 3.2 The process of the interspecific crossing of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' following by backcrossing with 'Westar' and selfing.

3.3.6 *Leptosphaeria maculans* isolates

One PG2 *L. maculans* isolate 03-15-03 (*AvrLm2*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*) was used to evaluate resistance to *L. maculans* in each generation. In addition to *L. maculans* isolate 03-15-03, three other *L. maculans* isolates including a PG3 isolate 3-42-6 (*AvrLm1*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*), a PGT isolate 09stonewall9553 (*AvrLm2*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*), and a PG4 isolate PG4-1-M (*AvrLm6*, *AvrLm11*, and *AvrLmJ11*) were used in the BC_3F_2 in this project to explore the interaction between *L. maculans* pathogen and resistance in plants. All of them were in the collection of the University of Manitoba except for 09stonewall9553, which was acquired from Dr. Coreen Franke (Crop Protection Services). All currently cloned avirulence genes in these four *L. maculans* isolates were completely sequenced and analyzed by Dr. Tengsheng Zhou (a post-doctor in our lab).

3.3.6.1 Preparation of isolates

All *L. maculans* isolates used for testing were obtained from single spore culture (Choi et al 1999). These isolates were stored with 15 % glycerol at - 20 ℃, then separately cultured on 20 % water agar medium in 100 mm × 15 mm sterile petri dishes (Fisher Scientific, Waltham, USA) under light at 23 ℃. Hyphae growing from a single spore were cut out individually under a binocular microscope with a very sharp needle (BD Eclipse, Franklin Lakes, USA). A small piece of the medium including the hyphae was transferred on the V8 medium in a 10 cm diameter petri dish under light at 23 ℃. The components of V8 medium included V8 juice (Campbell, Toronto, Canada) 200 mL/L, distilled water 800 mL/L, calcium carbonate 0.75 g/L, and agar 15 g/L. After culturing for two weeks, pycnidiospores sporulated and growth covered the entire plate. The pycnidiospores on the surface were

washed with 5 to 10 mL autoclaved distilled water and scraped gently with a microslide (Fisher Scientific, Waltham, USA). Subsequently, the pycnidiospore suspension was filtered through autoclaved filter paper (Fisher Scientific, Waltham, USA) into an autoclaved 50 mL cornial tube (Fisher Scientific, Waltham, USA). The pellet of the pycnidiospores was obtained by centrifuging at 4000 rpm for 10 min. The pycnidiospores were resuspended in autoclaved distilled water. The concentration of the pycnidiospore suspension was determined with a hemacytometer (Fisher Scientific, Waltham, USA). The concentration of the prepared *L. maculans* isolates was adjusted to 2×10^7 pycnidiospores per mL with autoclaved distilled water. The *L. maculans* isolates could be used immediately or stored at -20 ℃ with a higher concentration for less than three months.

3.3.6.2 Identification of isolates

More than twenty *L. maculans* isolates were evaluated on five *B. napus* accessions to identify pathogenicity groups (PG), and four of them were used in the project (described above). These five *B. napus* accessions included three cultivars 'Westar', 'Quinta', 'Glacier' and two testing lines '15C-92-11-2' with a single resistance gene *BLMR1* and 'G4A36-2-9' with another single resistance gene *Rlm2*. Seeds were planted in 96 cell plastic trays filled with growing mix (Sungro Horticulture, Agawam, Canada). Ninety-six plants in a tray were inoculated with 12 *L. maculans* isolates, and each isolate was tested on four plants of each of the five host accessions. The trays were maintained in a growth chamber at 20 ℃ / 18 ℃ (day/night) with a 16 hour photoperiod and 10 % humidity. The inoculation procedure was the same as described below.

3.3.7 Inoculation tests

3.3.7.1 Cotyledon inoculation

The inoculation with *L. maculans* isolates followed the method introduced by Williams et al (1979). In short, seedling cotyledons that had completely unfolded six or seven days after planting were inoculated with *L. maculans* isolates. The plants were watered before the day of the inoculation. The *L. maculans* isolates were diluted as described above and then placed on ice. Half of the cotyledon was wounded in the center with a homemade hole punch (one side of the point of 11.4 cm dissecting straight forceps was bent inward). Inoculations were performed using a pipette (Eppendorf, Hamburg, Germany) to drop 10 uL *L. maculans* spore suspension on the wound. The trays of the inoculated seedlings were place on a bench until the spore suspension dried on the cotyledons. Then the trays were placed back into a growth chamber at 20 ℃ / 18 ℃ (day/night) with a 16 hour photoperiod and 10 % humidity. The inoculated plants were not watered in the first 24 hours. In order to keep the cotyledons alive over fourteen days, true leaves were removed until the end of the survey of symptoms.

3.3.7.2 True leaf inoculation

Due to the use of the embryo rescue tissue culture in the production of the BC_1, true leaf inoculation was used to test seedling resistance to *L. maculans*. When two or three true leaves grew out after the seedlings were transferred from 1/2 MS medium into 8.5 × 8.5 × 8.5 cm pots, two younger true leaves of each individual plant were wounded in two points of each side separated by the main vein. The remainder of the procedure is the same as described above.

3.3.8 Disease score

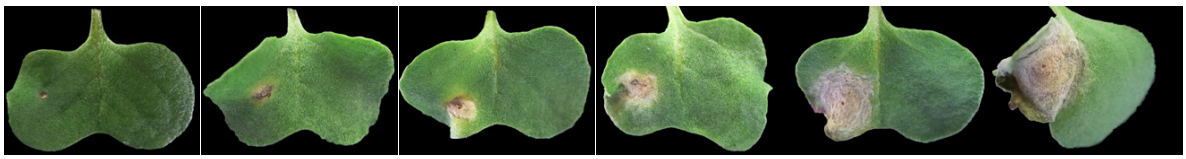
The severity of disease was classified into five categories, 0, 1, 2, 3, and 4, depending on the sizes of symptoms with or without sporulation and according to the classification of Williams et al (1979) (Table 3.1). In order to conveniently compare with previous tests in our lab, the classification of five categories (0, 1, 2, 3, and 4) was used for *L. maculans* isolates (03-15-03, 3-42-6, PGT-M, 09stonewall9553, and PG4-1-M) tests. The scores were recorded at 8, 11, and 14 days after cotyledon inoculation.

Disease scores for cotyledon inoculation tests were based on the size of a lesion with tissue collapse and necrosis and also symptoms of the control cultivar 'Westar'. The scores of cotyledon inoculation were recorded three times at 8, 11, and 14 days after inoculation using 0 to 9 scales (Williams et al 1979) (Table 3.2 and Figure 3.3). The scores recorded at 8 days after cotyledon inoculation were used in the analysis of the BC_3, BC_4, and BC_3F_2. Since the scores of the BC_1 and BC_2 were only recorded at 11 and 14 days after cotyledon inoculation, the scores recorded at 11 days were used in these two generations.

Plants with scores of three or less were classified as resistance. However, temperatures of growth chambers were not uniform for different times of cotyledon inoculation. Resistant and susceptible plants were also scored according to the development of the symptom on susceptible 'Westar'. Plants in the BC_3-1.1 and BC_3-4.1 with scores of one and zero were classified as resistance in comparison to the symptom developed on 'Westar'. The BC_3F_2 plants with a score of zero were classified as resistance due to the slow development of symptom inoculated with *L. maculans* isolate PG4-1-M.

The results of true leaf inoculation were record at 14 days after inoculations. The

resistant and susceptible plants of true leaf inoculation were classified according to the symptoms on 'Westar'. Susceptible plants showed large collapsed lesions with profuse sporulation. Resistant plants showed limited blacking around the wound and a faint chlorotic halo without sporulation (Figure 3.4).



0 1 3 5 7 9

Figure 3.3 Blackleg symptoms on *Brassica napus* cotyledons evaluated as 0, 1, 3, 5, 7, and 9 14 days after cotyledon inoculation with *Leptosphaeria maculans* isolate 03-15-03.

Resistant Intermediate Susceptible 'Westar'

Figure 3.4 Blackleg symptoms on *Brassica napus* true leaves evaluated as resistant, intermediate, and susceptible plants 14 days after true leaf inoculation with *Leptosphaeria maculans* isolate 03-15-03.

Table 3.1 Blackleg disease scores for the evaluation of *Leptosphaeria maculans*.

Disease scores	Disease description
0	No obvious visible symptom around the wound, no grayish-green lesion
1	Limited darkening tissue around the wound with faint halo, lesion diameter 0.5 – 1.5 mm without sporulation
2	Dark necrotic lesion diameter 1.5 – 3.0 mm without sporulation
3	Grayish-green tissue collapse or dark necrotic diameter 3.0 – 6.0 mm with minimal sporulation
4	Large tissue collapse (> 6.0 mm) with diffuse margins, along with profuse sporulation

3.3.8 Statistical analysis

Data was analyzed by using SAS software version 9.3 (SAS Institute Inc., 2008) using code for a chi-square test. The chi-square test for goodness of fit was performed to evaluate the segregation ratio of resistant and susceptible plants in each generation.

Table 3.2 Blackleg disease scores for the evaluation of cotyledon resistance (Williams et al 1979).

Disease scores	Disease description
0	Almost no darkening tissue around the wound, no grayish-green lesion and other symptoms
1	Limited blackening around the wound, lesion diameter 0.5 - 1.5 mm, faint chlorotic halo probably presented without sporulation
3	Dark necrotic lesion 1.5 - 3.0 mm diameter, faint chlorotic halo probably presented without sporulation
5	Lesion 3.0 - 6.0 mm diameter without sporulation, may show grayish-green tissue collapse or dark necrosis
7	Greyish-green tissue collapse 3.0 - 6.0 mm, sharply delimited, non-darkened margin
9	Rapid tissue collapse at about ten days, accompanied by profuse sporulation in large lesions (> 6.0 mm) with diffuse margins

3.4 Results

3.4.1 Evaluation of *Leptosphaeria maculans* isolates for virulence

The blackleg disease severity of five *L. maculans* isolates (03-15-03, 3-42-6, PGT-M, and PG4-1-M) was evaluated in five *B. napus* accessions 'Westar', 'Glacier', 'Quinta', '15C-92-11-2', and 'G4A36-2-9' (Table 3.3). The isolate PG4-1-M caused severe infection (disease score of 4.0) on all the five *B. napus* accessions at 14 days after inoculation (DAI).

PG4-1-M was one of the most aggressive isolates and caused collapsed lesions, but produced slow sporulation. The aggressiveness of isolates 3-42-6, PGT-M, and 09stonewall9553 were not significantly different based on the mean score at 14 DAI (Table 3.3). The isolate 03-15-03 was the least virulent among these five tested isolates with a mean score 1.47 across five *B. napus* accessions at 14 DAI, and was used to select resistant plants in early generations (Table 3.3).

Table 3.3 Blackleg disease severity (DS) caused by five *Leptosphaeria maculans* isolates in five *Brassica napus* accessions at 14 days after cotyledon inoculation.

Isolate	***B. napus* accession**					**$LSD_{(0.05)}$**	**Mean**
	Westar	**15C-92-11-2**	**G4A36-2-9**	**Glacier**	**Quinta**	**(Isolate)**	**(DS)**
03-15-03	4.00	4.00	0.00	0.00	0.00	0.00	1.47c
3-42-6	4.00	1.00	4.00	4.00	0.50	2.01	2.27b
PGT-M	4.00	4.00	0.00	0.50	4.00	0.73	2.42b
09stonewall9553	4.00	4.00	0.25	0.00	4.00	0.34	2.45b
PG4-1-M	4.00	4.00	4.00	4.00	4.00	0.00	4.00a
$LSD_{(0.05)}$	0.00	0.95	0.58	0.96	0.67		
Mean(DS)	4.00a	3.40b	0.87d	1.12d	2.50c		

DAI=days after inoculation

Mean values with the same letter in row and column of mean (DS) are not significantly different ($p \leq 0.05$).

3.4.2 Resistance to *Leptosphaeria maculans* in the F_1 of the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* 'T4001'

Only four F_1 seedlings were obtained in the cross of *B. napus* cultivar 'Westar' and *B. carinata* 'T4001'. All four plants showed a high level of blackleg resistance (with a score of 0)

similar to the parent *B. carinata* 'T4001' when they were inoculated with *L. maculans* isolate 03-15-03. The pollen grains of the hybrids were aborted due to the aneuploid chromosomes. Therefore, the pollen grains from four F_1 plants showed pink color compared with normal red color stained with acetocarmine staining solution. The results confirmed that all the four plants were true hybrids. All F_1 plants were used as the female to backcross to 'Westar' (Figure 3.1).

