Quantitative Trait Loci Controlling Sclerotinia Stem Rot Resistance and Seed Glucosinolate Content of Oilseed Rape (*Brassica napus* L.)

BY

JUN LIU

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA
FACULTY OF GRADUATE STUDIES
COPYRIGHT PERMISSION

Quantitative Trait Loci Controlling Sclerotinia Stem Rot Resistance
and Seed Glucosinolate Content of Oilseed Rape (*Brassica napus* L.)

BY

JUN LIU

A Thesis submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

Doctor of Philosophy

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to my advisor, Dr. Genyi Li for his aspiring guidance, constructive criticisms, friendly advice and constant encouragement during the five years of my graduate studies. His scientific knowledge, professional skills, keen insights and enthusiasm for *Brassica* genomic studies and value-added cultivar development have created excellent professional standards for me to follow.

I also would like to thank my committee members Dr. Peter B. E. McVetty, Dr. Mark Belmonte and Dr. Chunren Wu for their guidance on research, suggestions on professional development and patience when correcting papers. I am thankful to Dr. Wu for his ingenious initial suggestions, detailed advice on breeding operations, ideas on plant-pathogen interactions, and skillful research management, just to name a few.

I am grateful to Dr. Arvind H. Hirani, Dr. Xueping Liu, Adam Lorne and Dr. Carla Zelmer from the University of Manitoba, as well as Dr. Jonathan Jenkinson, Dr. Jed Christianson, Zhe Li, Steven Johnston, Judy Albrecht, and Dr. Issa Coulibaly from Monsanto for their technical assistance.

I really appreciate the full support and encouragement from Chris Anderson and other colleagues at Monsanto Canada for helping me manage my studies and work.

I greatly appreciate the patience and support of my wife, Chi, and my son, Yiran, who have seen very little of me during my doctoral studies.

Finally I would also like to thank all other people who helped me, one way or another, with my doctoral efforts!

TABLE OF CONTENTS

Page

ABBREVIATIONS

4C: four carbon aliphatic glucosinolates

5C: five carbon aliphatic glucosinolates

CIM: composite interval mapping

DH: doubled haploid

DPI: days post inoculation

GBC: glucobrassicin

GBN: glucobrassicanapin

GLS: glucoalyssin

GNP: gluconapin

GSL: glucosinolate

LG: linkage group

LOD: logarithm of odds

MIM: multiple interval mapping

MON: Monsanto

MPP: mycelium PDA/PGA plug

NGS: next generation sequencing

OA: Oxalic acid

PCR: polymerase chain reaction

PDA/PGA: potato dextrose (glucose) agar

PRO: progoitrin

QTL: quantitative trait locus

RAPD: random amplified polymorphic DNA

RFLP: restriction fragment length polymorphism

RIL: recombinant inbred line

ROI: reactive oxygen intermediate

SNP: single nucleotide polymorphism

SSR: simple sequence repeat (microsatellite)

TGC: total glucosinolate content

T-Ali: total aliphatic glucosinolates

UM: University of Manitoba

UP: unique SNP marker position

LIST OF TABLES

Table Page

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

APPENDICES

LIST OF FIGURES

Figure Page

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

APPENDICES

ABSTRACT

Liu, Jun. Ph.D., The University of Manitoba, December, 2015. Quantitative Trait Loci Controlling Sclerotinia Stem Rot Resistance and Glucosinolate Content in Oilseed Rape (*Brassica napus* L.). Major Professor; Genyi Li.

Canola/rapeseed (*Brassica napus* L.) is a major oilseed crop worldwide. However, its production is largely affected by the fungal disease Sclerotinia stem rot as well as seed glucosinolates. So far the genetic mechanisms controlling these two traits have been poorly understood. In the present study, three bi-parental doubled haploid *B. napus* populations M730, M692 and ZT were grown in either natural or artificial environments and genotyped using the Brassica 60K Infinium® SNPs and/or sequence related amplified polymorphisms. Three genetic linkage maps covered 2,597.7 cM, 2,474.1 cM and 1,731.6 cM in 19 chromosomes for M730, M692 and ZT, respectively. Plants were inoculated with *Sclerotinia sclerotiorum* mycelia on stems at the reproductive stage to evaluate their resistivity. Four aliphatic glucosinolates and one indolic glucosinolate were detected in the seeds using high-performance liquid chromatography. 4-hydroxy-3-indolylmethyl predominated over aliphatic glucosinolates in canola, but inversely constituted a small portion of total glucosinolate content in semi-winter rapeseed. In rapeseed, 2-hydroxy-3-butenyl predominated in 4C aliphatic glucosinolates, which in turn predominated in total aliphatic glucosinolates, which likewise predominated in total glucosinolate content. QTLs regulating major glucosinolates were located on chromosome A9 for high glucosinolate content populations M730 and ZT, and on chromosome C7 for low glucosinolate content population M692. Major QTLs for Sclerotinia stem rot resistance were located on chromosomes A7 and C6 in M730, on chromosomes A3 and A7 in ZT, while no major QTLs were found in M692. Additive

genetic effect was the major factor explaining phenotypic variations of the two traits. No direct genetic relationship was observed between Sclerotinia stem rot resistance in adult plants and seed glucosinolates in *B. napus*. The findings in the studies could be used to formulate breeding and research strategies in *B. napus* and the major QTLs controlling the two traits and their closely linked SNP markers could be validated over wide germplasm and used in marker assisted selection.

FORWARD

CHAPTER 1. QTLs Controlling Glucosinolate Content in Seeds of *Brassica napus* L. Aus. J. Crop Sci. 10(2):152-160, **CHAPTER 2. Quantitative Trait Locus Analyses of Seed Glucosinolates in *Brassica napus*** and **CHAPTER 4. Genetic Analyses of Sclerotinia Stem Rot Resistance in Canola (*Brassica napus*)**

J. Liu designed and performed experiments, analysed data and wrote the paper; A.H. Hirani developed lab protocols and reviewed the paper, Z. Li collected and analyzed data; C. Wu and G. Li selected parental lines; P.B.E. McVetty, C. Wu and G. Li designed experiments and reviewed the paper.

CHAPTER 3. Quantitative Trait Loci Controlling Sclerotinia Stem Rot Resistance in *Brassica napus* L.

J. Liu designed and performed experiments, analysed data and wrote the paper; X. Liu performed experiments; R.S. Behla and A.H. Hirani developed lab protocols and reviewed the paper, Z. Li collected and analyzed data; C. Wu and G. Li selected parental lines; P.B.E. McVetty, C. Wu and G. Li designed experiments and reviewed the paper.

INTRODUCTION

Canola/Rapeseed (*Brassica napus* L.) and Its Economical Importance

Brassica napus belongs to the *Brassicales*, the *Brassicaceae* (Cruciferae), the Brassiceae, the Brassicinae and the *Brassica.* It is believed to have originated naturally in northern Europe (Rakow 2004) or the Mediterranean region, western or northern Europe (Tsunoda 1980) about 7,500 years ago (Inaba and Nishio 2002; Rana et al. 2004). Yet, this species possesses a cultivation history of only 400 ~ 500 years (Prakash et al. 2011). *Brassica napus* was named by the Swedish botanist Carl Linnaeus in the mid of 18th century. It colineates, or shares the same order of genes, in genomic structures with other species in genera and subtribes of this family which has traditionally facilitated gene transfer and trait improvement in canola/rapeseed, such as the case for the artificial species Raparadish (RRCC) (Lelivelt et al. 1993).

Brassica napus is a model amphidiploid plant species expressing no significant inbreeding depression, strong heterosis from complementary parental combinations and polyploidy advantages over its diploid counterparts. After its seed quality was improved revolutionarily in the 1960-70's to create double low rapeseed, later called canola, quality improved rapeseed has become one of the three most economically important oilseed crops in the world along with soybean (*Glycine max* (L.) Merr.) and palm (*Elaeis* spp.) (USDA 2015).

A healthy oil profile and high oil content in seed, a rich and balanced protein profile in the oil-free meal, low glucosinolate content in the meal, a high quality feedstock for industrial purposes, and cost-effective production are main impetuses for the popularity of canola/rapeseed. As a matter of fact, canola seed is reported to have the most healthy

oil profile among the three major oilseed crops and excellently balanced protein composition (Sosulski 1979). On the other hand, high erucic acid, low glucosinolate content rapeseed is also a profitable industrial feedstock produced in western Canada boosted by the newly released high yielding hybrid HYHEAR 1 (McVetty et al. 2014).

Canada is the second largest canola/rapeseed producer in the world after the European Union (USDA 2015). Despite a short planting history of only 74 years (McInnis 2004) since rapeseed was introduced into this country, canola has become the most profitable crop in Canada. About one fourth of the farm land, or 8.19 Mha, grows canola (*http://www.agcanada.com/daily/statscan-raises-canola-acreage-but-actual-area-likely- lower*). Saskatchewan, Alberta and Manitoba, the three Prairie Provinces in western Canada account for 52.84%, 30.58% and 15.62% of the total acreage and 49.65%, 33.40% and 15.99% of the canola production in Canada, respectively (*http://www.canolacouncil.org/markets-stats/statistics/harvest-acreage/*). The canola industry contributed $19.3 billion to the Canadian economy in 2013 (LMC International 2013). Therefore, any factors that negatively affect canola yield, quality and production will have profound impact on local and national economy as well as human welfare worldwide. The fungal disease Sclerotinia stem rot and seed glucosinolates are two negative factors among these.

Sclerotinia Stem Rot

Sclerotinia stem rot on canola is also referred to as white mold. The stem is the plant organ that will exhibit the aptly named Sclerotinia stem rot symptoms on canola/rapeseed plants. Canola Sclerotinia stem rot is a common disease worldwide (Saharan and Mehta 2008) that has been identified as a devastating disease in rapeseed/canola by Bolton et al.

(2006) and Mei et al. (2011). As a rule of thumb, the percentage of canola yield lost is equal to half of canola Sclerotinia stem rot disease incidence (DI). For example, the canola Sclerotinia stem rot DI in Saskatchewan in 2014 averaged 14% (Canadian Plant Disease Survey, The Canadian Phytopathological Society, 2015) so a canola yield loss of approximately 7% would be expected. According to the report by del Río et al. (2007), 17% canola Sclerotinia stem rot incidence could cause canola yield losses to be equal to the cost of fungicide application. Canola yield losses caused by canola Sclerotinia stem rot were estimated at US$94 M from 1991 to 2002 in the United States (del Río et al. 2007). In UK, Sclerotinia stem rot damaged 16% of the winter oilseed crop in 2013 (Taylor et al. 2015). Canola Sclerotinia stem rot is the most prevalent disease in Saskatchewan and Manitoba (Canadian Plant Disease Survey, The Canadian Phytopathological Society 2015). In addition to yield losses, canola Sclerotinia stem rot also causes low oil content, modifies oil profile (Disi et al. 2014; Wei et al. 2014; Zhao and Meng 2003b) and results in inferior oil and meal quality (McCartney et al. 1999).

The severe damage from *S. sclerotiorum* has triggered great interest in controlling *Sclerotinia* diseases. Many methods have been recommended to control canola Sclerotinia stem rot including cultural controls (Duncan 2003; Gulya et al. 1997; Kurle et al. 2001; Morral and Dueck 1982; Williams and Stelfox 1980), physical control (Gilbert 1991; Lanoiselet et al. 2005; Teo et al. 1989); and biological control (Anas and Reeleder 1988; Fernando et al. 2007; Hu et al. 2005; Huang 1980; Huang and Erickson 2000; Huang and Kozub 1991; Li et al. 2006; Zhang 2004). However, many of these methods are not very effective or impossible to be implemented in western Canada. Fungicides are consistently the most common and effective means to control canola Sclerotinia stem rot

(Bardin and Huang 2001; Huang and Blackshaw 1995; Huang and Sun 1991; Koch et al. 2007). Despite their effectiveness, concerns with fungicide applications including their high cost and environmental impacts are prevalent even with the help of canola Sclerotinia stem rot forecast systems in the U.S. (Bradley et al. 2006b), in Canada (Turkington and Morrall 1993) or other methods being developed (Bom and Boland 2000; Makowskia et al. 2005).

Breeding disease resistant cultivars has been proven to be the most efficient and most cost-effective way to combat against disease epidemics in many crops. 'Zhongyou 821' is an excellent example with canola Sclerotinia stem rot partial resistance. It has been used to develop *B. napus* crops (Li et al. 1999) as the trait donor, an experimental sample, or as a positive control by many researchers (Ding et al. 2013; Fan et al. 2008; Jurke and Fernando 2008; Li et al. 2004; Liu et al. 2005; Sang et al. 2013; Yin et al. 2010; Zhao et al. 2009). However, the lack of complete canola Sclerotinia stem rot resistance sources and poor understanding of host resistance are large barriers to the successful breeding of rapeseed/canola cultivars which have strong field resistance to canola Sclerotinia stem rot worldwide.

Glucosinolates

Glucosinolates (*β*-thioglucoside-N-hydroxysulfates) are endogenous secondary metabolites in *Brassicales* plants. Sinigrin (2-propenyl) and sinalbin (4-hydroxybenzyl) were isolated as early as 1830s (Fahey et. al. 2001). Glucosinolates were found in mustard seeds in 1840 by Bussy (Bones and Rossiter 1996). Their correct chemical structures were first proposed by Ettlinger and Lundeen (1956). There are 135 glucosinolates identified in nature (Agerbirk and Olsen 2012). Three components

constitute a glucosinolate molecule: a β-thioglucose, a sulfonated oxime and an aglycone side chain (R) which specifies different glucosinolates.

Glucosinolates are divided into three general categories based on their origin and side chain structure. Aliphatic glucosinolates are derived from methionine (Met), alanine (Ala), leucine (Leu), isoleucine (Ileu), and valine (Val)) (Halkier and Gershenzon 2006); aromatic glucosinolates from phenylalanine (Phe) and tyrosine (Tyr); and indolic glucosinolates from tryptophan (Trp) (Zukalova and Vasak 2002) (Figure 1). Glucosinolates are stable and water soluble compounds existing in vacuoles (Weese et al. 2015) in parenchymatous tissues (Zukalova and Vasak 2002). Glucosinolate degradation enzymes such as myrosinases (β-thioglucosidase), on the other hand, exist in their own compartments called idioblasts (Zukalova and Vasak 2002) or myrosin cells (Drozdowska et al. 1992) separated from glucosinolates in plant cells. When plants are under biotic or abiotic stresses, plant defensive mechanisms are evoked. The two components, the anion glucosinolate substrates and myrosinases and their cofactors, come into contact with each other and trigger glucosinolates to degrade in the presence of water. The intermediate products of glucosinolates are not stable and undergo further degradation to produce sulphate and bio-active isothiocyanates (mustard oil), thiocyanates, nitriles and epithionitriles by cleaving thioglucoside linkages (Bones and Rossiter 1996) (Figure 1). Thiocyanates inhibit iodine uptake in the thyroid and have detrimental effects on the liver in animals. Campbell and Schöne (1998) reported that reductions in animal performance and impairment of normal thyroid function in animals, fetuses, embryos and liver haemorrhage mortalities were observed in hens overdosed with glucosinolates. In addition, the pungent flavor from degraded glucosinolates affects

livestock palatability. However, isothiocyanates are claimed to have anticarcinogenic effects in humans (Bones and Rossiter 1996).

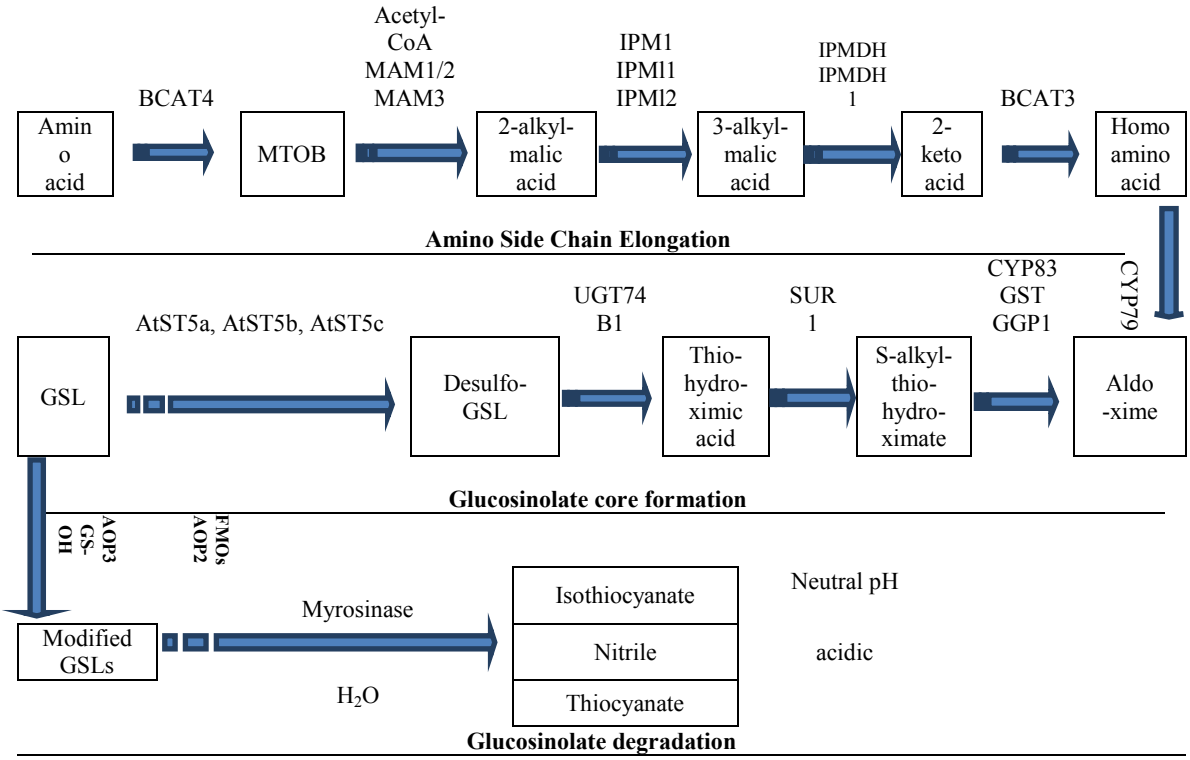


Figure 1. Glucosinolate (GSL) metabolic pathway modified from Fahey et al. (2001), Feng et al. (2012) and Hirani et al. (2012)

Since glucosinolates are involved in plant defense against biotic and abiotic stresses (Sotelo et al. 2014), continued efforts have been made to further reduce total glucosinolate content and optimize glucosinolate profile in rapeseed/canola seed worldwide following the development of low glucosinolate content canola cultivars. However, due to the complexity of the trait, intrinsic genetic mechanisms regulating glucosinolates are still poorly understood and successes are hardly achieved.

Studies on Sclerotinia Stem Rot Resistance and Glucosinolates in *B. napus*

Canola Sclerotinia stem rot resistance and seed glucosinolate content are quantitative traits and controlled by polygenes in plants. These genes are non-allelic and work in concert with different effects on the trait. Traditional breeding has encountered great obstacles preventing the development of promising commercial cultivars with strong canola Sclerotinia stem rot resistance and further reduced glucosinolate content in canola. Molecular breeding though has the potential to decipher the intrinsic genetic mechanisms regulating the two traits. Since the end of the last century, researchers have attempted to explain the two traits by using available technologies on plant materials with different genetic backgrounds under various environments. For example, *B. napus*, its two proposed diploid progenitors *B. rapa* and *B. oleracea,* the model plant *Arabidopsis thaliana* and their close relatives are major germplasm used in these studies. Hybridization-based RFLP, and PCR--based simple sequence repeats (SSR), random amplified polymorphic DNA (RAPD) (,amplified fragment length polymorphism (AFLP), SRAP sequence related amplified polymorphism (SRAP) and SNP single nucleotide polymorphism (SNP) with their developed analytical techniques are major technologies used in genetic linkage mapping, quantitative trait locus (QTL) identification, molecular marker development and gene introgression. Despite decades of studies on canola Sclerotinia stem rot resistance (Li et al. 2015; Mullins et al. 1995; Zhao and Meng 2003b) or on glucosinolates (Howell et al. 2003; Lu et al. 2014; Magrath et al. 1993; Uzunova et al. 1995), many fundamental questions still remain. Such questions concern the major regulating QTLs, where the QTLs are located in their genomes, and what is the nature of the relationship between canola Sclerotinia stem rot resistance and glucosinolates.

Hypotheses

The hypotheses for this experiment include: 1) polymorphic SNP markers are abundant in *B. napus*; 2) both plant Sclerotinia stem rot resistance and seed glucosinolates are controlled by QTLs; and 3) potential QTLs could be employed in marker assisted selection and marker assisted backcross to breed Sclerotinia stem rot resistance and low seed glucosinolate *B. napus*.

LITERATURE REVIEW

Brassica napus and Its AC Genome

Brassica napus can be divided into annuals such as spring oilseed rape and biennials including both semi-winter oilseed rape and winter oilseed rape, respectively, according to their life cycle. It has 3,709 divergent relatives in *Brassicaceae* (Warwick et al. 2006) and 38 closely related ones in *Brassica* (Warwick et al. 2009). *Brassicaceae* is the fifth largest monophyletic family (Gautam et al. 2014) and one of the ten most economically important families in plants (Rich 1991). The family includes scientifically and economically important diploid species such as *B. nigra* (L.) Koch (BB, 2n = 2x = 16), *B. oleracea* L. (CC, 2n = 2x = 18) and *B. rapa* L. (AA, 2n = 2x = 20), the amphidiploids (a species containing the summed chromosome numbers from two diploid parents) *B. carinata* Braun (BBCC, 2n = 4x = 34), *B. juncea* (L.) Czern (AABB, 2n = 2x = 36) and *B. napus* (AACC, 2n = 2x = 38) in *Brassica* (Nagaharu 1935), as well as diploids *Raphanus sativus* L., *Sinapis arvensis* L. and *Arabidopsis thaliana* (L.) Heynh. *Brassica napus* is a polyploid which is common for seed plant species on earth (Ming and Wai 2015). Allopolyploids including *B. napus* and *Triticum aestivum* have advantages in nature which explains the popularity and importance of these crops. *Brassica napus* is theorized to have originated from interspecific hybridization between *B. rapa* and *B. oleracea* (Morinaga 1934; Nagaharu 1935). Multiple hybridization cases transpired to form this species, with *B. rapa* being the most likely cytoplasm donor (Allender and King 2010). Ming and Wai (2015) attributed the formation of *B. napus* to unreduced A and C gametes, whereas Chalhoub et al. (2014) believed in chromosome doubling after the hybridization of reduced A and C gametes. The lack of wild counterparts, narrow germplasm background, successes in re-syntheses of *B. napus* (intact A and C subgenomes, Parkin et

al. 1995) from *B. rapa* and *B. oleracea*, and other cytogenetic, molecular analyses of organelles, genomic and fluorescence in situ hybridization (FISH) studies support the short history and allotetraploidy nature of this species (Snowdon et al. 2002; Snowdon et al. 2003; Snowdon 2007). Cai et al. (2014) reported that *B. napus* contained 70.1% *B. rapa* and *B. oleracea* genetic composition. This implies that less than one third of the *B. napus* genome is distinct from those of its proposed progenitors due to natural and artificial selection. Ming and Wai (2015) reported that a DNA region of 96,436 bp in the A subgenome shared colinearity with 104,516 bp in the C subgenome in *B. napus* giving further evidence supporting the homeologous nature of the A and C genomes.

Flow cytometry and Feulgen microdensity measurements determined that *B. napus* consists of approximately 1,200 Mbp (1,129 – 1,235 Mbp) (*http://www.brassica.info/info/reference/genome-sizes.php*). Its progenitors *B. rapa* and *B. oleracea* consist of 500 and 600 Mbp, respectively (Arumuganathan and Earle 1991). Michael and VanBuren (2015) reported the genome sizes and the number of genes for the three crops *B. rapa, B. oleracea* and *B. napus* to be 485 Mbp and 41,174, 630 Mbp and 45,758, and 1,130 Mbp and 101,040, respectively. This study indicates that *B. oleracea* has a much greater genome size and more genes than *B. rapa*. The genome size of *B. napus* is almost equal to the sum of the genomes of *B. rapa* and *B. oleracea* but has much more genes.