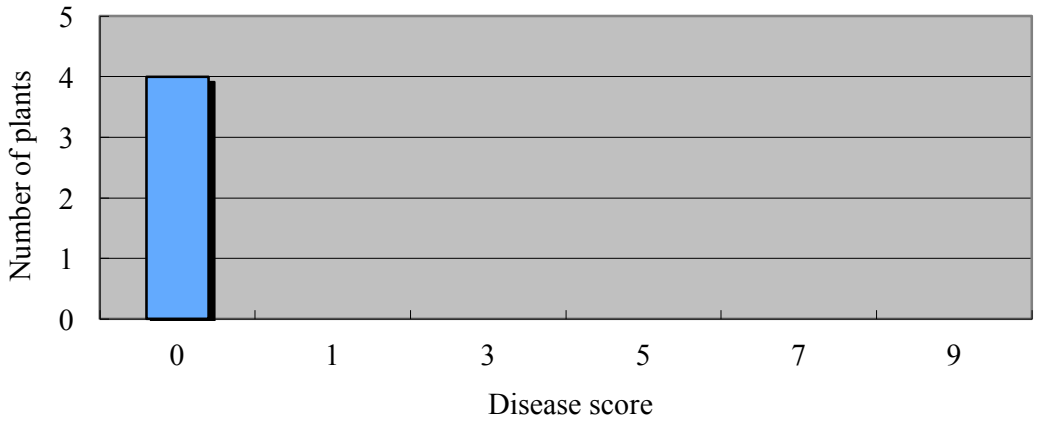


Figure 3.5 Blackleg disease scores for cotyledon inoculation of F_1 plants of the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2013.

3.4.3 Resistance to *Leptosphaeria maculans* in the BC_1, BC_2, BC_3, BC_4, and BC_3F_2

A total of 59 seedlings in the BC_1 were evaluated for cotyledon resistance to a *L. maculans* isolate 03-15-03. Forty-three plants were obtained from seeds directly while 16 plants were produced using tissue culture. Twenty-one out of 43 plants were resistant to *L. maculans*, and 22 were susceptible through cotyledon inoculation (Figure 3.6). For the true

leaf inoculation, 11 out of 16 plants displayed resistance to *L. maculans*, and five individual plants were susceptible. According to the results of the chi-square test for goodness of fit, both populations evaluated by cotyledon and true leaf inoculations followed a 1:1 segregation ratio of resistant and susceptible plants (Table 3.4). The segregation of resistant and susceptible plants in all BC_1 plants also fit a 1:1 ratio.

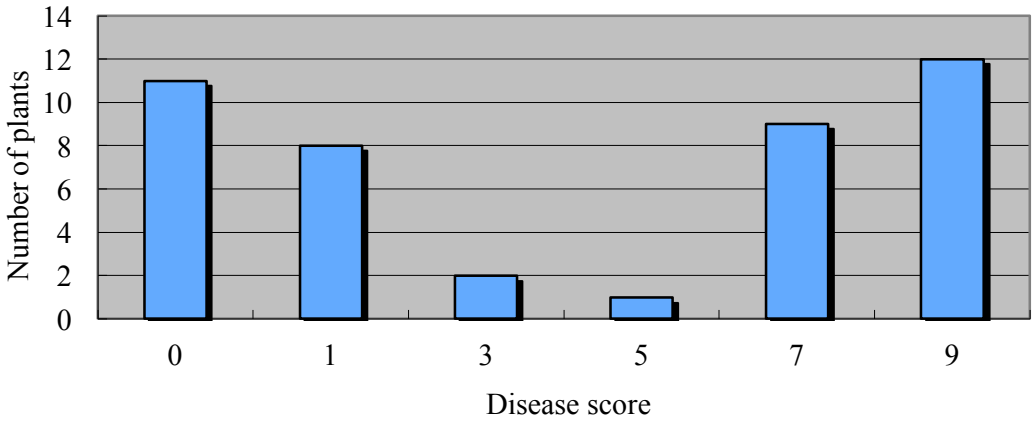


Figure 3.6 Blackleg disease scores for cotyledon inoculation of BC_1 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014.

In the BC_2, blackleg resistance of 106 seedlings from five families was evaluated using *L. maculans* isolate 03-15-03. The seed production for most families in the BC_1 was poor. Therefore, the numbers of plants were not enough to obtain a valid segregation ratio of resistant and susceptible plants. The scores of BC_2 plants are showed in Figure 3.7.

Table 3.4 Cotyledon and true leaf resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_1 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2014.

BC_1	**Phenotype**			**Disease scores**		**X^2 test for 1:1**	
	T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
Cotyledon	43	21	22	0.0-9.0	4.42±3.86	0.02	0.88
Westar	4			9.0-9.0	9.00±0.00		
True leaves	16	11	5			2.25	0.13
Total	59	32	27			0.42	0.52

T=total; R=resistant; S=susceptible

In the BC_2-1 family, only two plants were tested for blackleg resistance due to poor seed set. One was resistant and one was susceptible. No resistant plants were observed in nine seedlings of the BC_2-2 family. In the BC_2-3 family, only three out of 86 plants were evaluated as resistance to *L. maculans* isolate 03-15-03. Only one out of five plants was resistant to *L. maculans* in the BC_2-4 family. Two resistant and two susceptible plants were selected in the BC_2-5 family. The segregation of resistant and susceptible plants fit a 1:1 ratio, but the population size was not adequate. Accordingly, the segregation of resistant and susceptible plants in the BC_2 deviated from a 1:1 ratio. Only seven out of 106 plants were resistant to *L. maculans* while 99 were susceptible (Table 3.5).

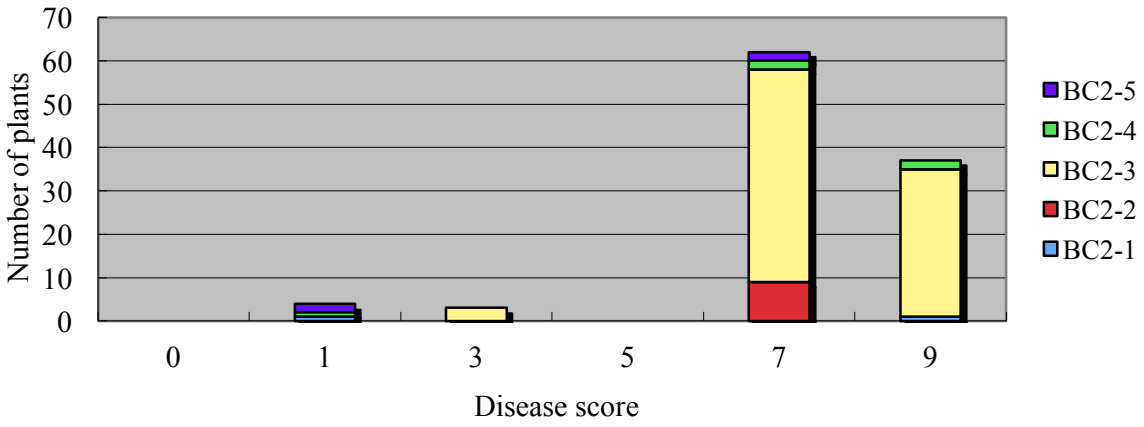


Figure 3.7 Blackleg disease scores for cotyledon inoculation of BC_2 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014.

Table 3.5 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_2 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2014.

BC_2	**Phenotype**			**Disease scores**		**X^2 test for 1:1**	
	T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_2-1	2	1	1	1.0-9.0	5.00±5.66	0.00	1.00
BC_2-2	9	0	9	7.0-7.0	7.00±0.00	0.00	
BC_2-3	86	3	83	3.0-9.0	7.67±1.25	74.42	<.0001
BC_2-4	5	1	4	1.0-9.0	6.60±3.29	1.80	0.18
BC_2-5	4	2	2	1.0-7.0	4.00±3.46	0.00	1.00
Total	106	7	99	1.0-9.0	7.38±1.73	79.85	<.0001
Westar	48			7.0-9.0	8.29±0.97		

T=total; R=resistant; S=susceptible

Three BC_2 families (BC_2-1, BC_2-4, and BC_2-5) produced the BC_3 which were evaluated for blackleg resistance with *L. maculans* isolate 03-15-03 (Figure 3.8). In this case, five out of 7 plants showed resistance to the isolate 03-15-03 in the BC_3-1.1 family, which fit a 1:1 segregation ratio of resistant and susceptible plants (Table 3.6). In the BC_3-4.1 family, six out of 15 were resistant and 9 were susceptible. The segregation of resistant and susceptible plants in the BC_3-4.1 family also fit a 1:1 ratio. Thirteen resistant and five susceptible seedlings were present in the BC_3-5.1 family. According to the result of the chi-square test at the 0.05 level, the segregation of resistant and susceptible plants in the BC_3-5.1 family fit a 1:1 ratio. Consequently, 24 resistant and 16 susceptible seedlings were selected by the isolate 03-15-03 in the BC_3 generation, which fit a 1:1 segregation ratio of resistant and susceptible plants (Table 3.6).

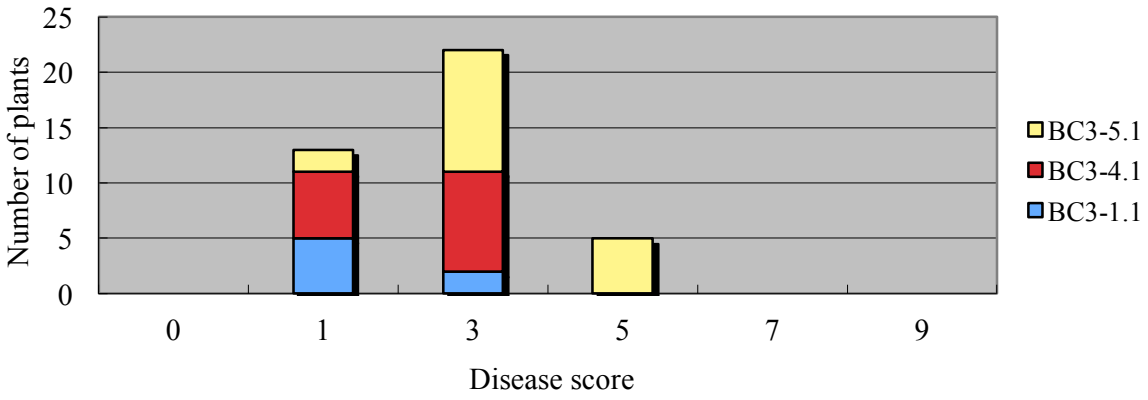


Figure 3.8 Blackleg disease scores for cotyledon inoculation of BC_3 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014.

Table 3.6 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_3 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2014.

BC_3	**Phenotype**			**Disease scores**		**X^2 test for 1:1**	
	T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_3-1.1	7	5	2	1.0-3.0	1.57±0.98	1.29	0.26
BC_3-4.1	15	6	9	1.0-3.0	2.20±1.01	0.06	0.44
BC_3-5.1	18	13	5	1.0-5.0	3.33±1.24	3.56	0.06
Total	40	24	16	1.0-5.0	2.60±1.30	1.60	0.21
Westar	28			3.0-5.0	4.21±0.99		

T=total; R=resistant; S=susceptible

Only one family (BC_3-1.1) was advanced to the BC_4. The plants were evaluated for blackleg resistance by *L. maculans* isolates 03-15-03 and PG4-1-M in the BC_4 (Figure 3.9). In this case, 23 plants were evaluated by the isolate 03-15-03 (Table 3.7). Eleven plants were resistant and 12 were susceptible, which fit a 1:1 segregation ratio. The same number of plants was tested for blackleg resistance using the isolate PG4-1-M. Similarly, ten plants showed resistance to this isolate and 13 were susceptible. The segregation of resistant and susceptible plants with cotyledon inoculation of PG4-1-M also fit a 1:1 ratio (Table 3.7).