A common ancestor with 24 conserved chromosomal blocks (Schranz et al. 2006) or one (n = 6) with at least 21 conserved blocks (Parkin et al. 2005) was proposed for the origin of the species in *Brassicaceae*. The model plant Arabidopsis diverged from *Brassica* c. 20 Mya (Yang et al. 1999). *Brassica* subgenomes (AA and CC) diverged 14.3 Mya (Cheung et al. 2009). Colinearity between *A. thaliana* and *B. napus* was reported by

Cavell et al. (1998). Compared to the genome in Arabidopsis, the genomes in *Brassica* species have undergone extensive duplications and triplications (Babula et al. 2003; Kowalski et al. 1994; Lagercrantz and Lydiate 1996; Lan et al. 2000; Lukens et al. 2003; Lysak et al. 2005; O'Neill and Bancroft 2000; Park et al. 2005; Parkin et al. 2003; Rana et al. 2004; Schmidt et al. 2001). Eleven segments in the Arabidopsis genome could be lined up with six segments in *B. napus* genome which resulted from gene deletions and chromosome rearrangements (Cheung et al. 2009). The number of genes proposed in *B. napus* compared to those in Arabidopsis is four-fold (Chalhoub et al. 2014). The colinearity of the genomes of Arabidopsis and *Brassica* species, especially *B. napus,* makes it possible to use the available information from Arabidopsis to study genomic structure, gene annotation and function, all necessary technologies in *B. napus* breeding. *Brassica napus* is low in genetic diversity (Hasan et al. 2006; Qian et al. 2006) so it can receive broad genetic input from its primary, secondary and tertiary gene pools for its trait improvement as done by Rahman et al. (2015). Modern molecular breeding based on the knowledge of detailed genetic composition of a species can meet the needs for gene transfer and trait introgression among these gene pools.

Sclerotinia sclerotiorum **and Sclerotinia Stem Rot**

Sclerotinia stem rot is caused by *S. sclerotiorum* (Lib.) de Bary. This fungus was first named by Madame Libert (Libert 1837). The current name was given by Purdy (1979) after it was renamed several times (Bardin and Huang 2001; de Bary 1886; Fuckel 1870; Wakefield 1924). *Sclerotinia sclerotiorum* belongs to the *Fungi*, the *Ascomycota*, the *Discomycetes*, the *Helotiales*, the *Sclerotiniaceae*, the *Sclerotinia* (Bolton et al. 2006). It has been reported to occur in all other continents except for Antarctica (Aeron et al. 2011;

Barari et al. 2011; Bardin and Huang 2001; Hu et al. 2011; Kirkegaard et al. 2006; Koch et al. 2007; Lamey 1995; Twengstrijm et al. 1998; Young and Werner 2012).

S. sclerotiorum is omnivorous and necrotrophic and infects more than 408 plant species in 278 genera of 75 families with over 100 of them in Canada reported by Boland and Hall (1994), or over 500 plant species newly reported by Sharma et al. (2015). Dicotyledonous plants are its major hosts including rapeseed, sunflower (*Helianthus annuus*), soybean and many other economically important vegetables such as lettuce (*Lactuca sativa*), carrot (*Daucus carota*), celery (*Apium graveolens*), potato (*Solanum tuberosum*), etc. The pathogen is also able to infect monocotyledonous plants such as onion (*Allium cepa* L.) and tulip (*Tulipa* spp.) (Boland and Hall 1994). Flax (*Linum usitatissimum* L.), originally resistant to it, was reported to be susceptible in Manitoba and Saskatchewan (Zhang 2004). *Sclerotinia sclerotiorum* is the most pathogenic fungus in this genus (Saharan and Mehta 2008).

S. sclerotiorum (n = 8, 2 nuclei/ascospore) occurs monocyclically. Sclerotia, the hyphal aggregates, are the form that the fungus adopts, or mycelia within plants reported by Yang et al. (2007), to survive overwinter. The longevity of sclerotia is reported to range from 4 to 10 years (Adams and Ayers 1979; Coley-Smith 1979; Coley-Smith and Cooke 1971; Duncan 2003; Kohli et al. 1995; Merriman 1976; Willetts and Wong 1980; Young and Werner 2012) depending on their local environmental conditions, interactions with plants and other micro-organisms and human activities. pH plays a significant role in sclerotium development (Bolton et al. 2006) where its development is inhibited in neutral or alkaline and encouraged in acidic environments (Rollins and Dickman 2001). Seeds

can transport mycelia (Bardin and Huang 2001) while wind, insects and wind-blown pollen can carry ascospores long distances to cause an epidemic.

Kohli et al. (1995) reported that *S. sclerotiorum* was homothallic and innately lacked recombination in its sexual process. Litholdo et al. (2011), however, reported that sexual reproduction dominated in tropical and subtropical regions whereas clonal reproduction dominated in temperate regions. Kohli and Kohn (1998) reported that new genotypes resulted occasionally from recombination and/or mutation. It explains the reason that only four populations of *S. sclerotiorum* have been found in North America (Phillips et al. 2002). In Canada, the population is heterogeneous (Kohn 1995; Kohn et al. 1991) and only a small number of the genotypes prevail (Bardin and Huang 2001). It has been reported that there was no difference in virulence between *S. sclerotiorum* samples obtained from soybean and rapeseed fields (Zhao et al. 2004) or from different canola fields (Sexton and Howlett 2004).

Early canola Sclerotinia stem rot infection on plants is caused by sclerotia myceliogenically (Li et al. 1999). However, infection caused by ascospores is the main explanation for the extended spread of the disease. Humidity is the major environmental factor contributing to the occurrence of this disease (Huang et al. 1998). Canola Sclerotinia stem rot optimally develops at 20 - 25 ºC and relative humiditygreater than 80% (Heran et al. 1999). When temperature is lower than 7 ºC or higher than 26 ºC, there is no mycelium development or canola Sclerotinia stem rot observed (Koch et al. 2007).

Jamaux et al. (1995) used scanning electron microscopy to observe *S. sclerotiorum* ascospore developmental stages on rapeseed petals, resulting in a general infection pathway: germination following adherence to the surface, penetration into the epidermal

layer by short germ tubes, then collapse of host epidermal cells. Mechanical pressure is the major force for initial penetration into plants for germinating mycelia (Zhang 2004). Enzymes involved in the degradation of plant cell walls and tissues include pectin methylesterase, polygalacturonase, hemicellulolytic and cellulolytic enzymes, pathotoxins and phototoxins (Riou et al. 1991). Oxalic acid (OA) is a major pathogenicity factor for canola Sclerotinia stem rot (Guo and Stotz 2010; Liu et al. 2015; Marciano et al. 1983; Muellenborn et al. 2011). *Sclerotinia sclerotiorum* produces OA which both poisons plants and facilitates enzyme capability to degrade plant tissues (Cessna et al. 2000). OA is required for *Sclerotinia* to colonize host plants and to suppress reactive oxygen intermediate (ROI) production (Walz et al. 2008). OA chelates divalent cations in cell walls and favors conversion of pectin to disintegrate plant tissues (Bateman 1972). Gene expression studies by Zhao et al. (2007) indicated that auxin, jasmonic acid and ethylene pathways were activated after *S. sclerotiorum* inoculation of *B. napus* plants. Jasmonic acid and ethylene related pathways are involved in plant defense against necrotrophic pathogens (Shokouhifar et al. 2011). Several groups of transporter genes for transporting nutrients including nuclear, amino acids, proteins, carbohydrates, ATP binding cassettes and magnesium are involved in the strengthening of plant defense systems (Zhao et al. 2007). The qualitative foundation of *B. napus* resistance to canola Sclerotinia stem rot was the lack of infectious appresoria or cushions forming after ascospore inoculation, hyphae growth retardation, hyphal cell wall disintegration and protoplast extrusion on the plants (Garg et al. 2010), or deposition of lignin in cortical cell walls to prevent pathogens from entering inner tissues (Uloth et al. 2015).

Genetic Map

A genetic map is a linear linkage of alleles on a chromosome and is required for accurate QTL identification. The closer the alleles to each other, the less likely these alleles experience recombination, and therefore greater chance to be inherited together into their progenies. Genetic maps show relative positions of genes or molecular markers on chromosomes. As such, they are critical in molecular breeding in crops and have been widely used in marker assisted selection (MAS), marker assisted backcrossing (MABC) and fingerprinting. The unit on a genetic map (m.u.) is termed as centiMorgan (cM) which is defined as the percent of neighboring alleles that will undergo recombination between them in 100 meioses. Genetic distances greater than 30 cM on chromosomes are considered gaps (Wu et al. 2015). The greater the gaps are, the more genes/markers are missing within these DNA stretches. It is important to minimize the gaps to increase the accuracy and precision for QTL identification and localization in order to efficiently and effectively use MAS/MABC or other tools in molecular breeding.

RFLP as a first-generation molecular marker system (Botstein et al. 1980) was initially used for genetic linkage mapping in humans. For *B. napus*, Landry et al. (1991) used 120 RFLP markers to construct a genetic map containing 19 linkage groups (LGs) which covered 1,413 cM in a F_2 population. In another study, Ferreira et al. (1994) assembled a linkage map covering 1,016 cM in 22 LGs and 6 pairs of linked markers using 132 RFLP markers in a DH population. Compared to the previous reported studies, Uzunova et al. (1995) used 204 RFLPs complimented with a few other markers to construct a linkage map covering 1,441 cM with 19 LGs in a DH population. They observed dominance, duplications and segregation distortions in their molecular markers. Although

codominance and high reproducibility based on prior sequence information were advantages of RFLP markers, its high cost, intensive labor requirement and low throughput made RFLP less attractive after PCR-based molecular marker systems made their debut in the 1990's. Second-generation molecular marker systems include RAPD (Welsh and McClelland 1990), SRAP (Li and Quiros 2001), AFLP (Eijk et al. 2003) and SSR. Sun et al. (2007) developed an ultra-dense linkage map in *B. napus* including both the A and C subgenomes with 13,551 SRAP markers in a 58-line DH population derived from the spring canola inbred line Westar and the semi-winter rapeseed var. Zhongyou 821. Their map covered 1,604.8 cM of the whole genome with an averaged marker density of 8.5 SRAPs per cM. It indicates that the SRAP is a powerful tool to dissect plant genomic structures and anchors genes or QTLs accurately. However, these SRAP markers were not evenly distributed and ranged from 5.1 markers per bin on chromosome C6 to 19.0 markers per bin on chromosome A8. Yin et al. (2010) used a combination of SSR, SRAP, RAPD, RFLP and expressed sequence tag (EST) markers to construct a genetic linkage map covering a distance of 1,746.5 cM in 20 LGs for a 72-line *B. napus* DH population. Their map had a marker density of 6.9 markers per cM. It indicates that various marker systems can be used complementally to reveal detailed genetic mechanisms for traits of interest. Wu et al. (2013) used 272 SSR markers in a *B. napus* DH population to construct a 1,579 cM genetic map containing 19 LGs. They observed 5.2 markers per cM on their genetic map and claimed even marker distribution on the genome. SSR markers are not only abundant and co-dominant, but also species-specific (Miah et al. 2013) hence can be used to anchor conserved genetic background. Cai et al. (2014) constructed a 2,477.4 cM genetic map for a 190-line *B. napus* DH population

using *Brassica* 6K Infinium® SNP and SSR markers. They used 1,667 SNPs and 448 SSRs and had a marker density of 1.3 markers per cM on the genetic map. The genetic maps developed from these studies varied in length due to differences in marker system used, number of markers and calculation environments. It can be seen from Cai's study that the SNP markers were densely populated on the genome. Genetic mapping has also been done for the progenitor of *B. napus*. For example, Lou et al. (2008) used over 300 AFLP and SSR markers in each of two DH populations and constructed two genetic maps covering about 700 cM in 10 LGs of the 10 chromosomes for each population with an integrated map covering 1,068 cM in *B. rapa*.

The SNP-marker system is currently the preferred system to be used for genetic mapping due to the abundance, wide distribution on genomes, the availability of automatic detection systems, high throughput and low cost of SNP markers. It has been reported that there is one SNP marker in every 600 bp (Edwards et al. 2007; Fourmann et al. 2002) or one SNP in 1,200/2,100 bp (Trick et al. 2009) in *B. napus*. Major technologies for SNP detection have been developed rapidly from simple and low throughput TagMan (Thermo Fisher Scientific, Waltham, USA), Goldengate (Illumina, San Diego, USA) and GeneChip (Affymetrix, Santa Clara, USA) to high throughput Infinium® 6K and 60K (Illumina, San Diego, USA). With the fast development of next generation sequencing (NGS) technologies, SNP markers have become more effective and efficient in genotyping, genetic map assembly, molecular marker development and gene annotation. Raman et al. (2014) used 6K *Brassica* Infinium® SNP chips to genotype a 175-line *B. napus* DH population and constructed a linkage map covering 2,514.8 cM with 613 SNP and 228 other PCR-based markers. One chromosome, C2, was not specified in their

analysis. The newly developed Illumina Infinium® 60K *Brassica* SNP technology has been used by Brown et al. (2014) to assemble a genetic map with 547 SNPs spanning 948.1 cM across 9 chromosomes of the C genome in a 150-$F_{2:3}$ families of a *B. oleracea* population and by Li et al. (2014) to assemble an association map with 24,256 SNP markers for a panel of 472 *B. napus* inbred lines. Li et al. (2014) found QTL peaks on chromosomes A9, C2, C7 and C9 associating with total glucosinolate content in seeds. These studies display the usefulness of genetic maps assembled from dense SNPs despite observations of uneven SNP marker distribution. Physical mapping of genetic components is the ultimate goal for genomic studies. The 849.7 Mbp physical map of the winter oilseed cultivar "Darmor-*bzh*" was published using NGS technology by Chalhoub et al. (2014). However, this map accounted for only 79% of the whole *B. napus* genome. There is still a long way to go to fill up the gaps.

Sclerotinia Stem Rot Resistance Evaluation and Seed Glucosinolates

Artificial Inoculation Methods to Assess Plant Resistance

Accurate phenotyping is fundamental for QTL studies and breeding practices. Artificial inoculation has been widely used to assess *S. sclerotiorum* resistance in crops in both indoor and outdoor nurseries by many researchers. Generally, the methods can be grouped into *S. sclerotiorum* mycelium inoculation, ascospore spray or OA assay.

S. sclerotiorum mycelium inoculation is the most common method adopted with various procedures to infect different organs of many crops. For example, mycelium inoculation was used on *B. napus* in procedures including mycelium PDA/PGA plug (MPP) (Barbetti et al. 2014; Li et al. 2009; Sharma et al. 2009; Wu et al. 2013; Yin et al. 2010; Zhao et al. 2009), mycelium infested toothpick (MTK) (Fan et al. 2008; Yin et al. 2010; Zhao et al.

2003b) and mycelium infested flower petal (MFP) (Yin et al. 2010) on living plant stems, MPP on detached stem (Wei et al. 2014), detached leaves (Bradley et al. 2006a; Dong et al. 2008; Wu et al. 2009; Wu et al. 2013; Zhao et al. 2003b), living petiole (Bradley et al. 2006a; Zhao et al. 2006; Zhao et al. 2007), MSS (mycelium suspension spray) (Li et al. 2007; Sharma et al. 2009) and SS (spread sclerotia) in nurseries (Dong et al. 2008; Li et al. 2007). In *B. oleracea*, Mei et al. (2013) and Disi et al. (2014) used MPP on both detached leaves and detached stems for canola Sclerotinia stem rot resistance assessment. In soybean, mycelium infested barley kernel (MBK) on living stems (Auclair et al. 2004), mycelium spray (MS) on living leaves and mycelium drops (MD) on living stems (Chen and Wang 2005) has been used.

Ascospore spray was reported to induce *S. sclerotiorum* disease on sunflower (Becelaere and Miller 2004), carrot (Finlayson et al. 1989) and lettuce (Young et al. 2004). OA assay was another method used to screen *B. napus* plants resistance to canola Sclerotinia stem rot (Bradley et al. 2006a; Liu et al. 1998; Liu et al. 2005) and tobacco plants resistance to *S. sclerotiorum* (Cessna et al. 2000).

Although there are many methods which can be used in screening for plant resistance against *S. sclerotiorum*, stem inoculated with MPP is the preferred method (Barbetti et al. 2014). Mycelium is the major form of pathogenic inoculum with stems being the main plant organs widely used by researchers and the western Canada canola/rapeseed recommending committee (WCC/RRC) to study the quantitatively featured *S. sclerotiorum* resistance.

Quantitative Trait Loci Controlling *Sclerotinia* Diseases

Brassica napus. QTLs are chromosome regions containing genes regulating quantitative traits which show continuous distribution of their phenotypes. QTLs are featured by multiple genes acting individually or epistatically on the same or different traits. For instance, three QTLs controlling leaf resistance in seedlings and another 3 QTLs controlling stem resistance in adult plants to canola Sclerotinia stem rot were identified in a $F_{2:3}$ population by Zhao and Meng (2003a) using a combination of RFLP, AFLP, SSR and RAPD markers. Five of the six QTLs were located on proposed chromosomes A3 and C7 for leaf resistance, and on A7, A10 and C5 for stem resistance but their positions could not be accurately located on the genetic map due to few markers on each chromosome and unassigned chromosomes. Zhao et al. (2006) identified 7 QTLs controlling stem lesion length (SLL) and 4 QTLs controlling days to wilt (DW) on chromosomes A2, A3, A5, C2, C4, C6, and C9 in one DH population and only two QTLs controlling these two measurements in another DH population. Furthermore, most of the QTLs could only be observed from either one measurement or not repeatedly observed in multiple replications. Zhao et al. (2007) observed hundreds of genes that might be involved in resistance to canola Sclerotinia stem rot by studying gene expression patterns in partially resistant and susceptible accessions. Yin et al. (2010) screened a DH population at different days post inoculation (DPI) with three inoculation methods and observed 10, 1 and 10 QTLs on chromosomes A1, A3, A4, A6, A7, A10, C1, C2, C7 and LG11 respectively for canola Sclerotinia stem rot resistance at a logarithm of odds (LOD) value of 2.5. LOD scores indicate the likelihood of the QTL-trait association. The greater LOD values are, the more likely the genotype is associated with the phenotype other than

by chance. In another report, ten QTLs on chromosomes A1, A2, A3, A6, A8, A9, C6, C7 and C8 responsible for stem resistance on adult plants and three QTLs on A3, A9 and C5 for leaf resistance in seedling plants in a DH population were identified by Wu et al. (2013). Li et al. (2015) integrated 35 QTLs reported by Mei et al. (2013), Wei et al. (2014), Wu et al. (2013), Zhao and Meng (2003b) and Zhao et al. (2006) respectively onto a physical map and found conserved QTLs within 5 Mb on chromosome A9 and within 6.5 Mb on chromosome C6 based on the physical map assembled by Chalhoub et al. (2014). These QTLs distributed over all other chromosomes on the genome except for chromosomes A4, A7 and A10. The C subgenome contained more QTLs than the A subgenome with chromosomes C9 and C6 being the most laden. Li et al. (2015) also reported 181 and 245 NBS-LRR (nucleotide binding site – leucine rich repeat) candidate genes respectively in the A and C subgenomes related to canola Sclerotinia stem rot resistance.

Other Crops. Mei et al. (2013) identified 18 QTLs controlling resistance to Sclerotinia stem rot, 12 on chromosomes C1, C3, C6 and C9 for leaf and 6 on chromosomes C4, C7 and C9 for stem resistance in a *B. oleracea* F_2 population using a combination of SSR, SRAP and AFLP markers. Micic et al. (2005), Rönicke et al. (2005) and Yue et al. (2008) used SSR and other markers to identify QTLs controlling *S. sclerotiorum* in segregating populations of sunflower. Li et al. (2010) also used SSR for QTL identification for white mold resistance in soybean. These studies show the complicated quantitative nature of the trait, and also indicate that plant resistance to this pathogen exists extensively in these crops.

Glucosinolates in *Brassica* Seeds

Glucosinolate accumulation and profile in canola and rapeseed seeds are major interests in research and in the industry. Glucosinolates are well characterized in plants (Bednarek et al. 2009; Daxenbichler et al. 1991; Fahey et al. 2001; Wittstock and Halkier 2000) when genotype is the major factor influencing glucosinolate content and profile compared to environmental conditions (Sotelo et al. 2014). *Brassica napus* seeds contain greater amount of glucosinolates than vegetative organs (EFSA 2008; Velasco et al. 2008). Rahman et al. (2014) reported that more than 100 μmol/g seed of total glucosinolates were contained in rapeseed seeds. It indicates that the *B. napus* seed is the most important sink among plant organs to store glucosinolates at late growth stage. Although freshly synthesized glucosinolates from nearby sources such as the stem and the pod explain the increase of seed glucosinolate content, the translocation of glucosinolates from aging leaves and other organs also contributes to the increase of seed glucosinolates. Aliphatic glucosinolates, especially progoitrin were reported to be predominant in *B. napus* seeds (Velasco et al. 2008). Sang and Salisbury (1988) used high-performance liquid chromotography (HPLC) to analyze *B. napus* and *B. rapa* seeds and observed that gluconapin, progoitrin, glucobrassicanapin, napoleiferin and glucobrassicin were predominant in rapeseed seeds while indole glucosinolate content was relatively high in the seed of low glucosinolate content *B. napus* lines.

Glucosinolates were also reported to have similar profile in *B. oleracea* seeds. Sotelo et al. (2014) observed that 4-methylsulfinylbutyl (GRA), gluconapin, and progoitrin were major glucosinolates which accounted for over 93% of total glucosinolate content. There were different glucosinolates, about 30 in *B. napus*, 16 in *B. rapa*, 12 in *B. oleracea* and

34 in Arabidopsis that have been identified (Lou et al. 2008). Among the 16 glucosinolates in *B. rapa*, 4C aliphatic glucosinolate gluconapin and 5C aliphatic glucosinolate glucobrassicanapin predominated.

The Defensive Effects of Glucosinolates. Glucosinolates have long been recognized as anti-herbivore substances in *Brassicales* plants (Lou et al. 2008). Bones and Rossiter (1996) reported that glucosinolates were involved in plant defense against pests including animals, insects, other plants and fungi as well as playing roles in the regulation of plant growth. It is well documented that there are negative effects on livestock when fed with rapeseed meal containing a high concentration of glucosinolates. Thyroid, liver and kidney damage and low palatability are commonly reported problems in livestock caused by glucosinolates intake (Walker and Booth 2001). Glucosinolates were associated with bacterial and fungal disease resistance (Bednarek et al. 2009; Brader et al. 2006) and had direct influence on fungi (Bressan et al. 2009) in Arabidopsis. The hydrolytic products from propenyl glucosinolates were most toxic to fungi (Mithen et al. 1986). High leaf alkenyl glucosinolate content related to resistance to the fungus *Leptosphaeria maculans* has also been reported (Mithen et al. 1987).

However in one study, it was reported that no correlation was found between alkenyl glucosinolates and disease resistance in their synthesized *B. napus* plants (Mithen and Magrath 1992).

Quantitative Trait Loci Regulating Glucosinolates. In *B. napus,* glucosinolate content is regulated by QTLs (Rahman et al. 2014). Uzunova et al. (1995) and Toroser et al. (1995) individually reported 4 and 5 QTLs for total seed glucosinolate content in 4 and 5 LGs for two DH populations using RFLP markers. Two of the QTLs aforementioned

were confirmed to be major ones by de Quiroz and Mithen (1996). One major QTL controlling total GSL content on chromosome C9 was reported by Schatzki et al. (2014) using 149 AFLP and 80 SSR markers. Major QTLs were defined as the QTLs explaining greater than 10% of phenotypic variation by Collard et al. (2005). Rahman et al. (2001) determined that four genes were involved in the regulation of total glucosinolate content and that these four genes must be in their homozygous recessive states to obtain low glucosinolate *B. napus* plants. Zhao and Meng (2003a) observed three QTLs for total glucosinolate content and 3 to 15 QTLs for different glucosinolate content in seeds. Multiple QTLs regulating total seed glucosinolate content were identified by Howell et al. (2003) and Lu et al. (2014). Feng et al. (2012) used 786 markers including SSR, SNP, sequence-tagged site (STS), single-strand conformation polymorphism (SSCP), RFLP, cleaved amplified polymorphic sequence (CAP), AFLP and MS-AFLP and observed 436 QTLs on 18 chromosomes controlling total, aliphatic, indolyl, and aromatic glucosinolates in seeds and leaves for a bi-parental DH population. SNP clusters controlling total glucosinolate content were reported by Li et al. (2014) on chromosomes A9, C2, C7 and C9 using the Infinium® *Brassica* 60K SNPs.