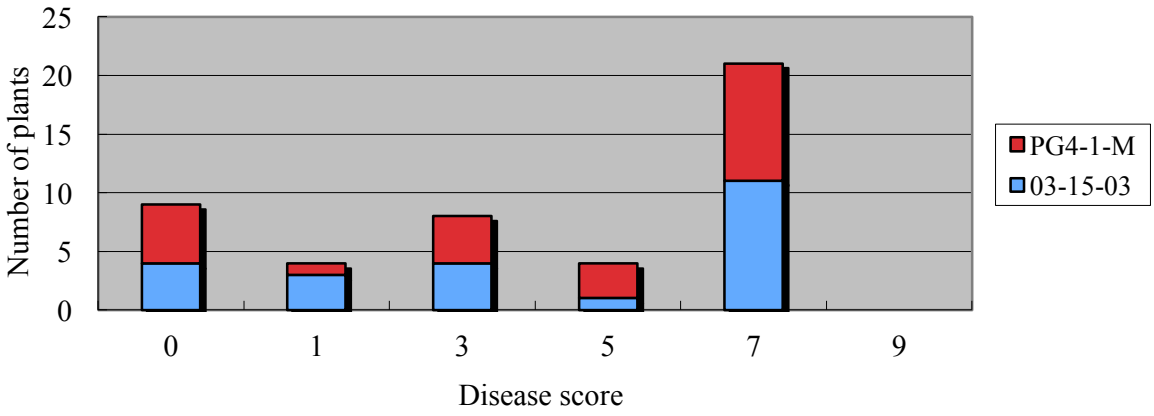


Figure 3.9 Blackleg disease scores for cotyledon inoculation of BC_4 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03 and PG4-1-M in a greenhouse in Winnipeg, MB in 2015.

Table 3.7 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03 and PG4-1-M in BC_4 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2015.

BC_4	**Isolate**	**Phenotype**			**Disease scores**		**X^2 test for 1:1**	
		T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_4-1.1.1	03-15-03	23	11	12	0.0-7.0	4.22±2.97	0.04	0.83
Westar	03-15-03	4			5.0-7.0	6.50±1.00		
BC_4-1.1.1	PG4-1-M	23	10	13	0.0-7.0	4.26±2.90	0.39	0.53
Westar	PG4-1-M	4			7.0-7.0	7.00±0.00		

T=total; R=resistant; S=susceptible

Two BC_3F_2 families (BC_3F_2-1.1.1s and BC_3F_2-1.1.2s) were obtained from the BC_3-1.1

family. They were evaluated for blackleg resistance using four *L. maculans* isolates (03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M) (Figure 3.10 and Figure 3.11). In the BC_3F_2-1.1.1s family, seventy-four out of 93 seedlings showed resistance to *L. maculans* isolate 03-15-03. The data fit a 3:1 segregation ratio of resistant and susceptible plants. The segregation of resistant and susceptible plants also fit a 3:1 ratio in the BC_3F_2-1.1.1s family, which was inoculated with *L. maculans* isolate 09stonewall9553. Sixty-five out of 87 seedlings were resistant to this isolate and 22 were susceptible. Even though the symptoms developed slowly at the primary stage, the differences could be distinguished between 'Westar' and plants in the BC_3F_2-1.1.1s family inoculated with PG4-1-M. The segregation of resistant and susceptible plants also fit a 3:1 ratio. Sixty-six out of 93 plants were resistant to PG4-1-M and 27 were susceptible (Table 3.8).

In the BC_3F_2-1.1.2s family, 57 out of 76 plants showed resistance to the isolate 03-15-03 and 19 were susceptible. This also fit a 3:1 segregation ratio of resistant and susceptible plants. Sixty resistant and 18 susceptible plants were observed when the plants were evaluated with *L. maculans* isolate 3-42-6, which also fit a 3:1 segregation ratio of resistant and susceptible plants. In 81 tested seedlings, 60 plants were resistant and 21 were susceptible when the plants were inoculated with the isolate PG4-1-M. The data also fit a 3:1 resistant-to-susceptible segregation ratio (Table 3.8).

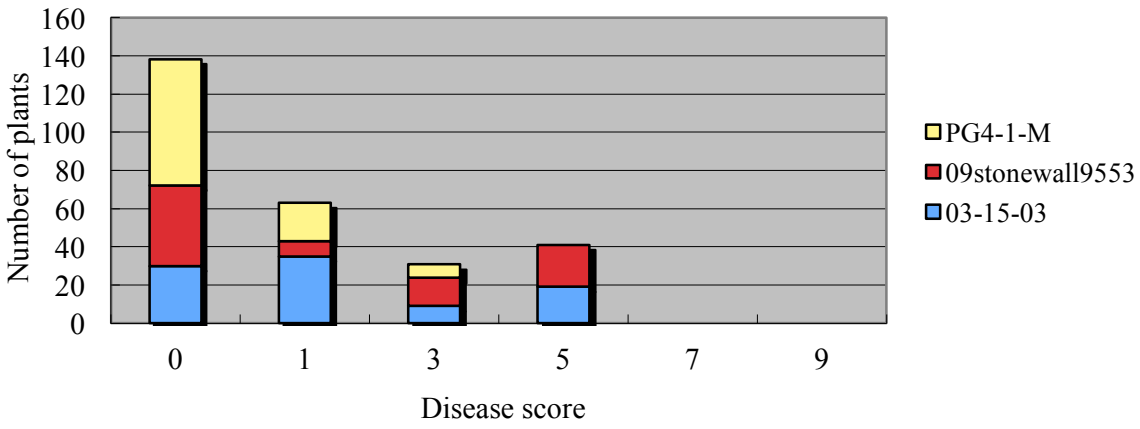


Figure 3.10 Blackleg disease scores for cotyledon inoculation of BC_3F_2-1.1.1s plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015.

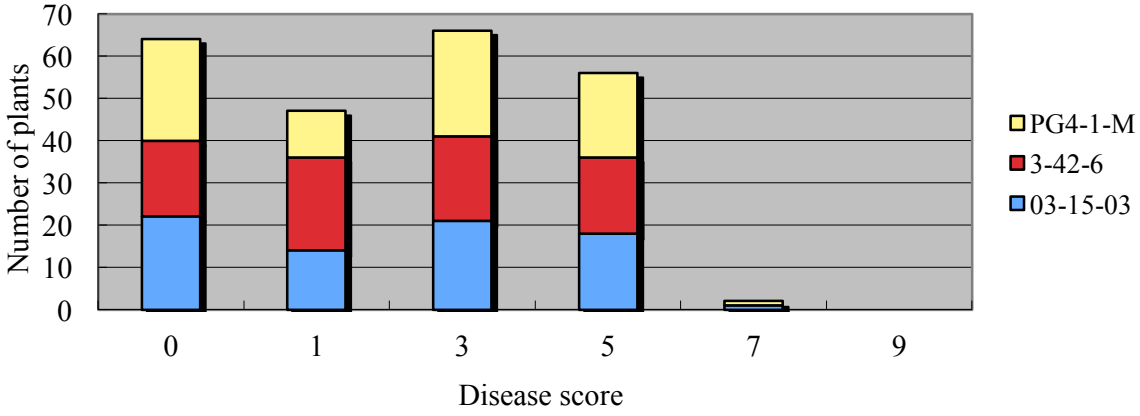


Figure 3.11 Blackleg disease scores for cotyledon inoculation of BC_3F_2-1.1.2s plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015.

Table 3.8 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in BC_3F_2 plants following the cross of *Brassica napus* cultivar 'Westar' and *Brassica carinata* cultivar 'T4001' in a greenhouse in Winnipeg, MB in 2015.

BC_3F_2	**Isolate**	**Phenotype**			**Disease scores**		**X^2 test for 3:1**	
		T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_3F_2-1.1.1s	03-15-03	93	74	19	0.0-5.0	1.68±1.88	1.04	0.31
BC_3F_2-1.1.1s	09stonewall9553	87	65	22	0.0-5.0	1.87±2.12	0.00	0.95
BC_3F_2-1.1.1s	PG4-1-M	93	66	27	0.0-3.0	0.44±0.84	0.81	0.37
BC_3F_2-1.1.2s	03-15-03	76	57	19	0.0-5.0	2.29±2.01	0.00	1.00
BC_3F_2-1.1.2s	3-42-6	78	60	18	0.0-5.0	2.21±1.88	0.15	0.69
BC_3F_2-1.1.2s	PG4-1-M	81	60	21	0.0-5.0	2.38±2.02	0.04	0.85
Westar	03-15-03	4			3.0-5.0	4.50±0.93		
Westar	09stonewall9553	4			5.0-7.0	6.00±1.15		
Westar	3-42-6	4			5.0-5.0	5.00±0.00		
Westar	PG4-1-M	4			1.0-5.0	2.50±1.77		

T=total; R=resistant; S=susceptible

3.5 Discussion

Blackleg resistance from *B. carinata* cultivar 'T4001' (BBCC) was transferred into *B. napus* (AACC) through interspecific hybridization. F_1 plants (ABCC) were aborted and confirmed through the acetocarmine staining of pollen grains. Mason et al (2010) reported that 88 % of pollen mother cells carried homologous chromosome pairings in the two C genomes from *B. napus* and *B. carinata* respectively in interspecific hybridization. The

homeologous pairings of A-C, A-B, and B-C were rare in the cross of *B. napus* and *B. carinata*, which constituted ABCC genomes (Mason et al 2010; Sacristan et al 1985). A frequent phenomenon was that an additional chromosome was involved in a homologous chromosome pairing in ABCC hybrids (Mason et al 2010). The additional chromosome resulted in the strong meiotic disturbances, which caused the low fertility of plants (Struss et al 1991). The sterility of plants in the F_1 and the low seed set and even sterility of some plants in later backcrossing and self-pollinated generations in my study suggested the presence of an additional chromosome.

Considerable allosyndesis was identified in the A-C genome association compared with the low association of the A-B and B-C genomes (Mason et al 2010). Therefore, the A and C genomes showed a higher level of pairing than the AB and BC genomes (Attia et al 1986; 1987). A higher level of autosyndesis is reported in the haploid A and C genomes than that in the haploid B genome (Mason et al 2010). The B genome chromosomes are rarely paired with the A or C genome chromosomes and are suggested to be additional chromosomes during meiosis (Attia et al 1986; Navabi et al 2011; Struss et al 1991). It was considerably easy to lose the B genome chromosomes that were supposed to carry a high level of resistance to *L. maculans* in interspecific crosses (Li et al 2004; Roy 1978b; Sacristan et al 1986; Sjodin et al 1988). Navabi et al (2010) reported that only five percent of the 60 randomly selected doubled haploid lines derived from three or four plants in the BC_2S_3 following the cross of *B. carinata* and *B. napus* carried the whole or part of the B genome chromosomes. The elimination of the B genome probably explained the quick loss of resistance to *L. maculans* in the BC_2, which resulted in the deviation of an expected 1:1

segregation ratio of resistant and susceptible plants. Only 7 out of 106 plants were resistant to *L. maculans* isolate 03-15-03 in the BC_2. Sacristan et al (1986) also reported that blackleg resistance was almost totally lost in the BC_1 following the cross of *B. carinata* and *B. napus* through inoculation of the crown region.

In order to obtain a stable introgression of desirable traits, the regular chromosome pairing and homeologous recombination through allosyndesis should be obtained in the cross between two *Brassica* species (Mason et al 2010; Parkin et al 1997). Duplication, translocation, insertion, deletion, and inversions have occurred in the *Brassica* A, B, and C genomes (Long et al 2011; Mayerhofer et al 2005; Parkin et al 2003). Accordingly, a number of homeologous sections exist among the A, B, and C genomes, especially between the A and C genomes (Parkin et al 2003, 2005). The homeologous segments from the linkage groups of the A and C genomes also were coordinated with the linkage groups in the B genome (Lagercrantz et al 1996). Panjabi et al (2008) used intro polymorphism (IP) markers to compare the mapping of *B. juncea* and Arabidopsis, and also observed the homeologous segments of the *Brassica* A, B, and C genomes. The similarity among the *Brassica* A, B, and C genomes provides an opportunity for the introgression of blackleg resistance gene(s) from the B genome into the A or C genomes (Panjabi et al 2008; Sacristan et al 1986; Sjodin et al 1988). Navabi et al (2011) reported that one segment of B5 from *B. carinata* appeared to be translocated to the A or C genome of *B. napus*. Therefore, it may be possible to achieve stable blackleg resistance derived from the B genome of *B. carinata* in a *B. napus* background using interspecific hybridization.

In the BC_1 and BC_3, the segregation of resistant and susceptible plants fit a 1:1 ratio,

which suggests that a single locus controls this resistance to *L. maculans*. In addition, the segregation of resistant and susceptible plants in the BC_4 and BC_3F_2 also fit a 1:1 and 3:1 ratio, respectively, using four different *L. maculans* isolates (03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M). These results confirm that the transferred blackleg resistance is controlled by a single locus. Rahman et al (2007) suggested that at least two blackleg resistance genes had been transferred from *B. carinata* accession #98-14513 into *B. napus*. The results were based on the molecular markers located in the A and C genomes and co-segregated with blackleg resistance (Rahman et al 2007). In my study, a single resistance locus in different plants may be the same, but it is possible to have more than one locus controlling blackleg resistance in *B. carinata*.