In *B. rapa,* Lou et al. (2008) used AFLP and SSR markers and reported 16 QTLs regulating aliphatic glucosinolate content, three QTLs regulating indolic glucosinolate content and three QTLs regulating aromatic glucosinolate content in leaves. These QTLs were scattered over 10 LGs in two DH populations, with major QTLs located on chromosomes A3 and A10. Hirani et al. (2012) identified a major QTL on chromosome A3 controlling 5C aliphatic glucosinolate content for a *B. rapa* recombinant inbred line (RIL) population. Rahman et al. (2014) reported three QTLs on chromosomes A2, A7

and A9 for F_1, F_2, backcrosses and RIL populations and specified the QTL on A9 as the major one.

In *B. oleracea*, Sotelo et al. (2014) used SSR and RFLP markers and observed 82 major QTLs controlling seed, leaf or bud glucosinolate content in a DH population which were located on 8 of the 9 chromosomes except for C1.

In the amphidiploid *B. juncea,* Cheung et al. (1998) identified 5 QTLs controlling seed 2-propenyl and 3-butenyl glucosinolates in 18 LGs for a DH population using RFLP markers. Mahmood et al. (2003) used RFLP markers and identified two QTLs regulating 3-butenyl, three QTLs regulating 2-propenyl and five QTLs regulating total aliphatic glucosinolates in a DH population.

In *A. thaliana,* Kliebenstein et al. (2001) identified QTLs controlling total aliphatic, total indolic and benzylic glucosinolates, and epistatic interactions among the QTLs.

All the QTLs identified in *B. napus* and their close relatives are useful in comparative studies of the phenotypic traits to infer their intrinsic genetic mechanisms in future forward breeding.

.

CHAPTER 1

QTLs Controlling Glucosinolate Content in Seeds of *Brassica napus* L.

Jun Liu[1], Arvind H. Hirani[2], Zhe Li[1], Chunren Wu[1], Peter B.E. McVetty[2] and Genyi Li[2*]

[1] Monsanto Canada Inc., 900 - One Research Road, Winnipeg, Manitoba R3T6E3 Canada
[2] Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2 Canada

* Corresponding email: genyi.li@umanitoba.ca

Abstract

Glucosinolates are a group of endogenous secondary metabolites commonly found in *Brassica* plants. Oilseed rape (*Brassica napus* L.) contains toxic glucosinolates in its otherwise potentially high quality meal. Molecular breeding has many advantages over traditional breeding and may provide the solution to further decrease glucosinolate content and/or optimize glucosinolate profiles in the meal. In the present study, the *Brassica* 60K SNP (single nucleotide polymorphism) Infinium® microarrays supplemented with sequence related amplified polymorphism (SRAP) markers were used to map the whole *B. napus* genome of a DH population derived from an F_1. Eight thousand eight hundred and thirty-nine SNP and 35 SRAP markers were organized into 1,220 bins covering 2,597.7 centiMorgans (cM) on 19 chromosomes from 88 DH lines. The average bin density was 0.47 bin/cM. Nine quantitative trait loci (QTLs) controlling total and major glucosinolate components were localized on chromosomes A1, A8, A9, C2, C3 and C9 with the most abundant and significant QTLs residing on A9. The total phenotypic variances explained by the QTLs ranged from 7.84% for progoitrin to 40.10% for 4C aliphatic glucosinolate content. It was worthwhile to use the co-segregating flanking markers of these QTLs for marker assisted selection in practical canola/rapeseed breeding.

Keywords: glucosinolate, quantitative trait loci (QTLs), *Brassica napus,* molecular marker, SRAP, SNP.

Introduction

Glucosinolates (β-thioglucoside-N-hydroxysulfates) are a group of endogenous allelochemicals, a subset of secondary metabolites mainly found in plants of order *Brassicales*. There are over 132 different glucosinolates found in nature (Baskar et al. 2012). Their enzyme-mediated hydrolytic products function as defense substances against natural enemies (Fahey et al. 2001; Uzunova et al. 1995) and abiotic stresses. Some glucosinolates may have detrimental effects on livestock palatability and health, while others are beneficial and may be used as anti-carcinogens (Fahey et al. 2001) or condiments (Lou et al. 2008), which has triggered tremendous interest in glucosinolate research in Brassica crops. Great success was achieved by converting rapeseed into low erucic acid and low glucosinolate canola cultivars in the 1960-70s, which paved a foundation for quality improved rapeseed to become one of the most three important oilseed crops in the world (*http://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf*). However, further decreasing glucosinolate content and modifying glucosinolate profiles in *B. napus* seed meal has been a long term breeders' objective which has been hard to achieve using traditional breeding because of the complexity of the mechanisms involved in glucosinolate synthesis and regulation. Molecular breeding is well suited, on the other hand, to investigate intrinsic genetic structure, especially quantitative trait loci (QTLs) controlling the traits. In *B. napus*, Uzunova et al. (1995) and Toroser et al. (1995) used first-generation hybridization-based restriction fragment length polymorphism (RFLP) maps to identify 4 QTLs which explained 61.7% of phenotypic variation for total glucosinolate content and 5 QTLs which explained 71.0% of phenotypic variation for total aliphatic glucosinolate content in separate linkage groups (LGs) of the genomes

from seeds of two DH (doubled haploid) populations, respectively. In another study, two of these previously detected 4 QTLs for total seed glucosinolate content were proposed to be the same as the two major QTLs in a set of spring and winter cultivars and lines described by de Quiroz and Mithen (1996). Zhao and Meng (2003a) used mainly RFLP markers and identified 3 QTLs controlling total glucosinolate content, and 3 to 15 QTLs for different glucosinolates in seeds of an F_3 population. One locus associated with aliphatic glucosinolates and another with indole glucosinolates were linked to plant resistance to *Sclerotinia* stem rot. Howell et al. (2003) used traditional quantitative genetics and Harper et al. (2012) used associative transcriptomics to report 3 QTLs controlling total glucosinolate content on LG A9, C2 and C9 in seeds of *B. napus*. Li et al. (2014) used association mapping to screen a diverse panel of 472 accessions with the 60K Infinium® (Illumina Inc., San Diego, CA, USA) microarray and found SNP clusters associated with total seed glucosinolate content on chromosomes A9, C2, C7 and C9. The close relatives of *B. napus*, especially its proposed progenitors *B. rapa* and *B. oleracea*, also attracted interest for their glucosinolate traits. In *B. rapa* Lou et al. (2008) used amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers to identify 6 QTLs for aliphatic, 3 QTLs for indolic and 3 QTLs for aromatic glucosinolate content in leaves of 2 DH populations. Hirani et al., (2013) discovered a major QTL controlling 5C aliphatic glucosinolate content on chromosome A3 in a recombinant inbred line (RIL) population and validated through homeologous gene replacement through interspecific hybridization. In *B. oleracea*, Sotelo et al. (2014) identified 82 major QTLs controlling the synthesis of various glucosinolates in a 155-line DH population by using SSR and RFLP markers. Eighteen of these QTLs were consensus

ones found in seeds, leaves and buds of the plants. Epistatic effects were observed for the consensus QTLs. Seven candidate loci, 3 for aliphatic and 4 for indolic glucosinolate content were proposed in *Brassicaceae* by the authors. There were also studies in *B. juncea* (Cheung et al. 1998; Mahmood et al. 2003; Ramchiary et al. 2007) and in *Arabidopsis thaliana* Kliebenstein et al. (2001) which reported QTLs functioning differently for either total glucosinolate content or for glucosinolate components.

The genetic mechanisms regulating to the biosynthesis of glucosinolates and their components are still poorly understood in *B. napus.* In the present study, we used the newly developed *Brassica* 60K Infinium® SNP microarray and SRAP molecular markers for a bi-parental *B. napus* DH population and found 9 QTLs distributed on 6 chromosomes using 8,839 SNP and 35 SRAP markers. Two thousand five hundred and ninety-eight cM of genetic distance were covered. Chromosome A9 had more QTLs which were highly significant and played a major role in the biosynthesis and regulation of major glucosinolate components.

Materials and Methods

Population and Environments

The spring canola inbred line M77 was pollinated by the semi-winter rapeseed inbred line M23 to produce F_1 from which the doubled haploid (DH) lines were generated using microspore culture and subsequent chromosome doubling techniques described by Weber et al. (2005). Eighty-nine DH lines along with their parents were grown in a growth room under 22/16 °C and artificial lighting 16/8 h day/night. Liquid fertilizer (20-8-20) at 200 ppm mixed with magnesium sulfate 8.74 g/l was applied once a day with watering. The

plants were grown in 10 cm pots, one plant per pot in Sunshine® #4 mixed soil (Sun Gro Horticulture Canada Ltd). Plant leaf tissues were sampled at 3-leaf stage for genotyping. After harvesting mature seeds from each DH line, seeds were dried up at 35°C overnight prior to glucosinolate extraction.

Detection of SNP and SRAP Markers

Brassica 60K Infinium® SNP microarray BeadChips (Illumina Inc., USA) were used for the whole genome genotyping of the 88 DH lines along with their parents following the manufacture's protocols (Illumina Inc., USA) for library preparation and marker identification. To generate SRAP markers, DNA was extracted by CTAB (Cetyltrimethyl ammonium bromide) method according Li and Quiros (2003). Twenty-nine primer pairs, eight fluorescently labeled forward primers with FAM (blue), NED (yellow), PET (red) and VIC (green) fluorescent dyes and 24 unlabeled reverse primers were used. PCR (polymerase chain reaction) was programmed according to Sun et al., (2007) on a PCR machine (Eppendof®, ON, Canada). The PCR products were mixed and denatured in formamide (Hi-Di™, Life technologies, USA) with the size standard dye Liz-500 (Life technologies, USA). The denatured DNA was loaded onto the Genetic Analyzer (3130xl Genetic analyzer, Life technologies, USA) to separate PCR products. ABI GeneScan 3.7 (Life technologies, USA) was used to analyze the data. Genographer® v1.6.0 was used to score SRAP markers.

Genetic Mapping and QTL Identification

The JoinMap® 3.0 (Van Ooijen and Voorrips 2001) was used to group and localize the SNP and SRAP markers which were translated into homozygous values acceptable by WinQTLCart v2.5_011 (Statistical Genetics, NCSU, USA, 2012). To facilitate QTL

analysis, the markers at the same position or within 0.5 cM on the same chromosome were grouped into the same bin. The first marker was used to represent the bin for testing. The calculation environments were set up as follows: Composite Interval Mapping (CIM) with a significance level of 0.05 and 1,000 times of permutation , at walking speed 1 cM by Model 6, Kosambi function, five control markers, window size 10.0 cM, backward and forward regression method both with probability for into and out of 0.1. The threshold for QTL declaration was set up at LOD (logarithm of odds) 3.

Glucosinolate Analysis

Two hundred mg of seed of each DH line were ground in liquid nitrogen. Glucosinolate extraction protocol was used according to Kliebenstein et al. (2001) and Liu et al. (2012) with minor modifications. Total glucosinolate was eluted by Sephadex DEAE and de-sulfonated by sulfatase before quantification. Eighty microliters of the glucosinolate samples from each line were analyzed using high-performance liquid chromatography (HPLC) (Hewlett-Packard 1100) with a 5 mm column (Lichrocart 250-4 RP18e, Fisher Scientific, Canada) in which acetonitrile and water was used as solvents. The composition of each glucosinolate samples was determined at wavelength 229 nm. The running program was set up with acetonitrile gradient 1.5 -7% (*v*/*v*) 8 min, 7 – 15% 4 min, 15 – 55% 18 min, 55 – 92% 5 min, 92% 5 min, 92 – 1.5% 5 min, 1.5% 3 min and 0% 4 min. The peaks detected were identified by referring to (Liu et al. 2011). The areas under the major peaks were converted to $\mu molg^{-1}$ seed by using the response factors from (Vinjamoori et al. 2004).

Results

Glucosinolate Identification and Quantification

Five major glucosinolates (Figure 1), aliphatic 2-hydroxy-3-butenyl (progoitrin, PRO), 3-butenyl (gluconapin, GNP), 5-methylsulfinylpentyl (glucoalyssin, GLS), 4-pentenyl (glucobrassicanapin, GBN) and indolic 3-indolylmethyl (glucobrassicin, GBC) were identified. The total glucosinolate content of the 2 parental lines were 7.35 μmol/g seed for the low glucosinolate line M77 and 95.10 μmol/g seed for the high glucosinolate line M23 (Table 1).