Additionally, the resistance transferred from *B. carinata* into *B. napus* showed the same reaction to all four *L. maculans* isolates, including 03-15-03 (*AvrLm2*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*), 3-42-6 (*AvrLm1*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*), 09stonewall9553 (*AvrLm2*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*), and PG4-1-M (*AvrLm6*, *AvrLm11*, and *AvrLmJ11*). In the BC_4, the segregation of resistant and susceptible plants in the BC_4-1.1.1 family fit a 1:1 ratio when the plants were inoculated with both *L. maculans* isolates 03-15-03 and PG4-1-M. In the BC_3F_2, the segregation of resistance and susceptible plants in two families followed a 3:1 ratio when the plants were inoculated with all the four *L. maculans* isolates. Since the avirulence genes in these four *L. maculans* isolates are different, it is unclear which *L. maculans* avirulence gene(s) correspond to the *B. carinata* resistance genes.

Numerous studies have been attempted to transfer a high level of blackleg resistance

from *B. carinata* and *B. juncea* into *B. napus* (Chevre et al 1996, 1997; Fredua-Agyeman et al 2014; Navabi et al 2010; Rahman 2012; Roy 1978b, 1984; Sacristan et al 1986; Struss et al 1996;). However, low frequency of A-B or B-C chromosome pairing results in sterility as well as quick loss of blackleg resistance (Mason et al 2010; Sacristan et al 1985; Struss et al 1991), which also happened in most families of early generations in my study. These issues barricade a further research on blackleg resistance originated in *B. carinata* (Fredua-Agyeman et al 2014; Rahman et al 2013;). Accordingly, the resistant plants that have been remained in my study can be utilized as precious materials to further study blackleg resistance derived from *B. carinata*.

Chapter 4. Transferring blackleg resistance from 'Meng' (AABBCC) into *Brassica napus* (AACC)

4.1 Abstract

Blackleg resistance was introgressed into canola through the interspecific hybridization between synthetic hexaploid *Brassica* species 'Meng' (AABBCC) and *Brassica napus* L. cultivar 'Westar' (AACC). All F_1 plants were obtained following tissue culture. One *Leptosphaeria maculans* (Desm.) Ces. & De Not. isolate was used to evaluate and select resistance in the following backcrossing and selfing generations. A 1:1 segregation ratio of resistant and susceptible plants did not show up in most BC_1 and BC_2 families while two families in each of the two backcrossing generations followed a 1:1 segregation ratio of resistant and susceptible plants. The BC_1-3.1 family with the 1:1 ratio was used in the next two self-pollinated generations to obtain the BC_1F_2 and BC_1F_3 families. The segregation of resistant and susceptible plants in these two self-pollinated generations fit a 3:1 ratio inoculated with four *L. maculans* isolates (03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M). Meanwhile, the BC_2-3.1.1 family also followed a 1:1 segregation ratio of resistant and susceptible plants inoculated with the isolate 03-15-03. The results suggest that a single blackleg resistance gene was introgressed from synthetic hexaploid *Brassica* species 'Meng' into *B. napus*.

4.2 Introduction

Blackleg disease caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. & De Not. causes major yield loses in canola (*Brassica napus* L.) fields in Canada, Australia, and Europe (Fitt et al 2006; Howlett 2004). The ascospores of *L. maculans* released from

pseudothecia in crop residue can infect plants by penetrating cotyledons and true leaves, and cause leaf lesions (Hall 1992; Hammond et al 1985). Conidia produced from pycnidia on leaf lesions are ascribed to the secondary spread of this fungus through rain splash (Barbetti 1975; Travadon et la 2007; Vanniasingham et al 1989). Then the fungus produces hyphae and grows down to the stem, which causes a stem canker at the base of the stem (Hammond et al 1985; Sexton et al 2001). Stem cankers cause the most damage in infected plants due to the restriction of water and nutrients (Gugel et al 1992). Blackleg resistant cultivars are considered to restrict fungal growth through rapid collapse of their guard cells (Chen et al 1996). The utilization of resistant cultivars is an effective strategy to manage blackleg, especially in Canada (Peng et al 2015).

Brassica napus L. is an important cultivated *Brassica* species with high yield and superior quality, which has been cultivated for approximately four hundred years (Gomez-Campo et al 1999; Harlan 1971). However, the short history of domestication, local distribution, and limited traditional breeding methods led to a narrow genetic background in this species (Li et al 2007; Rahman 2013; Rahman et al 2014a; Xiao et al 2010). Therefore, it is necessary to broaden the genetic diversity by introgressing desirable characteristics for seed yield and quality (Rahman 2013). However, these interspecific crosses can not be used directly for two different *Brassica* species, like *B. carinata* A. Braun and *B. napus*, due to abnormal chromosome pairings during meiosis (Li et al 2006). Accordingly, synthetic *Brassica* species were developed to solve the problem of poor seed set following interspecific crosses (Li et al 2004, 2005, 2006, 2007; Meng et al 1998; Rahman 2001; Xiao et al 2010).

Meng et al (1998) and Rahman (2001) used synthetic hexaploid *Brassica* species

(AABBCC) as a bridge to combine yellow seed coat genes located on the A, B, and C subgenomes into *B. napus*. The hexaploid *Brassica* species were derived from the chromosome doubling of a trigenomic hybrid (ABC) with 27 chromosomes (Meng et al 1998; Rahman 2001). The genomic construction in the F_1 of the cross of the hexaploid hybrid and *B. napu*s was expected as AABCC (Meng et al 1998). In addition to the combination of yellow seed coat genes, the trigenomic synthetic *Brassic*a species were also utilized to introduce more genetic sources into traditional *B. napus*. The synthetic *Brassica* species actually combined the A genome from *B. rapa* L. and C genome from *B. carinata* (Li et al 2004). The trigenomic synthetic *Brassica* species were useful materials as a bridge to make it possible to transfer the desirable genes between two *Brassica* species (Chen et al 2011). To date, no report has described the transfer of blackleg resistance from the trigenomic synthetic *Brassica* species into *B. napus*. The introgression of blackleg resistance from the synthetic *Brassica* species into *B. napus* is an approach to widen blackleg resistant sources in the limited background of *B. napus*.

The objective of this research was to transfer blackleg resistance from synthetic hexaploid *Brassica* species 'Meng' into *B. napus*. Four *L. maculans* isolates were used to investigate the interaction between *L. maculans* and *B. napus*. The inheritance of the introgressed blackleg resistance from hexaploid *Brassica* species 'Meng' was explored following cotyledon inoculation.

4.3 Materials and methods

4.3.1 Plant materials

The parental lines used in the cross were *B. napus* cultivar 'Westar' and synthetic

Brassica species ‘Meng’, which was obtained from Dr. Jinling Meng at Huazhong Agricultural University, China. ‘Meng’ with different numbers were derived from different seed types shown in Table 4.1. ‘Meng 2’, ‘Meng 4’, ‘Meng 5’, ‘Meng 7’, and ‘Meng 8’ were used in the project, which showed complete resistance to *L. maculans*. ‘Meng 2’, ‘Meng 4’, and ‘Meng 8’ were derived from self-pollinated hexaploid plants. ‘Meng 5’ was obtained from the hybrid between two different hexaploid plants. ‘Meng 7’ was from the cross between a hexaploid plant and mustard. The hexaploid plants (AABBCC) were derived from the chromosome doubling of the trigenomic hybrids (ABC).

Each of ‘Meng 2’, ‘Meng 4’, ‘Meng 5’, ‘Meng 7’, ‘Meng 8’ and ‘Westar’ were planted in eight cells in a 54 × 27 × 6 cm (length × width × height) tray composed of 96 4 × 3 × 5 cm cells. The LA4 potting mixture was used for seed germination (Sungro Horticulture, Agawam, Canada). The plants were kept in a growth chamber at 20 ℃ / 18 ℃ (day/night) with a 16 hour photoperiod and 10 % humidity. However, some plants in the BC_2F_2 and BC_1F_3 (Figure 4.1) were kept in a growth chamber and growth room at 23 ℃ / 18 ℃ and 35 % humidity. Resistant plants in all generations were transferred into 15 × 15 cm (diameter × deep) plastic pots with a potting mixture (sand : peat : soil = 2 : 2 : 1 with 16-20-0-14 Milorganite fertilizer with 4% iron) and placed in a greenhouse at 20 - 25 ℃ with a 16 hour photoperiod. The plants were treated with the pesticides imidacloprid and spinosyn (Bayer Corporation, Robinson Township, USA; Dow Agrosciences, Indianapolis, USA) to control aphids and thrips, one week after being transferred to pots. The plants were watered daily and fertilized every second week with 20 - 20 - 20 (N - P - K) water soluble fertilizer (Plant-Prod, Leamington, Canada).

Table 4.1 Name, seed type, generation, and parents of synthetic hexaploid *Brassica* species 'Meng'.

No.	Name	Seed type	Gene ration	Parent of Ethiopia Mustard	Parent of Chinese Cabbage
Meng 2	NA	Hexaploid selfing	NA	NA	NA
Meng 4	C15	Hexaploid selfing	S8	CGN03953	Baiguotianyoucai
Meng 5	C21×C28	Hexaploid× Hexaploid	F4	NA	NA
	C21	Hexaploid parent	NA	CGN03983	Wulitianyoucai
	C28	Hexaploid parent	NA	CGN03995	BaijianNo.13
Meng 7	NA	Hexaploid× Mustard	NA	NA	NA
Meng 8	C15	Hexaploid selfing	S6	CGN03953	Baiguotianyoucai

4.3.2 Crossing procedure

Flower buds of female plants were carefully opened using forceps, and all stamens were completely removed without damaging the pistil (Branca 2008). Then stamens of a male plant with opened anthers were used to dust fresh pollen on the pistil of a female plant. Plants were used as female and male shown in Figure 4.1. The crossing procedure was performed when numerous pollen grains were mature on anthers. After pollination, the branches used for crossing were protected with pollen bags and labeled with a tag. The bags were removed one to two weeks after pollination.

4.3.3 Production of F_1 plants with tissue culture

The plants in the F_1 were obtained through tissue culture. Tissue culture was performed on siliques growing for two or three weeks after pollination. Siliques were surface sterilized with 75 % ethanol for 10 min in a 50 mL tube (Fisher Scientific, Waltham, USA), and washed with autoclaved distal water twice. Subsequently, siliques were dissected from the dehiscence

zone in a new plate by using a surface sterilized surgical blade and forceps. The developing ovules were excised and transferred on 1/2 Murashige & Skoog (1962) (MS) medium (2.2 g MS/L, 10 g sucrose/L, 8 g Agar/L, pH =5.8). These plates were sealed with parafilm (Vwr, Radnor, USA) and put in a tissue culture room. Seedlings with growing roots were transferred into 8.5 × 8.5 × 8.5 cm (length × width × height) pots with growing mix (Sungro Horiculture, Agawan, Canada) and covered with transparent plastic covers for two or three days.

4.3.4 Production of backcrossing and selfing generations

Following inoculation (described below), the resistant plants in the F_1 were backcrossed with *B. napus* cultivar 'Westar' to obtain the BC_1, BC_2, and BC_3 (Figure 4.1). Additionally, resistant plants in the BC_1 were self-pollinated to produce the BC_1F_2. The BC_2 and BC_1F_2 were self-pollinated to obtain the BC_2F_2 and BC_1F_3, respectively (Figure 4.1).

'Westar' (AACC) × 'Meng' (AABBCC)

↓

F_1 (AABCC) × 'Westar'

↓

'Westar' × BC_1

↓ ↘self-pollinated

BC_2 × 'Westar' BC_1F_2

self-pollinated↓ ↓ ↓self-pollinated

BC_2F_2 BC_3 BC_1F_3

Figure 4.1 The process of the interspecific crossing of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng' following by backcrossing with 'Westar' and selfing.

4.3.5 *Leptosphaeria maculans* isolates

Four *L. maculans* isolates 03-15-03, 3-42-6, 09stonewall9553 and PG4-1-M were previously assigned in pathogenicity groups (PG) PG2, PG3, PGT and PG4 (Koch et al 1991; Mengistu et al 1991), respectively. Using all currently cloned avirulence genes (Balesdent et al 2013; Fudal et al 2007; Gout et al 2006; Ghanbarnia et al 2015; Plissonneau et al 2015; Parlange et al 2009; Van de Wouw et al 2014), all four isolates were analyzed through sequencing by Dr. Tengsheng Zhou (a post-doctor in our lab). The PG2 *L. maculans* isolate 03-15-03 contained four avirulence genes *AvrLm2*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*, the PG3 isolate 3-42-6 has four avirulence genes *AvrLm1*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1*, the PGT isolate 09stonewall9553 consists four avirulence genes *AvrLm2*, *AvrLm6*, *AvrLm11*, and *AvrLmJ1* while the PG4 isolate PG4-1-M has three avirulence genes *AvrLm6*, *AvrLm11*, and *AvrLmJ1*. The isolate 03-15-03 was used to evaluate blackleg resistance derived from 'Meng' in each generation. Other three isolates were also used in the BC_1F_3 and BC_2F_2 to explore the interaction between *L. maculans* pathogen and blackleg resistance of plants. All the isolates were in the collection of the University of Manitoba except for 09stonewall9553 obtained from Dr. Coreen Franke (Crop Protection Services).

4.3.5.1 Preparation of isolates

Leptosphaeria maculans isolates were derived from the single spore culture (Choi et al 1999). These original isolates were stored with 15 % glycerol at - 20 ℃. These isolates were cultured on 20 % water agar medium in 100 mm × 15 mm sterile petri dishes (Fisher Scientific, Waltham, USA) under light at 23 ℃. A sterile needle (Bd Eclipse, Frandlin Lake, USA) was used to transfer hyphae from 20% water agar medium on V8 medium (V8 juice

(Campbell, Toronto, Canada) 200 mL/L, distilled water 800 mL/L, calcium carbonate 0.75 g/L, and agar 15 g/L). These 10 cm diameter petri dishes were sealed with parafilm (Vwr, Radnor, USA) and placed under light at 23 ℃. After they were grown on V8 medium for two weeks, the pycnidiospores on the surface were scraped gently with a flamed glass slide and flooded with 5 to 10 mL autoclaved distilled water. Subsequently, the pycnidiospore suspension was filtered through an autoclaved filter paper (Fisher Scientific, Waltham, USA) into an autoclaved 15 mL cornial tube (Fisher Scientific, Waltham, USA) and centrifuged at 4000 rpm for 10 min. The spore pellet was resuspended in autoclaved distilled water. The concentration of the pycnidiospore suspension was determined with a hemacytometer (Fisher Scientific, Waltham, USA). The pycnidiospore suspension was separated into 1 mL sterile tubes (Fisher Scientific, Waltham, USA) and stored at - 20 ℃ with a higher concentration for less than three months.

4.3.5.2 Identification of isolates

Five *B. napus* accessions were used to identify *L. maculans* isolates. They included three *B. napus* cultivars 'Westar', 'Quinta', 'Glacier' and two testing lines '15C-92-11-2' with a resistance gene *BLMR1*and 'G4A36-2-9' with the *Rlm2* resistance gene. They were planted in 96 cell plastic trays with growing mix (Sungro Horiculture, Agawan, Canada). Ninety-six plants in a tray were inoculated with 12 *L. maculans* isolates. Four plants of each of the five *B. napus* accessions were used to be inoculated with one isolate. Plants were kept in a growth chamber at 20 ℃ / 18 ℃ (day/night) with a 16 hour photoperiod and 10 % humidity. The inoculation procedure was the same as described below.

4.3.6 Inoculation tests

4.3.6.1 Cotyledon inoculation

Cotyledon inoculation was performed in the BC_1, BC_2, BC_1F_2, BC_1F_3, and BC_2F_2 at seven days after planting. The inoculation with *L. maculans* isolates followed the method introduced by Williams et al (1979). When cotyledons completely unfolded, it was time to perform cotyledon inoculation with *L. maculans* isolates. The *L. maculans* isolates were diluted to the concentration of 2×10^7 pycnidiospores per mL and then placed on ice. A homemade hole punch (one side of the point of 11.4 cm dissecting straight forceps was bent inward) was used to make a wound in the center of half of a cotyledon. A pipette (Eppendorf, Hamburg, Germany) was used to drop 10 uL *L. maculans* spore suspension on the wound. The trays of the inoculated seedlings were kept on a bench overnight. Then the trays were placed back into a growth chamber at 20 ℃ / 18 ℃ (day/night) with a 16 hour photoperiod and 10 % humidity. True leaves were removed at the first ten days in order to keep cotyledons alive.

4.3.6.2 True leaf inoculation

All plants in the F_1 were obtained through embryo rescue tissue culture. Therefore, the plants were evaluated through true leaf inoculation at the two or three leaf stage using *L. maculans* isolate 03-15-03. Seedlings were transferred from 1/2 MS medium into 8.5 × 8.5 × 8.5 cm pots. Two younger true leaves were wounded in four points. The remainder of procedure is the same as cotyledon inoculation.

4.3.7 Disease score

The severity of disease was classified into five categories, 0, 1, 2, 3, and 4, depending

on the sizes of symptom with or without sporulation (Williams et al 1979) (Table 3.1). This classification was only used for *L. maculans* isolates (03-15-03, 3-42-6, PGT-M, 09stonewall9553, and PG4-1-M) tests. The scores were recorded at 8, 11, and 14 days after cotyledon inoculation.

Disease scores for cotyledon inoculation depended on the size of the lesion, degree of the necrosis, and the presence of sporulation using a 0 - 9 rating scales (0, 1, 3, 5, 7, and 9) (Williams et al 1979) (Table 3.2). Plants with scores of three or less were classified as resistance. The classification of resistant and susceptible plants was also strictly in accordance with the symptoms of 'Westar' through inoculation at the same time under the same conditions. The BC_1F_3 plants with scores of one and zero were recorded as resistant plants due to the slow development of symptoms inoculated with *L. maculans* isolate PG4-1-M. No obvious visible disease symptoms on cotyledons of plants were scored as zero. Plants with a score of 9 showed large spread and dark lesions with sporulation (Figure 3.3).

The results of true leaf inoculation in the F_1 were record at 14 days after true leaf inoculations. The symptom of 'Westar' was an important standard for the classification of resistant and susceptible plants through true leaf inoculation. Susceptible plants showed large collapse lesions with profuse sporulation. Resistant plants showed limited blacking around the wound and faint chlorotic halo without sporulation, which was similar with the symptom of 'Westar' (Figure 3.4).

4.3.8 Statistical analysis

Data was analyzed by using SAS software version 9.3 (SAS Institute Inc., 2008) for a chi-square test. The chi-square test for goodness of fit was performed to determine the

segregation of resistant and susceptible plants in each generation.

4.4 Results

4.4.1 Resistance to *Leptosphaeria maculans* in the F_1 of the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'

Twenty-four F_1 plants were obtained through plant tissue culture and evaluated for blackleg resistance by *L. maculans* isolate 03-15-03. Six families in the F_1 showed a high level of resistance to *L. maculans* isolate 03-15-03, except for one family (F_1-2). Seven out of ten plants were classified as resistant plants while three were classified as susceptible plants according to true leaf inoculation (Table 4.2). Three 'Westar' were also inoculated as controls with *L. maculans* isolate 03-15-03 on the true leaves and were rated as susceptible plants (Table 4.2).

4.4.2 Resistance to *Leptosphaeria maculans* in the BC_1, BC_2, BC_1F_2, BC_2F_2, and BC_1F_3

In the BC_1, 668 plants that belonged to eleven families were tested using *L. maculans* isolate 03-15-03. According to the results of the chi-square test, only two families (BC_1-7.1, and BC_1-7.3) followed a 1:1 segregation ratio of resistant and susceptible plants. Two families had over ten resistant plants, but most lost resistance completely following the BC_1 (Figure 4.2).

Table 4.2 True leaf resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in F_1 plants of the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng' in a greenhouse in Winnipeg, MB in 2014.

F_1	**Phenotype**		
	T	**R**	**S**
F_1-1	1	1	0
F_1-2	10	7	3
F_1-3	2	2	0
F_1-4	1	1	0
F_1-5	4	4	0
F_1-6	1	1	0
F_1-7	5	5	0
Total	24	21	3
Westar	3	0	3

T=total; R=resistant; S=susceptible

F_1-1=Westar×Meng 2-1; F_1-2= Westar×Meng 2-2; F_1-3= Westar×Meng 4-5; F_1-4= Westar×Meng 5-5; F_1-5= Westar×Meng 7-2; F_1-6= Westar×Meng 7-3; F_1-7= Westar×Meng 8-4.

In the BC_1-2.1 family, only one out of 55 plants displayed resistance while 54 were susceptible (Table 4.3). However, all 50 seedlings were susceptible in the BC_1-2.2 family. Only one resistant plant out of 47 plants was identified in the BC_1-2.3 family when inoculated with *L. maculans* isolate 03-15-03. Only three out of 132 plants in the BC_1-2.4 family were evaluated as resistant. In the BC_1-2.5 family, only one out of 91 plants exhibited blackleg resistance while 90 were susceptible. Thirteen resistant and 34 susceptible plants were

evaluated in the BC_1-3.1 family, and this did not fit a 1:1 segregation ratio of resistant and susceptible plants (Table 4.3). Fifty-three plants were susceptible in the BC_1-5.1 family. In the BC_1-5.2 family, 9 out of 77 plants were resistant. The segregation of resistant and susceptible plants in the BC_1-7.1 family did follow a 1:1 ratio, but the population was relatively small (7 plants). Two out of seven were resistant plants while five were susceptible. The segregation of resistant and susceptible plants in the BC_1-7.3 family fit a 1:1 ratio, which included 28 resistant and 39 susceptible plants. Finally, only six out of 42 were resistant and 36 were susceptible in the BC_1-7.2 family, which deviated from a 1:1 segregation ratio of resistant and susceptible plants (Table 4.3).

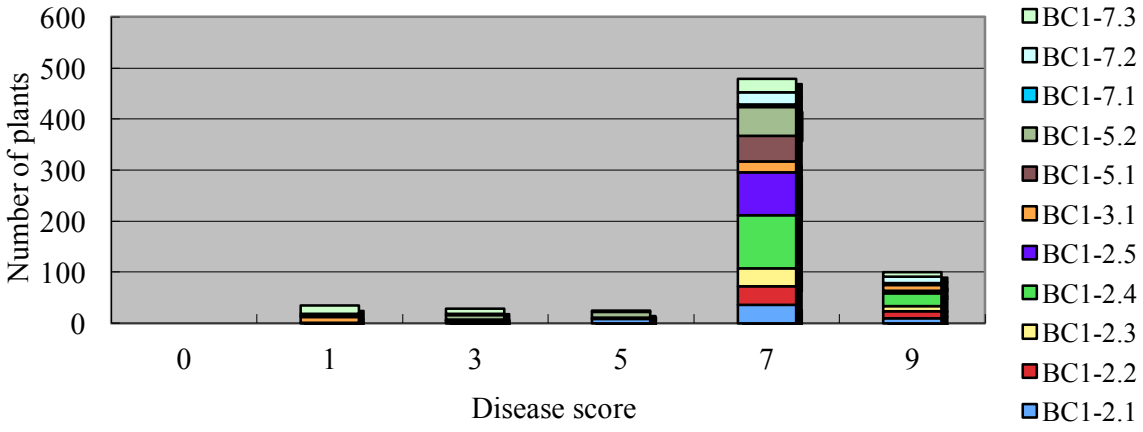


Figure 4.2 Blackleg disease scores for cotyledon inoculation of BC_1 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014.

Table 4.3 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_1 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng' in a greenhouse in Winnipeg, MB in 2014.

BC_1	**Phenotype**			**Disease scores**		**X^2 test for 1:1**	
	T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_1-2.1	55	1	54	3.0-9.0	7.00±1.28	51.07	<.0001
BC_1-2.2	50	0	50	7.0-9.0	7.56±0.91	0.00	
BC_1-2.3	47	1	46	7.0-9.0	7.34±1.05	43.09	<.0001
BC_1-2.4	132	3	129	1.0-9.0	7.24±1.11	120.27	<.0001
BC_1-2.5	91	1	90	3.0-9.0	7.09±0.66	87.04	<.0001
BC_1-3.1	47	13	34	1.0-9.0	5.81±3.00	9.38	0.002
BC_1-5.1	53	0	53	7.0-9.0	7.11±0.47	0.00	
BC_1-5.2	77	9	68	3.0-7.0	6.25±1.38	45.21	<.0001
BC_1-7.1	7	2	5	1.0-9.0	5.43±2.73	1.29	0.26
BC_1-7.2	42	6	36	1.0-9.0	6.64±2.36	21.43	<.0001
BC_1-7.3	67	28	39	1.0-9.0	5.00±2.93	1.81	0.18
Total	668	64	604	1.0-9.0	6.72±1.80	437.49	<.0001
Westar	122			7.0-9.0	7.98±1.00		

T=total; R=resistant; S=susceptible

Seven families in the BC_2 were evaluated for resistance to *L. maculans* isolate 03-15-03. One hundred and twenty-two out of 394 were resistant and 272 were susceptible, which did not follow a 1:1 segregation ratio of resistant and susceptible plants. However, two families (BC_2-3.1.1 and BC_2-7.1.1) in the BC_2 fit a 1:1 segregation ratio of resistant and susceptible plants (Figure 4.3).