Total glucosinolate content (TGC) varied from 2.6 to 105.7 μmol/g seed and had a range of 103.1 μmol/g seed. There was only one line having TGC lower than M77. However, there were 14 lines having TGC higher than M23. The average TGC was 67.8 μmol/g seeds and the median 76.3 μmol/g seed from the 89 DH lines was skewed toward the high glucosinolate end (Table 1S). Aliphatic glucosinolates (4C, four carbon + 5C, five carbons, T-ALI, total aliphatic) were the dominant glucosinolates in the seed with an average 93.49% of the TGC. This is in agreement with the results from Velasco et al. (2008). In contrast, the detected indolic GBC only accounted for 6.50% of TGC. It was quite consistent that over 90% of TGC in almost all the seed samples was from T-ALI. On the other hand, four-carbon aliphatic glucosinolates predominated in T-ALI ranging from 73.22 to 100% of T-ALI. Similarly PRO accounted for the largest proportion of 4C with a mean of 68.39%. There were only four lines having more than 50% GNP among the 88 DH lines. There was a trend when TGC was less than 30 μmol/g seed, where the proportion of aliphatic glucosinolates decreased and indolic glucosinolates increased with the decrease of TGC.

Correlation coefficients (r) reflected the relationships among the different glucosinolate components. For example, PRO had strong positive relationship with 4C, T-ALI and TGC with r = 0.9541, 0.9551 and 0.9514, respectively. To a lesser extent GNP also had positive relationship with 4C, T-ALI and TGC by r = 0.8588, 0.8435 and 0.8472. GLS and GBN had similar patterns in that the relationships were all positive; each of their relationships with 5C was stronger than that with T-ALI and TGC such as r = 0.7739 and 0.9153 for both with 5C, respectively, and 0.3788, 0.3888 for GLS and 0.7516, 0.7476 for GBN with T-ALI and TGC. Four carbon aliphatic glucosinolates had r = 0.9945 and 0.9935 with T-ALI and TGC but the 5Cs' corresponding r = 0.7053 and 0.7069. T-ALI was a good representative for TGC with r = 0.9993 but GBC was not with r = -0.51.21 (Figure 3).

Construction of Genetic Map with SNP and SRAP Markers

Eight thousand eight hundred and thirty-nine polymorphic SNP markers were generated and mapped onto 19 chromosomes. One thousand one hundred and eighty-five SNP bins were grouped with 35 SRAP markers to assembly a genetic map which covered genetic distance of 2597.7 cM in the 19 chromosomes (Table 2). The average genetic density was 0.47 bins/cM and 0.91 unique SNP positions per cM. Chromosome A3 had the most bin number of 108 and unique SNP positions of 280, while chromosome C6 had the fewest bins of 24 and fewest unique SNP positions of 37. There were 690 SNP bins, 3,855 SNP markers, 1,431 unique SNP positions and 1351.5 cM of genetic distance was covered in subgenome A. The corresponding values were 495 SNP bins, 4,984 SNP markers, 965 unique SNP positions and 1,246.2 cM in subgenome C. The average densities of bins and

unique SNP positions were 0.53/cM and 1.09/cM in subgenome A and 0.40/cM and 0.74/cM in subgenome C.

QTL Identification for Glucosinolate Related Traits

There were 17 QTLs regulating the glucosinolate traits PRO, GNP, 4C, GLS, GBN, 5C, T-ALI, GBC and TGC individually on 6 chromosomes (Table 3). Among them, chromosome A9 had the most QTLs with 8. Chromosomes A1 and C3 anchored 3 QTLs each. There was only 1 QTL on each of chromosome A8, C2 and C9, respectively (Figure 3 and Figure 4).

Chromosome A9 not only had more QTLs than other chromosomes, the QTLs on this chromosome also had significant effects on glucosinolate traits. For example, LOD values of the QTLs on chromosome A9 ranked the highest for 5 QTLs among all of the 17 QTLs and explained the highest phenotypic variances ranging from 23.87% to 40.10% with the average of 30.12%. Four of the 5 QTLs on chromosome A9 had positive additive effects and contributed to increases of 4C with 40.10%, PRO with 29.63%, T-Ali with 28.96% and TGC with 28.05% of their corresponding phenotypic variances. One QTL, on the other hand, had negative genetic effect and contributed to the decrease of GBC with 23.87% of phenotypic variance. The other three QTLs had positive genetic effects on 5C, GBN and GLS with LOD values ranging from 3.52 to 6.03 and explained 14.18 to 18.78% of phenotypic variances for their traits.

Two QTLs for 5C and GLS on chromosome A1 were located at the same position, 102.0 cM from the left end and had positive genetic effects explaining 14.93% and 14.94% of phenotypic variances. One QTL controlling 4C was located at 33.1 cM and had negative genetic effect on 4C explaining 11.45% of phenotypic variance.

The 3 QTLs on chromosome C3 were located at approximately the same position and controlled 3 different glucosinolate traits 4C, TGC and T-Ali with negatively additive effects represented by R^2 = 12.18%, 9.99% and 9.70% respectively. There was one QTLs located at 62.8 cM on chromosome A8 controlling PRO, one at 122.8 cM on C2 controlling GNP and one at 53.9 cM on C9 controlling PRO explaining 16.59%, 14.88% and 7.84% of phenotypic variances.

Three QTLs on chromosome A8, A9 and C9 controlling PRO or another 3 QTLs on chromosome A1, A9 and C3 controlling 4C explained their compound phenotypic variances 54.06% or 16.47%. Two QTLs on chromosome A1 and A9 controlling GLS and 5C, another 2 QTLs on chromosome A9 and C3 controlling T-Ali or TGC explained their corresponding compound phenotypic variances 29.12%, 33.71%, 19.26% or 18.06%. There was only 1 QTL each controlling GNP, GBN and GBC and explained their phenotypic variances 14.88%, 16.29% and 23.87%.

Discussion

In *B. napus* germplasm, about 30 different glucosinolates were reported (Lou et al. 2008), however a few glucosinolates predominate total glucosinolate content in seeds such as progoitrin, gluconapin, glucobrassicanapin etc. Most glucosinolate compounds exit in small quantity which is difficult to quantify for QTL mapping to find meaningful QTL. In this study, five major glucosinolates including both aliphatic and indolic glucosinolates in the seed of a biparental *B. napus* DH population were assessed for QTL mapping. The difference of 87.7 μmol/g seed for total glucosinolate content between the spring-type canola parent line, M77 and the semi-winter-type rapeseed parent line, M23 was a good

indicator to use their progenies for QTL mapping. There was only one DH line having lower total glucosinolate content than line, M77 while 14 DH lines had higher total glucosinolate content than line M23, indicating that over-expression patterns and multi-gene inheritance for glucosinolates and glucosinolate content predominated. Line M77 carried low-glucosinolate alleles, similarly Howell et al. (2003) had only 2.6 μmol/g seed total glucosinolate content in their analysis. The low-glucosinolate line M77 also possessed alleles contributing to increased total glucosinolate content of the 14 DH lines which had higher total glucosinolate content than line M23, suggesting that epistatic effects of the QTLs were also involved in glucosinolate content over-expression. Total glucosinolate content was mainly determined by 4C aliphatic glucosinolates, predominately PRO. Indolic glucosinolates only accounted for a small proportion of total glucosinolate content especially when the total glucosinolate content was high. Interestingly, when total glucosinolate content was lower than a given threshold level there was a trend showing that indolic glucosinolate content increased with a decrease in TGC. This suggests that although the genetic effects of the QTL controlling GBC were minor, these QTL or QTLs were difficult to eliminate. It was apparent that 4C aliphatic glucosinolates were more important than 5C aliphatic glucosinolates in rapeseed seeds since they predominantly contributed to total aliphatic or total glucosinolate content.

It was possible to develop dense genetic maps which more accurately located functional genes or QTLs and their co-segregating markers in this study using microarray chips. The 60K *Brassica* Infinium® chips were a powerful tool with dual-color imaging system for genetic studies and breeding since they targeted the whole genome. However, there were

some gaps between neighboring SNP markers. Therefore, SRAP marker supplements helped pinpoint the tentative QTLs more accurately as was the case for chromosome A9.

In this study, 17 QTLs regulating 9 glucosinolate traits individually distributed over 6 chromosomes.

Two QTLs on chromosome A1 with their peaks at exactly the same position 102.0 cM were specified by the same SNPs SNP0076, SNP0078 and SNP0079 and shared the 95% confidence interval (CI) 11.3 cM from left border (LB) 94.7 cM to right border (RB) 106.0 cM, so they might be the same QTL controlling two related traits GLS and 5C. Two QTLs on chromosome A9 with peaks at 42.5 cM and 44.5 cM were also specified by the same markers SNP0679, SRAP273 and SRAP105 and shared the 95% CI 9.8 cM from LB 35.4 cM to RB 45.2 cM, so they might be the same QTL controlling two traits PRO and GBC. Four QTLs on chromosome A9 with their peaks at the same position 49.2 cM were located by the same markers SRAP105, SNP0680 and SNP0681 and shared the 95% CI 6.9 cM from LB 46.1 cM to RB 53.0 cM and might be the same QTL regulating 4C, GBN, T-Ali and TGC. QTLs on the same chromosome A9 with peaks at 53.5 cM and 55.4 cM were identified by the same markers SNP0680, SNP 0681 and SRAP141 and shared 95% CI 7.1 cM from LB 51.5 cM to RB 58.6 cM regulating two related traits GLS and 5C. Similarly three QTLs on chromosome C3 with their peaks at exactly the same position 71.5 cM were indicated by the same SNPs SNP0970, SNP0971 and SNP0972 and shared 95% CI 7.5 cM from LB 65.3 cM to RB 72.8 cM regulating three related traits 4C, T-Ali and TGC might be the same QTL as well. It agreed with Sotelo et al. (2014) that one QTL could regulate more than one glucosinolates.

Chromosome A9 harbored the largest number and the most significant QTLs controlling 8 out of the 9 glucosinolate traits in this study. Previous studies also detected significant QTLs for total glucosinolate content on chromosome A9 in *B. napus* (Harper et al. 2012; Howell et al. 2003; Li et al. 2014). Similarly, Rahman et al. (2014) reported common and significant QTLs for total glucosinolate content on A9 linkage group from *B. rapa* populations. Detection of a large number of QTL on chromosome A9 may be explained by the existence of several genes with multiple copies that are involved in glucosinolate biosynthesis pathway in *Brassica* species. Finding of many QTL on chromosome A9 is supported by whole genome sequence data and its comparative analysis with Arabidopsis, Wang et al. (2011b) revealed 3 transcription factors, 7 side chain elongation and 5 side chain modification genes involved in the biosynthesis pathways of glucosinolates in comparative analysis of whole genome with Arabidopsis. Furthermore, total 102 putative genes identified are involved in glucosinolate biosynthesis through genome wide comparative analysis with 52 ortholog of Arabidopsis (Wang et al. 2011b). It suggests that the glucosinolate biosynthesis pathway is complex itself in Arabidopsis, which make it complex in *B. rapa* due to duplication or triplication events during evolutionally separation in diploid species, and even more complex in allotetraploid species such as *B. napus*.

Epistatic effect existed in the interactions of the QTLs (Sotelo et al. 2014). Therefore, it was more desirable to identify each single component of glucosinolate content to find major QTLs for practical crop breeding.

QTLs controlling total glucosinolate content in *B. napus* seeds were also identified on LG C2 and C9 (Harper et al. 2012; Howell et al. 2003), on C2, C7 and C9 (Li et al. 2014).

Rahman et al., (2014) found QTLs on A2 and A7 from *B. rapa*. In this study, we located QTLs on A1, A8, C2, C3 and C9 in addition to those QTLs on A9. Some QTLs might be the same QTLs as those previously reported while some QTLs might be new ones. Also, the QTL for GNP on C2 might be the same as those reported by Li et al. (2014). This indicated that QTLs for glucosinolate traits widely distributed over both the A and C genomes and these QTLs might be controlled by the genes in the biosynthesis pathway of glucosinolates (Wang et al. 2011b). Future studies with more emphasis on individual glucosinolates using genetically diverse germplasm will be required to validate and fine map QTLs for glucosinolate traits.