In the BC_2-2.1.1 family, only one seedling showed resistance while other 15 were susceptible. All 14 plants in the BC_2-2.4.1 family were susceptible. The segregation of

resistant and susceptible plants in the BC_2-3.1.1 family followed a 1:1 ratio, which included 34 resistant and 27 susceptible plants (Table 4.4). In the BC_2-5.2.1 family, four out of 69 plants exhibited resistance while 65 were susceptible. Two resistant and two susceptible plants were identified in the BC_2-7.1.1 family. In the BC_2-7.2.1 family, 14 plants and 27 plants were resistant and susceptible respectively, which did not fit a 1:1 resistant-to-susceptible segregation ratio (Table 4.4). In total, 67 out of 189 were resistant and 122 were susceptible, which deviated from a 1:1 segregation ratio of resistant to susceptible plants (Table 4.4).

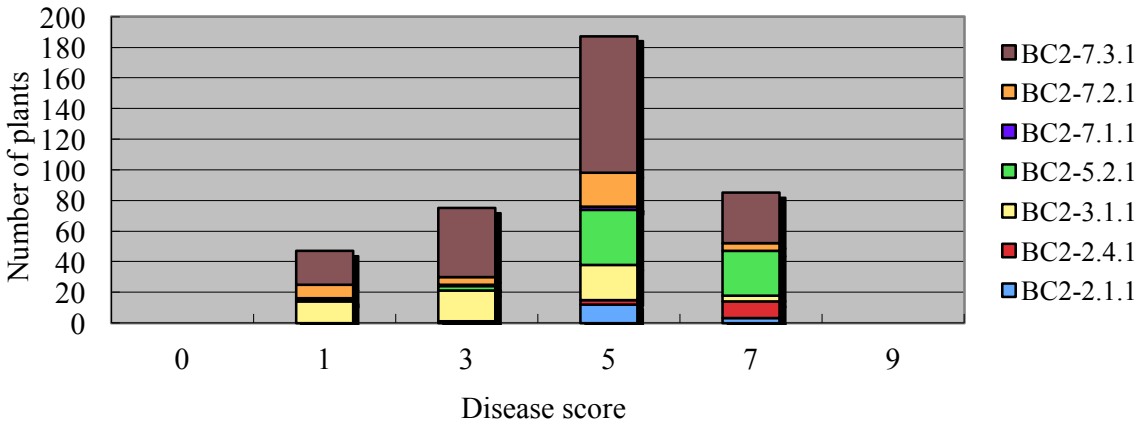


Figure 4.3 Blackleg disease scores for cotyledon inoculation of BC_2 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014.

The families in the BC_1 were used to produce seven families in the BC_1F_2 and BC_2, respectively. A total of 401 individual plants were evaluated for blackleg resistance using *L.*

maculans isolate 03-15-03, and 210 plants were resistant in the BC_1F_2 (Figure 4.4 and Table 4.5). The segregation of resistant and susceptible plants of the BC_1F_2 generation deviated from a 3:1 ratio (Table 4.5).

Table 4.4 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_2 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng' in a greenhouse in Winnipeg, MB in 2014.

BC_2	**Phenotype**			**Disease scores**		**X^2 test for 1:1**	
	T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_2-2.1.1	16	1	15	3.0-7.0	5.25±1.00	12.25	0.00
BC_2-2.4.1	14	0	14	5.0-7.0	6.57±0.85	0.00	
BC_2-3.1.1	61	34	27	1.0-7.0	3.56±1.79	0.80	0.37
BC_2-5.2.1	69	4	65	1.0-7.0	5.70±1.28	53.93	<.0001
BC_2-7.1.1	4	2	2	1.0-5.0	3.50±1.91	0.00	1.00
BC_2-7.2.1	41	14	27	1.0-7.0	4.12±1.95	4.12	0.04
BC_2-7.3.1	189	67	122	1.0-7.0	4.41±1.78	16.00	<.0001
Total	394	122	272	1.0-7.0	4.57±1.83	57.11	<.0001
Westar	93			3.0-7.0	5.71±1.09		

T=total; R=resistant; S=susceptible

No resistant plants were presented in the BC_1F_2-2.1.1s family composed of 16 plants. Only one resistant plant was observed in 29 plants in the BC_1F_2-2.4.1s family. The segregation of resistant and susceptible plants in the BC_1F_2-3.1.1.1s family fit a 3:1 ratio; Forty-seven plants were resistant and 16 were susceptible. In the BC_1F_2-5.2.1s family, 11 out of 59 seedlings were resistant while 48 were susceptible. In the BC_1F_2-7.1.1s family, five

plants were resistant while six were susceptible. Even though the number of resistant plants was higher than that of susceptible plants in the BC_1F_2-7.2.1s and BC_1F_2-7.3.1s families, the segregation of resistant and susceptible plants did not follow a 3:1 ratio. In the BC_1F_2-7.2.1s family, 14 out of 25 plants were resistant while 11 were susceptible to *L. maculans*. One hundred and thirty-two out of 198 were resistant and 66 were susceptible in the BC_1F_2-7.3.1s family (Table 4.5).

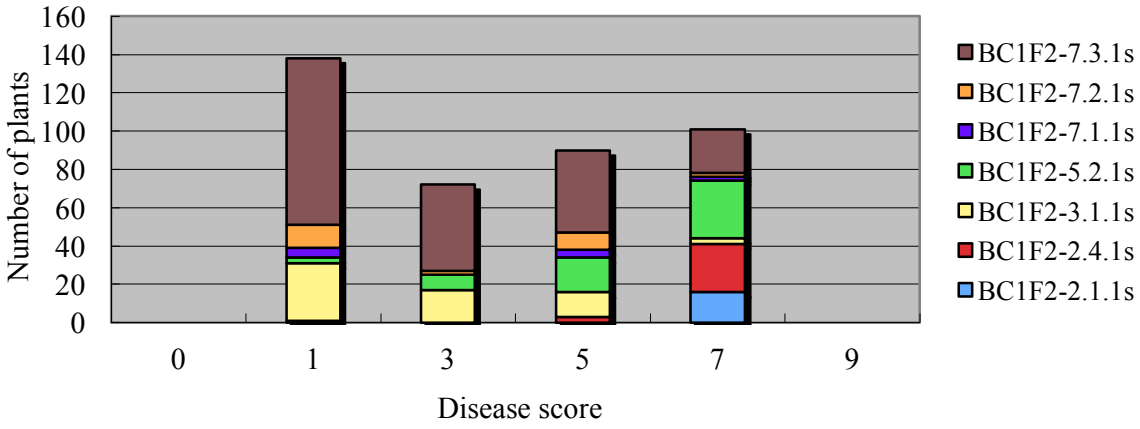


Figure 4.4 Blackleg disease scores for cotyledon inoculation of BC_1F_2 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolate 03-15-03 in a greenhouse in Winnipeg, MB in 2014.

Only the BC_2F_2-3.1.1.1s family was evaluated for blackleg resistance using three *L. maculans* isolates 03-15-03, PG4-1-M and 3-42-6 in the BC_2F_2 (Figure 4.5). Seventy resistant and 16 susceptible plants were identified following cotyledon inoculation with *L. maculans* isolate 03-15-03, which followed a 3:1 segregation ratio of resistant to susceptible plants

(Table 4.6). The segregation of resistant and susceptible plants in the BC_2F_2-3.1.1.1s family inoculated with *L. maculans* isolate 3-42-6 also followed a 3:1 ratio. In this case, 72 out of 86 were resistant to the isolate 3-42-6 (Table 4.6). However, the results of cotyledon inoculation with *L. maculans* isolate PG4-1-M showed that 46 out of the 72 plants were resistant, which did not fit a 3:1 resistant-to-susceptible ratio (Table 4.6).

Table 4.5 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolate 03-15-03 in BC_1F_2 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng' in a greenhouse in Winnipeg, MB in 2014.

BC_1F_2	**Phenotype**			**Disease scores**		**X^2 test for 3:1**	
	T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_1F_2-2.1.1s	16	0	16	7.0-7.0	7.00±0.00	0.00	
BC_1F_2-2.4.1s	29	1	28	1.0-7.0	6.59±1.24	79.18	<.0001
BC_1F_2-3.1.1s	63	47	16	1.0-7.0	2.65±1.85	0.00	0.94
BC_1F_2-5.2.1s	59	11	48	1.0-7.0	5.54±1.77	99.94	<.0001
BC_1F_2-7.1.1s	11	5	6	1.0-7.0	3.55±2.54	5.12	0.02
BC_1F_2-7.2.1s	25	14	11	1.0-7.0	3.08±2.20	4.81	0.03
BC_1F_2-7.3.1s	198	132	66	1.0-7.0	3.02±2.12	7.33	0.01
Total	401	210	191	1.0-7.0	3.77±2.39	109.53	<.0001
Westar	97			3.0-7.0	5.12±1.35		

T=total; R=resistant; S=susceptible

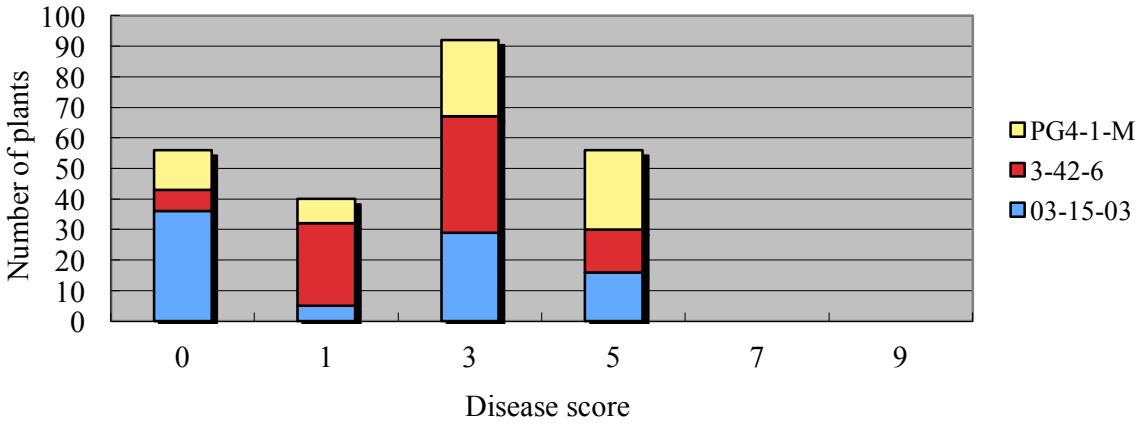


Figure 4.5 Blackleg disease scores for cotyledon inoculation of BC_2F_2 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015.

Table 4.6 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in the BC_2F_2 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng' in a greenhouse in Winnipeg, MB in 2015.

BC_2F_2	**Isolate**	**Phenotype**			**Disease scores**		**X^2 test for 3:1**	
		T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_2F_2-3.1.1.1s	03-15-03	86	70	16	0.0-5.0	2.00±1.95	1.88	0.17
Westar	03-15-03	4			3.0-5.0	4.00±1.15		
BC_2F_2-3.1.1.1s	3-24-6	86	72	14	0.0-5.0	2.45±1.54	3.49	0.06
Westar	3-42-6	4			3.0-5.0	4.00±1.15		
BC_2F_2-3.1.1.1s	PG4-1-M	72	46	26	0.0-5.0	2.96±1.89	4.74	0.03
Westar	PG4-1-M	3			3.0-5.0	4.33±1.15		

T=total; R=resistant; S=susceptible

Three families were evaluated for blackleg resistance using cotyledon inoculation and four *L. maculans* isolates 03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M in the BC_1F_3 (Figure 4.6, 4.7, and 4.8). Both BC_1F_3-3.1.1.1ss and BC_1F_3-3.1.1.2ss families followed a 3:1 segregation ratio of resistant and susceptible plants under cotyledon inoculations with three *L. maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M (Table 4.7). However, the segregation of resistant and susceptible plants of the BC_1F_3-7.3.1.1ss family deviated from a 3:1 ratio when inoculated with the isolate 03-15-03 (Table 4.7).

As described in Table 4.7, 69 out of 84 seedlings in the BC_1F_3-3.1.1.1ss family inoculated with *L. maculans* isolate 03-15-03 were resistant while 15 were susceptible. The segregation of resistant and susceptible plants of the BC_1F_3-3.1.1.1ss family inoculated with the isolate 09stonewall9553 fit a 3:1 ratio, which consisted of 63 resistant and 21 susceptible plants. Moreover, 65 out of 92 plants were resistant and 27 were susceptible in the BC_1F_3-3.1.1.1ss family tested with the isolate PG4-1-M (Table 4.7). In the BC_1F_3-3.1.1.2ss family, 59 out of 78 seedlings were resistant when inoculated with the isolate 03-15-03; 51 out of 73 plants were classified as resistant when inoculated with the isolate 09stonewall9553, and 69 out of 82 plants were resistant when inoculated with the isolate PG4-1-M (Table 4.7).

However, the segregation of resistant and susceptible plants in the BC_1F_3-7.3.1.1ss family did not follow a 3:1 ratio when the isolate 03-15-03 was used to evaluate blackleg resistance. Almost all plants were resistant to *L. maculans* isolate 03-15-03, except for two susceptible plants. Sixty-one out of 78 plants in the BC_1F_3-7.3.1.1ss family inoculated with the isolate 3-42-6 were resistant, and 72 out of 90 plants were resistant when inoculated with the isolate PG4-1-M (Table 4.7).

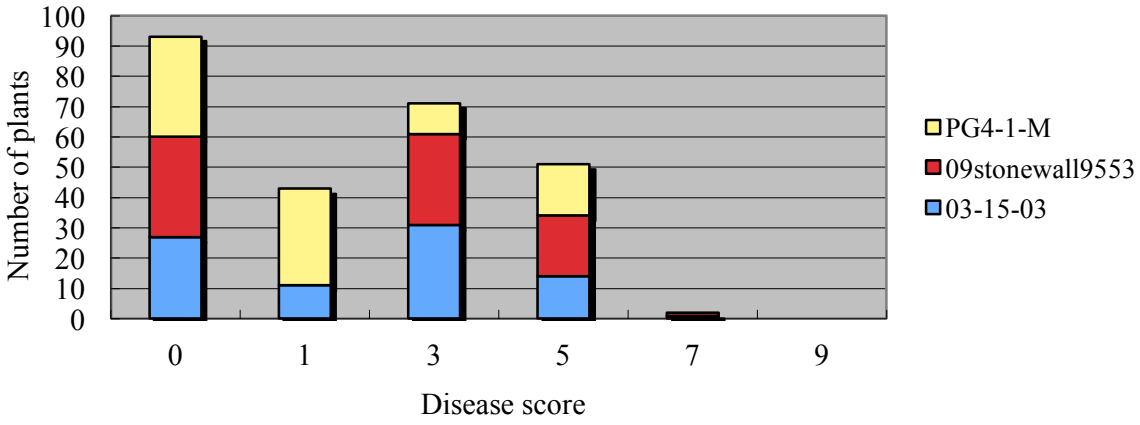


Figure 4.6 Blackleg disease scores for cotyledon inoculation of BC_1F_3-3.1.1.1ss plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015.

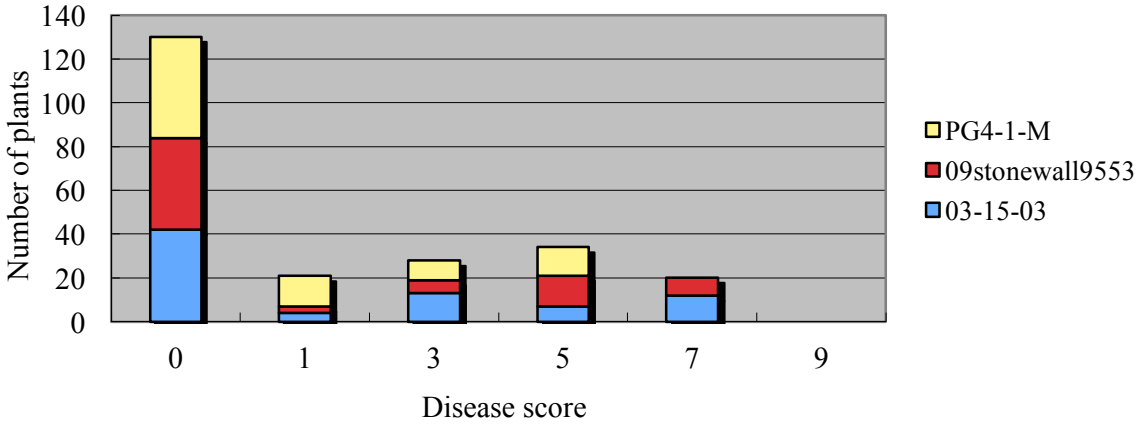


Figure 4.7 Blackleg disease scores for cotyledon inoculation of BC_1F_3-3.1.1.2ss plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 09stonewall9553, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015.

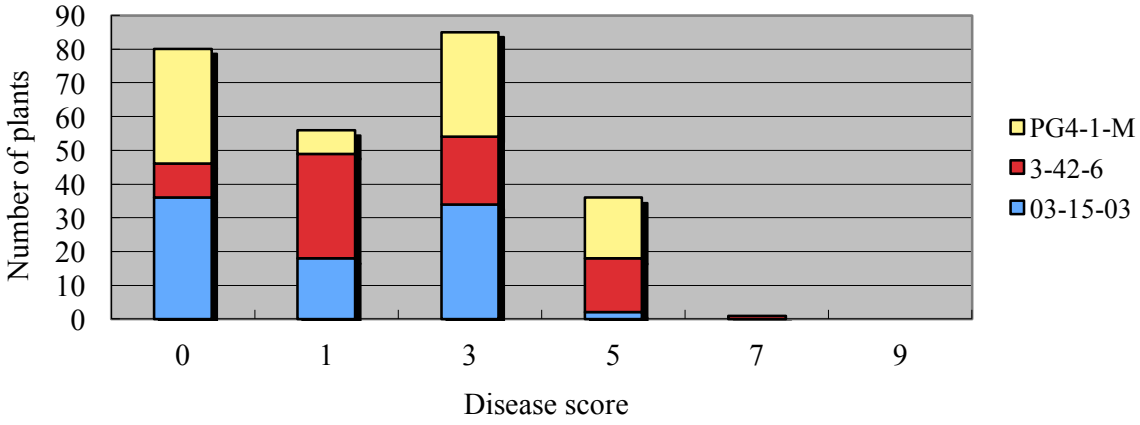


Figure 4.8 Blackleg disease scores for cotyledon inoculation of BC_1F_3-7.3.1.1ss plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng'. Plants were inoculated with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, and PG4-1-M in a greenhouse in Winnipeg, MB in 2015.

4.5 Discussion

The synthetic hexaploid *Brassica* species (AABBCC) were derived from the hybrid (ABC) between *B. carinata* (BBCC) and *B. rapa* L. (AA) (Meng et al 1998; Li et al 2004, 2006, 2007). It is difficult to obtain seeds in the cross of *B. napus* and the synthetic hexaploid *Brassica* species. In my study, no seed was produced in the crosses of *B. napus* cultivar 'Westar' and the synthetic hexaploid *Brassica* species 'Meng'. Embryo rescue tissue culture is necessary to obtain F_1 plants. Rahman (2001) also reported that no seed was produced in the cross of *B. napus* and the hexaploid *Brassica* species (AABBCC). However, three seeds per pollination were obtained in the reciprocal cross (Rahman 2001) and Li et al (2004) also obtained 0.3 seeds per pollination when a synthetic hexaploid *Brassica* species was used as female to cross with *B. napus*.

Table 4.7 Cotyledon resistance following inoculation with *Leptosphaeria maculans* isolates 03-15-03, 3-42-6, 09stonewall9553, and PG4-1-M in BC_1F_3 plants following the cross of *Brassica napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng' in a greenhouse in Winnipeg, MB in 2015.

BC_1F_3	**Isolate**	**Phenotype**			**Disease scores**		**X^2 test for 3:1**	
		T	**R**	**S**	**Range**	**Mean ± SE**	**X^2**	***P***
BC_1F_3-3.1.1.1ss	03-15-03	84	69	15	0.0-5.0	2.15±1.90	2.29	0.13
BC_1F_3-3.1.1.1ss	09stonewall9553	84	63	21	0.0-5.0	2.35±2.07	0.00	1.00
BC_1F_3-3.1.1.1ss	PG4-1-M	92	65	27	0.0-5.0	1.60±1.85	0.93	0.34
BC_1F_3-3.1.1.2ss	03-15-03	78	59	19	0.0-7.0	2.08±2.67	0.02	0.90
BC_1F_3-3.1.1.2ss	09stonewall9553	73	51	22	0.0-7.0	2.01±2.64	1.03	0.31
BC_1F_3-3.1.1.2ss	PG4-1-M	82	69	13	0.0-5.0	1.29±1.87	3.66	0.06
BC_1F_3-7.3.1.1ss	03-15-03	90	88	2	0.0-5.0	1.44±1.44	24.90	<.0001
BC_1F_3-7.3.1.1ss	3-42-6	78	61	17	0.0-5.0	2.28±1.82	0.43	0.51
BC_1F_3-7.3.1.1ss	PG4-1-M	90	72	18	0.0-5.0	2.11±1.94	1.20	0.27
Westar	03-15-03	8			3.0-7.0	5.17±1.34		
Westar	09stonewall9553	8			5.0-7.0	6.00±1.15		
Westar	3-42-6	4			3.0-5.0	4.00±1.15		
Westar	PG4-1-M	8			1.0-5.0	3.55±1.81		

T=total; R=resistant; S=susceptible

The synthetic hexaploid *Brassica* species contains the A, B, and C subgenomes (Li et al 2004; Meng et al 1998; Rahman et al 2001). This genetic resource should provide increased genetic diversity within *B. napus* (Li et al 2007; Xiao et al 2010). The pentaploid hybrid in the F_1 of the cross of synthetic *Brassica* species and *B. napus* had the genomic construction AABCC (Li et al 2004; Meng et al 1998), and the blackleg resistance gene from *B. carinata*

was located in the B genome as suggested in the previous report (Sjodin et al 1988). Accordingly, all F_1 plants with AABCC in my study should be resistant to *L. maculans* isolates. However, three seedlings in the F_1-2 family showed susceptible symptoms to *L. maculans* isolate 03-15-03 based on true leaf inoculation. Synthetic hexaploid *Brassica* species 'Meng' used in my study was derived from different seed types. The F_1-2 family was obtained from the cross of *B. napus* cultivar 'Westar' and 'Meng 2', which was from selfing of a synthetic hexaploid *Brassica* species. The genomic structure of synthetic hexaploid *Brassica* species are not stable (Meng et al 1998; Struss et al 1991), and their numbers of chromosomes were not confirmed through a cytological analysis in current study. The chromosomes carrying blackleg resistance may be lost during the cross of *B. napus* 'Westar' and the synthetic hexaploid *Brassica* species 'Meng', which may have resulted in the presence of susceptible plants in the F_1.

Rahman (2001) reported that most plants in the F_1 (AABCC) of the cross of the synthetic *Brassica* species (AABBCC) and *B. napus* did not yield seed following selfing, and only 0.07 seeds per pollinated flower could be obtained manually. In addition, 19 or even more pairings were observed in the 80% pollen mother cells of the pentaploid hybrids (AABCC) (Rahman 2001). This indicated that the 10 chromosomes of the A subgenome in the pentaploid hybrids paired with the A subgenome chromosomes from *B. napus*, and this also occurred for the C subgenome chromosomes (Li et al 2004). The B subgenome chromosomes often existed as univalents based on the studies of PMCs (pollen mother cells), and tended to be eliminated during meiosis in the pentaploid hybrids due to the laggard action in anaphase (Li et al 2004, 2005, 2006, 2007; Meng et al 1998; Xiao et al 2010). Since the B

genome chromosomes are suggested to carry the high level of blackleg resistance in *B. carinata* (Sjodin et al 1988), the absence of the B subgenome chromosomes results in the loss of blackleg resistance in the primary backcrosses and self-pollinated generations, which is most likely the case in the current study. In the BC_1, only 64 out of 668 plants showed resistance to *L. maculans* isolate 03-15-03. Chi-square analysis demonstrated that most families in the BC_1 and BC_2 did not follow the expected 1:1 segregation ratios of resistant and susceptible plants and more susceptible plants were displayed in the BC_1 and BC_2.