Conclusion

Glucosinolate is one of the most important plant secondary metabolites found in the *Brassica* species, which play an important role in agriculture and animal industries. It is important to manipulation different glucosinolate compounds in canola/rapeseed and other vegetable crops for effective applications, such as reduction of detrimental glucosinolate compounds in seed meal and increasing those glucosinolate compounds which possess anticancer properties in *Brassica* vegetables. It is therefore important to identify genes/QTL involve in glucosinolate biosynthesis. In this study QTL mapping was carried out for individual glucosinolates and 9 QTL detected on different chromosomes. A large number of QTL identified on chromosome A9 which is known to hold several glucosinolate biosynthesis genes including transcription factors, side chain elongation and side chain modification. Flanking markers can be deployed in marker

assisted selection in canola/rapeseed breeding to manipulate glucosinolate profile and content.

Acknowledgment

This research was financially supported by the Monsanto Canada Inc. and NSERC

Table 1. Glucosinolates (μmol/g Seed) in a Doubled Haploid *Brassica napus* Population M730 and Their Parents*

Cont	PRO	GNP	4C	PRO/ 4C%	GNP/ 4C%	GLS	GBN	5C	GLS/ 5C%	GBN/ 5C%	T-Ali	4C/T-Ali%	5C/T-Ali%	GBC	TGC	T-Ali/ TGC%	GBC/ TGC%
Min	0.6	0	0.6	33.65	0	0	0	0	-	-	0.6	73.22	0	0.4	2.6	23.48	0.43
M77	2.0	0.9	2.9	68.97	31.03	0.4	0.2	0.6	65.08	34.90	3.5	82.20	17.90	3.8	7.4	48.03	51.97
μ	39.5	18.9	58.5	68.39	31.61	3.1	3.9	7.0	48.06	51.93	65.5	89.00	11.00	2.3	67.8	93.49	6.50
Med	45.7	18.1	67.9	69.67	30.35	2.7	3.2	6.5	49.36	50.53	73.3	89.27	10.71	2.1	76.3	96.71	3.29
M23	56.7	29.0	86.0	66.17	33.83	2.6	5.3	7.9	33.08	66.80	93.6	91.60	8.46	1.5	95.1	98.39	1.61
Rg	64.9	45.7	93.7	66.35	66.35	8.8	13.5	18.5	65.84	65.72	104.3	26.78	26.78	5.0	103.1	76.09	75.71
Max	65.5	45.7	94.3	100	66.35	8.8	13.5	18.5	85.36	80.48	104.9	100	26.78	5.4	105.7	99.57	76.14

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin; and TGC, total glucosinolate content.

Table 2. Bin Assignments for SNP and SRAP Markers on the 19 Chromosomes of *Brassica napus*[*]

Chromosome	#Bin	#Bin (SNP)	#SNP	UP[1]	#SRAP	GD[2] (cM)	Bin/cM	UP/cM
A1	71	71	374	135	0	131.2	0.54	1.03
A2	47	47	220	93	0	164.5	0.29	0.57
A3	108	108	752	280	0	163.1	0.66	1.72
A4	62	59	400	136	3	100.4	0.62	1.35
A5	76	76	418	142	0	141.1	0.54	1.01
A6	69	67	206	108	2	179.7	0.38	0.60
A7	82	81	507	214	1	118.8	0.69	1.80
A8	38	38	258	58	0	76.2	0.50	0.76
A9	90	85	360	141	5	177.8	0.51	0.79
A10	58	58	360	124	0	98.7	0.59	1.26
Subgenome A	701	690	3,855	1,431	11	1351.5	0.53	1.09
C1	50	45	395	103	5	135.8	0.37	0.76
C2	60	60	1823	149	0	141.5	0.42	1.05
C3	99	97	794	209	2	199.5	0.50	1.05
C4	87	80	633	128	7	166.7	0.52	0.77
C5	38	38	158	58	0	138.5	0.27	0.42
C6	24	24	198	37	0	75.0	0.32	0.49
C7	67	66	419	114	1	135.5	0.49	0.84
C8	59	55	409	119	4	134.7	0.44	0.88
C9	35	30	155	48	5	119.0	0.29	0.40
Subgenome C	519	495	4,984	965	24	1246.2	0.40	0.74
Genome AC	1,220	1,185	8,839	2,396	35	2597.7	0.47	0.91

[*]: SNP, single nucleotide polymorphism; SRAP: sequence related amplified polymorphism.
[1]: UP, Unique SNP Position.
[2]: GD, Genetic Distance.

Table 3. Quantitative Trait Loci Regulating Glucosinolates (GSLs) Detected Using Composite Interval Mapping with Single Nucleotide Polymorphism (SNP) and Sequence-Related Amplified Polymorphism (SRAP) Markers from a *Brassica napus* Doubled Haploid Population M730*

Ch	QTL	Pos	Peak	C.I.	LB	RB	LMK	LcM	RMK	RcM	LOD	Add.	R^2	GSL
9	*Bna7G5-1*	49.1	49.2	6.9	46.1	53.0	*SRAP105*	45.2	SNP0681	53.4	12.0	18.01	0.4010	4C
13	*Bna7G8-1*	69.8	71.5	7.7	65.3	73.0	SNP0970	65.0	SNP0972	71.7	4.5	-8.97	0.1218	4C
1	*Bna7G1*	33.1	33.1	9.8	26.6	36.4	SNP0022	32.1	SNP0024	34.5	4.3	-12.40	0.1145	4C
													0.1647	4C
9	*Bna7G6-1*	53.5	53.5	11.4	47.2	58.6	SNP0680	49.1	*SRAP141*	56.3	6.0	2.44	0.1878	5C
1	*Bna7G2-1*	102.0	102.0	11.6	94.7	106.3	SNP0076	100.2	SNP0079	107.6	4.9	1.52	0.1493	5C
													0.3371	5C
9	*Bna7G4-2*	44.3	44.5	14.6	34.0	48.6	SNP0679	29.8	*SRAP105*	45.2	7.2	-0.63	0.2387	GBC
9	*Bna7G5-2*	49.1	49.2	14.7	41.2	55.9	*SRAP105*	45.2	SNP0681	53.4	5.2	1.10	0.1629	GBN
1	*Bna7G2-2*	102.0	102.0	11.3	94.7	106.0	SNP0076	100.2	SNP0079	107.6	4.0	0.69	0.1494	GLS
9	*Bna7G6-2*	55.4	55.4	7.1	51.5	58.6	SNP0680	49.1	*SRAP141*	56.3	3.5	1.05	0.1418	GLS
													0.2912	GLS
12	*Bna7G7*	122.7	123.0	17.6	119.5	137.1	SNP0936	121.5	SNP0938	123.7	4.7	3.95	0.1488	GNP
9	*Bna7G4-1*	42.4	42.5	9.8	35.4	45.2	SNP0679	29.8	*SRAP105*	45.2	10.0	9.84	0.2963	PRO
8	*Bna7G3*	62.8	62.8	9.4	55.4	64.8	SNP0647	61.8	SNP0648	64.8	6.3	9.93	0.1659	PRO
19	*Bna7G9*	53.9	-	-	-	-	-	-	SRAP271	56.5	3.2	4.86	0.0784	PRO
													0.5406	PRO
9	*Bna7G5-3*	49.1	49.2	8.1	45.4	53.5	*SRAP105*	45.2	SNP0681	53.4	8.9	15.53	0.2896	T-Ali
13	*Bna7G8-2*	70.6	71.5	9.2	63.6	72.8	SNP0970	65.0	SNP0972	71.7	3.5	-15.90	0.0970	T-Ali
													0.1926	T-Ali
9	*Bna7G5-4*	49.1	49.2	8.1	45.4	53.5	*SRAP105*	45.2	SNP0681	53.4	8.6	14.93	0.2805	TGC
13	*Bna7G8-3*	70.6	71.5	9.2	63.6	72.8	SNP0970	65.0	SNP0972	71.7	3.5	-15.75	0.0999	TGC
													0.1806	TGC

*: Ch, chromosome; Pos, position in cM from left telomere; Peak, in cM from left telomere; C.I., 95% confidence interval for the detected QTL; LB, left border of the 95% confidence interval; RB, right border of the 95% confidence interval; LMk, left marker; LcM, left marker position in cM; RMk, right marker; RcM; right marker position in cM; LOD, logarithm of odds; Add., additive genetic effect; GSL, glucosinolate; PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content.

Table S1. Glucosinolate Content (μmol/g Seed) of a *Brassica napus* Doubled Haploid Population M730 and Their Parents*

Line	PRO	GNP	4C	GLS	GBC	5C	TAl	GBC	TGC	Line	PRO	GNP	4C	GLS	GBC	5C	TAl	GBC	TGC
75	1	0	1	0	0	0	1	2	3	80	49	20	69	2	3	5	74	3	77
M77	2	1	3	0	0	1	4	4	7	34	47	21	68	4	4	8	76	3	79
49	4	1	5	0	0	0	5	3	8	24	49	18	67	4	6	10	77	4	81
83	4	1	5	1	0	1	6	4	10	66	47	24	71	3	7	10	81	0	81
45	5	2	7	1	0	1	8	4	12	39	50	18	68	7	4	11	79	3	81
71	6	2	8	1	0	1	9	5	14	64	57	17	74	3	4	7	81	1	82
46	9	3	11	1	1	2	13	4	18	67	52	22	74	3	3	6	80	3	83
40	9	3	12	2	1	3	15	5	20	33	45	25	70	5	6	11	81	3	83
100	7	8	15	2	0	2	17	3	20	8	50	18	68	8	6	14	82	2	84
32	10	4	13	3	2	4	18	4	22	2	51	18	69	5	8	13	82	2	84
53	12	10	23	1	0	2	24	4	29	55	52	23	75	3	6	9	84	1	84
56	13	9	22	2	1	3	25	5	30	69	53	17	70	6	6	12	82	3	85
87	12	12	24	4	2	6	30	3	32	28	49	25	74	5	4	9	83	2	85
107	24	5	29	2	1	3	32	2	34	17	47	25	72	6	4	10	82	4	85
102	21	8	30	1	1	3	32	3	35	42	48	30	78	2	4	6	83	3	86
105	23	9	32	2	1	3	36	3	38	25	50	23	74	4	7	11	84	2	86
90	24	11	34	2	1	3	38	2	40	98	52	28	80	2	3	6	86	2	87
106	23	13	36	3	2	6	41	1	42	11	56	20	76	4	6	10	87	1	87
82	25	11	37	2	2	5	41	2	43	31	59	23	81	2	4	6	87	1	88
81	25	12	37	2	2	4	41	2	43	79	60	17	77	4	6	10	88	2	89
73	31	7	38	3	3	6	43	2	46	21	52	30	82	2	2	4	87	3	89
99	27	10	37	4	3	7	43	3	46	77	52	31	83	2	4	5	88	2	90
72	32	13	45	2	2	4	49	2	50	23	66	16	81	3	4	8	89	1	90
14	32	13	45	3	2	5	50	2	52	54	53	23	77	5	8	12	89	1	90
59	31	6	37	9	5	14	50	2	52	94	62	20	82	4	3	7	89	2	90
91	31	16	47	2	3	4	52	1	52	78	53	30	83	2	4	7	89	2	91
13	23	17	40	7	1	9	49	5	54	92	59	25	84	3	4	6	90	2	92
86	33	13	47	4	3	6	53	2	55	68	50	35	85	4	3	7	92	2	94
44	32	19	51	2	1	2	53	2	55	37	40	46	86	2	6	7	93	2	95
63	38	10	48	4	2	6	54	2	56	20	44	39	83	3	7	11	93	2	95
48	37	13	50	3	3	6	55	1	57	M23	57	29	86	3	5	8	94	2	95
76	30	16	46	4	4	9	55	2	57	30	47	35	82	4	8	12	94	1	95
52	26	21	47	4	2	6	53	5	57	22	61	24	85	3	6	9	94	1	96
62	35	17	52	2	3	5	57	2	59	12	60	25	85	4	5	9	94	2	96
18	30	18	48	4	3	7	55	4	59	16	59	26	85	2	9	11	95	0	96
6	16	31	46	3	8	10	57	4	61	10	49	38	87	2	7	8	95	1	96
57	43	17	59	3	3	5	65	1	66	35	55	35	90	1	3	4	94	2	96
1	32	23	56	3	2	6	61	5	66	5	65	21	86	3	7	10	96	1	96
70	44	12	57	5	2	7	64	3	67	41	58	25	83	5	8	12	95	2	97
19	46	18	64	2	2	4	68	1	70	15	51	36	87	3	6	9	96	1	97
43	42	18	60	5	6	11	70	3	73	36	55	35	90	2	5	7	97	1	98
50	53	14	67	2	3	5	71	2	74	4	59	20	80	5	14	19	98	1	99
61	50	9	60	5	8	13	72	3	75	51	54	37	91	2	6	8	99	1	100
26	43	24	68	2	2	4	71	3	75	7	51	34	84	5	10	15	99	2	100
29	39	27	65	4	3	6	72	4	76	3	63	32	94	3	8	11	105	1	106
101	50	15	64	5	4	9	73	3	76										

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBC, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; TAl, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content.