In my study, the fertility of most plants in the advanced generations improved based on the seed set. Meng et al (1998) and Rahman (2001) also obtained populations with normal fertility in the backcross following the cross of the synthetic hexaploid *Brassica* species and *B. napus*. The sterile plants would be phased out due to the elimination of univalent chromosomes during meiosis (Meng et al 1998). The normal meiosis behavior, pollen germination, and embryo sac development would be derived from the normal chromosome pairing during meiosis, which might be obtained from the complete loss of the B subgenome chromosomes in the backcross of the hybrids (AABCC) and *B. napus* (AACC) (Li et al 2007). Therefore, the B subgenome in the plants with normal seed set may have been completely lost following two backcrosses and selfing in my study. This should be confirmed through cytogenetic study and genome specific molecular markers or whole genome sequencing. However, the plants with good seed set also showed good resistance to *L. maculans*. The segregation of resistant and susceptible plants of the BC_2-3.1.1 family followed a 1:1 ratio and the BC_1F_2-3.1.1s family followed a 3:1 segregation ratio of resistant and susceptible plants. Subsequently, two families in the selfing generations, BC_1F_3 and BC_2F_2 that were

derived from the BC_1F_2 and BC_2 generations, also fit a 3:1 resistant-to-susceptible ratio.

A study on the mother pollen cell of the trigenomic hybrid (ABC) showed the absence of some univalent B subgenome chromosomes during meiosis, which proved the involvement of the B subgenome chromosomes in pairing with the A and C subgenome chromosomes (Choudhary et al 2000). Li et al (2004, 2005) also reported that the homeologous B subgenome chromosomes also paired with the bivalents between the A or C subgenome chromosomes to form a trivalent or even quadrivalent. Based on this, it appears possible to have homeologous recombination. This may be the reason why most plants showing resistance to *L. maculans* had normal fertility and seed set in my study. Accordingly, it indicated that the blackleg resistance from synthetic hexaploid *Brassica* species 'Meng' could be introgressed into *B. napus*.

To the best of our knowledge, this is the first study on transferring blackleg resistance from synthetic hexaploid *Brassica* species into *B. napus*. Previously, synthetic hexaploid *Brassica* species was used to transfer yellow seed color genes from *B. rapa* and *B. carinata* into *B. napus* (Meng et al 1998; Rahman 2011). The resistant plants in the current project provide excellent materials to understand blackleg resistance derived from synthetic *Brassica* species in the future.

Chapter 5. General discussion

Transfer blackleg resistance from *Brassica* relatives into *Brassica napus* L. is brought with interspecific hybridization following backcrossing and selfing. However, it is difficult to obtain normal seed set in the hybrids from crosses or even in the backcrosses and selfing generations due to the affects of aneuploid chromosomes during meiosis. In my research, seeds of the cross between *B. napus* cultivar 'Westar' and *B. carinata* A. Braun cultivar 'T4001' were difficult to obtain. *Brassica napus* has 38 chromosomes with the AACC genomes while *B. carinata* contains 34 chromosomes with the BBCC genomes. The F_1 should contain the ABCC genomes (Mason et al 2011; Sacristan et al 1986). In the interspecific hybrid between *B. carinata* and *B. napus*, Mason et al (2010) reported that 88% of homologous chromosome pairings came from the C subgenome. Homeologous chromosome pairings between the A, B, and C subgenomes in the hybrid were infrequent (Mason et al 2010). Inability to establish chromosome pairings during meiosis was an obstacle for successful development of fertilized ovules to into viable seeds (Meng et al 1998).

Specifically, all plants in the F_1 of the cross between *B. napus* cultivar 'Westar' and *B. carinata* 'T4001' were sterile because of abnormal synapsis during meiosis. Similarly, no seed was directly obtained from the crosses of *B. napus* cultivar 'Westar' and synthetic hexaploid *Brassica* species 'Meng', which contained the AACC genome with 38 chromosomes and the AABBCC genome with 54 chromosomes, respectively. The genome construction of the F_1 of this cross should be AABCC with 46 chromosomes. Li et al (2004) showed that 196 out of 355 seedlings in the F_1 of the cross of the synthetic hexaploid

Brassica species (AABBCC) and *B. napus* (AACC) contained 46 chromosomes. The low fertility of pollen grains and poor seed set in the F_1 was attributed to unpaired chromosomes of the B subgenome resulting in meiotic abnormalities (Li et al 2004).

Most blackleg resistance genes that have been mapped are located in linkage group N7 and N10 of the A subgenome in *B. napus* and the A genome in *B. rapa* (Delourme et al 2004; Yu et al 2005, 2008; Long et al 2001). No blackleg resistance genes have been identified in the C genome of *B. oleracea* L. based on the testing of several *B. oleracea* associations (Mithen et al 1987). However, the B genome containing *Brassica* species, *B. carinata*, *B. juncea* (L.) Czem. & Coss, and *B. nigra* (L.) Koch were identified with a high level of blackleg resistance (Roy 1984; Rimmer et al 1992; Sacristan et al 1986; Sjodin et al 1988). Several blackleg resistance genes have been mapped in linkage group B4 of *B. nigra* (Chevre et al 1996; Delourme et al 2008; Eber et al 2011) and linkage groups B3 and B8 in *B. juncea* (Chevre et al 1997; Balesdent et al 2002; Christianson et al 2006; Raman et al 2013). Conversely, in *B. carinata* little is known about blackleg resistance genes in the B genome. Song et al (1994) indicated that a number of homeologous chromosomes present in the three B genomes of *B. nigra*, *B. juncea*, and *B. carinata* in according to the distinction at the level of linkage groups and DNA. Strong signals of hybridization between a DNA probe from *B. nigra* and the B genome chromosomes in *B. carinata* were also detected by Li et al (2004). Furthermore, the B subgenome of *B. carinata* was observed to contain a high level of resistance to *Leptosphaeria maculans* (Desm.) Ces. & De Not. (Sacristan et al 1986). Recently, cotyledon resistance to *L. maculans*, derived from *B. carinata*, was detected throughout the B3 chromosome in the doubled haploid (DH) 1, which was derived from the

families in the BC_2S_3 population following the cross of *B. napus* and *B. carinata* (Fredua-Agyeman et al 2014). Also, blackleg resistance was detected in regions of the B3 and B8 chromosomes in the DH2 population, arising from the F_1 cross of *B. napus* with DH1 (Fredua-Agyeman et al 2014). In current study it is suggested that the B genome from *B. carinata* was eliminated in backcrosses and selfing generations due to abnormal chromosome pairing during meiosis. Accordingly, the blackleg resistance was lost quickly in the BC_2 following the cross of *B. napus* and *B. carinata*.

Li et al (2004) reported that 15% of self-pollinated progeny of the pentaploid plants (AABCC) contained 38 chromosomes. Furthermore, a subsequent cytogenetic study showed that most of the plants with 38 chromosomes had AACC genomes (Li et al 2004). These results revealed that the B subgenome was completely eliminated during meiosis in the self-pollinated generations (Li et al 2004; Xiao et al 2010). One SSR markers (sN9756) co-segregated with cotyledon resistance to *L. maculans* from *B. carinata* and was identified in the C subgenome of *B. napus*, which suggested that blackleg resistance was transferred from the B subgenome of *B. carinata* into the C subgenome of *B. napus* (Rahman et al 2007, 2014b). Alternatively, blackleg resistance was originally derived from the C subgenome of *B. carinata* (Rahman et al 2007). Struss et al (1996) also inferred that intergenomic translocation was present between the B subgenome chromosomes and A or C subgenome chromosomes in the hybrids with 38 chromosomes, obtained from the reciprocal crosses of *B. nigra* and *B. napus*, *B. rapa* and *B. carinata*, and *B. oleracea* and *B. juncea* following selfing and backcrossing with *B. napus*. According to the presence of blackleg resistant plants in each generation studied in my research findings, there is a strong possibility that blackleg

resistance from the B genome in *B. carinata* was introgressed into the A or C subgenomes. If the B subgenome persisted in the resistant plants as whole B chromosomes, the meiosis in these plants would be adversely affected by these additional chromosomes, resulting in poor seed set. Even though the seed set was not directly recorded (number of seed per silique), the total amount of seed was equal to the seed set of 'Westar' (for resistant plants in self-pollinated generations derived from the cross of *B. napus* cultivar 'Westar' and *B. carinata* 'T4001' and the cross of *B. napus* 'Westar' and synthetic hexaploid 'Meng'). The observed normal seed set suggested that these plants probably contained 38 chromosomes (like *B. napus*) without the additional B chromosomes.

A few homeologous chromosome pairings could be present during meiosis, as recorded by Busso et al (1987) and Panjabi et al (2008). These results suggest that there could be opportunity for blackleg resistance gene(s) to be transferred from the B subgenome into the A or C subgenomes. However, the high number of susceptible plants observed in my results infers that the loss of the B subgenome led to susceptible progeny, as observed by others (Roy 1984; Rimmer et al 1992; Sacristan & Gerdemann 1986; Sjodin et al 1988). Thus, susceptible plants were more common in the BC_1, BC_2, and BC_1F_2 following the cross of 'Westar' and the synthetic hexaploid 'Meng'. Segregation of resistant and susceptible plants in early generations did not follow a 1:1 or 3:1 ratio. Further backcrosses will be necessary to eliminate the genetic background of *B. carinata* and synthetic hexaploid 'Meng' in order to obtain blackleg resistant *B. napus* which does not contain undesirable agronomic traits introduced from *B. carinata* and hexaploid 'Meng'.

Chevre et al (1997) reported that blackleg resistance in *B. juncea* was controlled by a

single gene, which was consistent with reports from Rimmer and van den Berg (1992). Rahman (2012) showed that the segregation of resistant and susceptible plants of seven BC_1 families deviated from a 1:1 ratio, making it difficult to infer the number of blackleg resistance genes in *B. carinata*. In my study, the segregation of resistant and susceptible plants of BC_4 and BC_3F_2 generations in the cross of *B. napus* 'Westar' and *B. carinata* 'T4001' fit a 1:1 and 3:1 ratio, respectively. These results demonstrate that blackleg resistance transferred from *B. carinata* is controlled by a single gene. In the progeny of *B. napus* and synthetic hexaploid *Brassica* species 'Meng', the segregation of resistant and susceptible plants in the BC_1F_2-3.1.1.1s, BC_2F_2-3.1.1.1s, and BC_1F_3-3.1.1.1ss families fit a 3:1 resistant-to-susceptible ratio suggesting that a single blackleg resistance gene was possibly introgressed from the synthetic *Brassica* species 'Meng' into *B. napus*.

Finally, the synthetic *Brassica* species 'Meng' used in my study was derived from the trigenomic hybrids in the cross of *B. rapa* and *B. carinata*. Since *B. rapa* is susceptible, blackleg resistance in synthetic *Brassica* species 'Meng' was originally located in the B subgenome as *B. carinata*. With the seed set higher in the BC_1 of *B. napus* and the synthetic *Brassica* species 'Meng' than the BC_1 of *B. napus* and *B. carinata*, the synthetic *Brassica* species 'Meng' might provide a bridge to improve the transfer of blackleg resistance from *B. carinata* into *B. napus*. Such resistance could be extremely useful when introduced into commercial canola cultivars.

Chapter 6 Future research

Blackleg resistance was successfully transferred from *Brassica carinata* A. Braun and synthetic hexaploid *Brassica* species into *B. napus* L. in this research. Good seed set of blackleg resistant plants in the advanced backcross and selfing generations indicates that the B genome chromosomes are most likely lost during meiosis in these plants. For future research, cytological analysis is recommended to detect the number of chromosomes in these plants. Further backcross and selfing generations are necessary to break a large segment of chromosomes and obtain stable introgressed blackleg resistance derived from the synthetic hexaploid *Brassica* species 'Meng'. Additionally, the utilization of molecular markers for *Brassica* A, B, and C genomes and sequencing of introgression lines using next generation sequencing will help to detect successful introgression of segments from *B. carinata* and 'Meng' into *B. napus*. Furthermore, blackleg resistance derived from *B. carinata* and 'Meng' may be better understood.

Since the segregation of resistant and susceptible plants fit a 1:1 ratio in the advanced backcross generations following the cross between *B. napus* cultivar 'Westar' and *B. carinata* cultivar 'T4001', transferred blackleg resistance in *B. napus* is probably controlled by a single gene. Since a single locus in different resistant plants is not analyzed, the single locus is possible to be the same or distinct. Mapping and sequencing of this resistance gene(s) will facilitate comparison with other known blackleg resistance genes which have previously been cloned. If this resistance gene(s) differ from known blackleg resistance genes used in most commercial cultivars in Canada, it will be beneficial to develop *B. napus* cultivars with introduced novel resistance so as achieve more effective resistance to *L. maculans*.

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