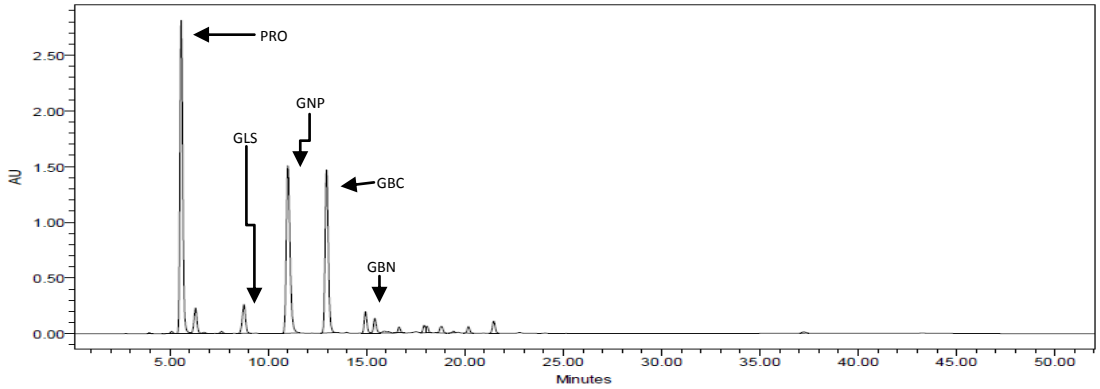


Figure 1. Five Major Glucosinolates in Seed of a Doubled Haploid *Brassica napus* Population M730*

*: AU, absorbance units; Minutes, retention time on HPLC; PRO (progoitrin); GLS (glucoalyssin); GNP (gluconapin); GBC (glucobrassicin) and GBN (glucobrassicanapin).

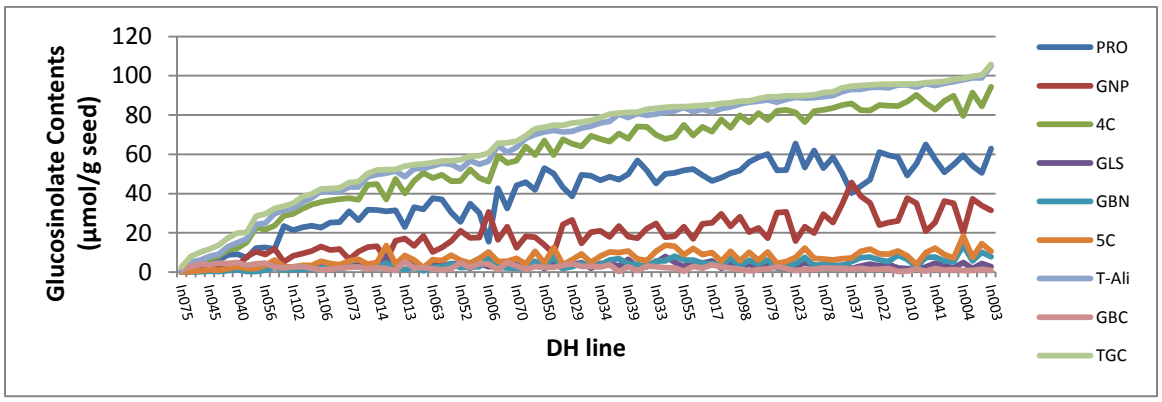


Figure 2. The Distribution of Content of Glucosinolate Components in Seed of a *Brassica napus* Doubled Haploid Population M730*

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin; and TGC, total glucosinolate content.

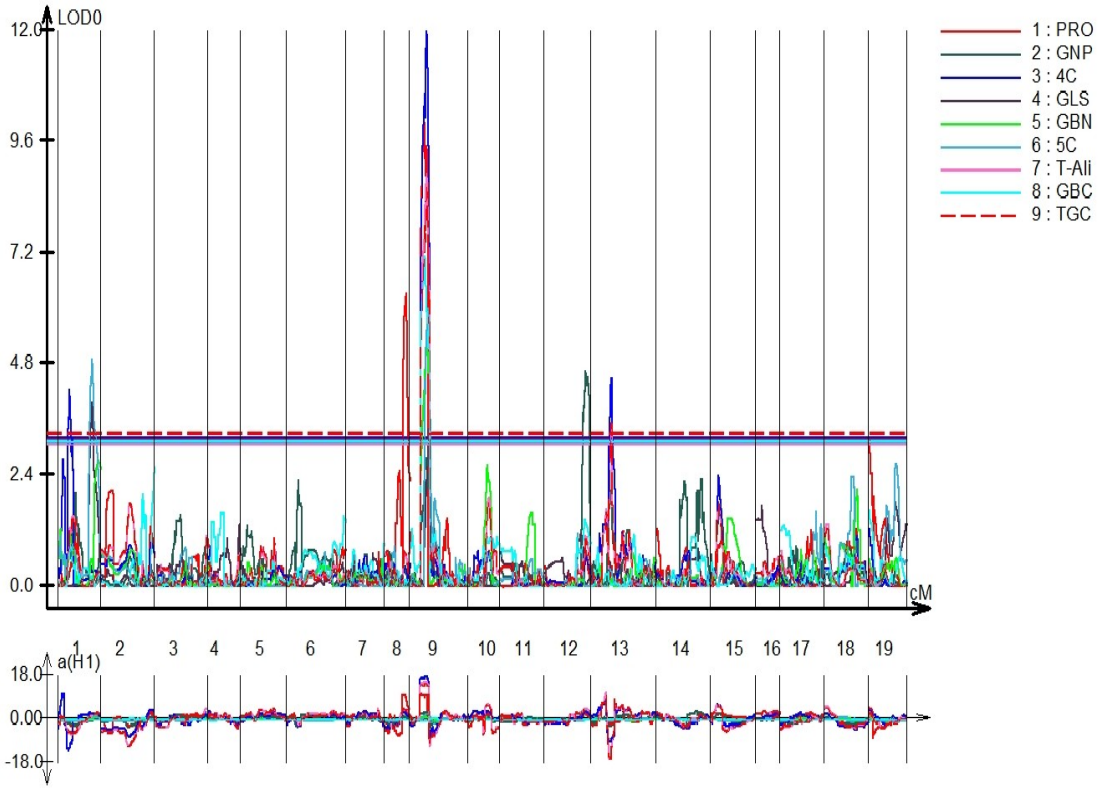


Figure 3. QTL Identification with Composite Interval Mapping Using Single Nucleotide Polymorphism (SNP) and Sequence-Related Amplified Polymorphism (SRAP) Markers for Glucosinolate Traits in Seed of a *Brassica napus* Doubled Haploid Population M730*

*: PRO (progoitrin), GNP (gluconapin), 4C (four carbon aliphatic glucosinolates), GLS (glucoalyssin), GBN (glucobrassicanapin); 5C (five carbon aliphatic glucosinolates), T-Ali (total aliphatic glucosinolates), GBC (glucobrassicin) and TGC (total glucosinolate content). **Upper graph**: QTLs identified by LOD (logarithm of odds). Horizontal axis indicates chromosome number; **Lower graph**: additive effects of the QTLs on each chromosome.

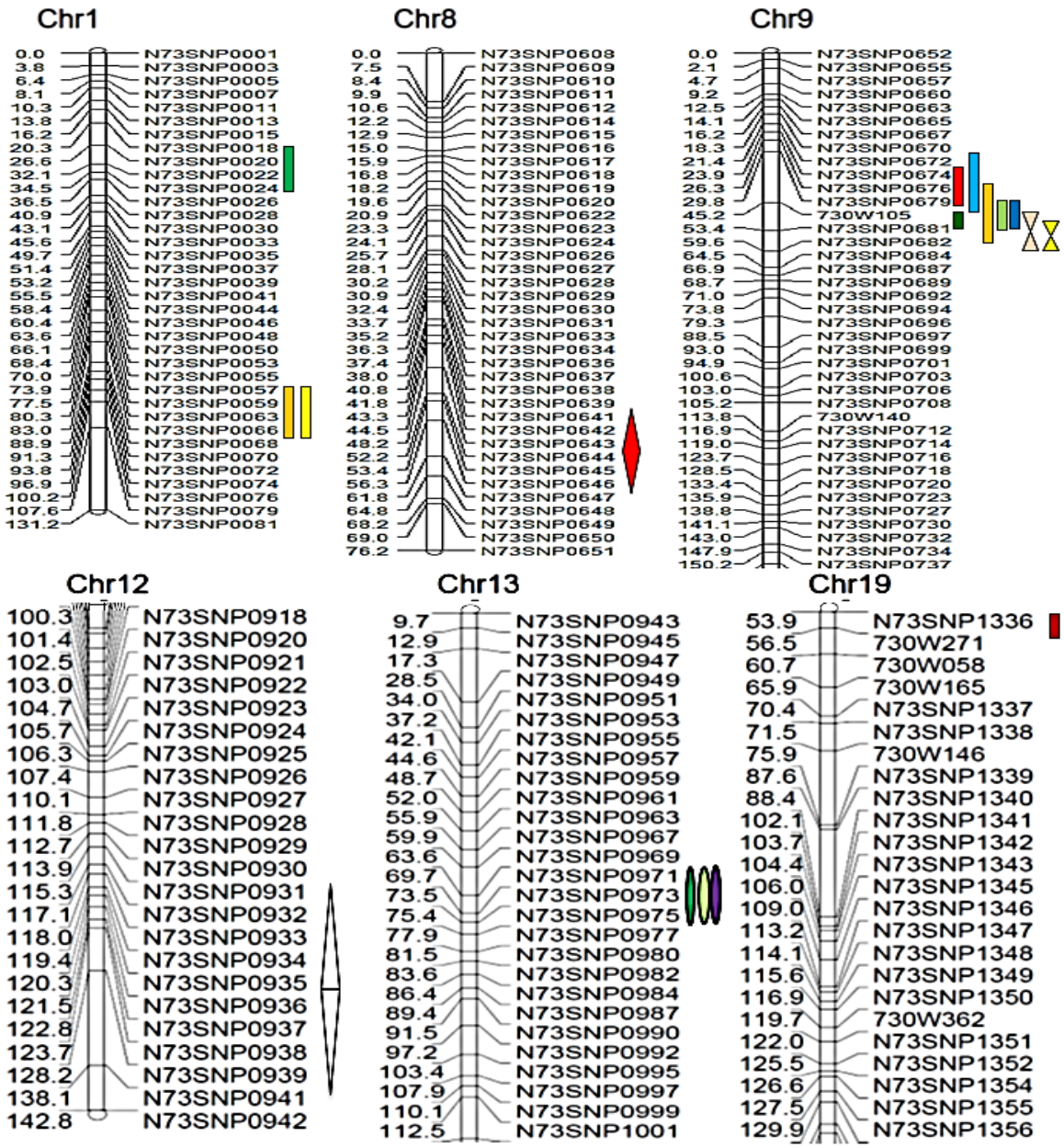


Figure 4. QTLs on the Genetic Map Identified with Composite Interval Mapping Using Single Nucleotide Polymorphism (SNP) and Sequence-Related Amplified Polymorphism (SRAP) Markers from Seed of a Doubled Haploid *Brassica napus* Population M730*

*: Rectangle bar, round-corner rectangle bar, diamond, parallelogram, double-sided border bar, two triangle, diamond with a cross, ellipse were QTL 1-8. Dark green, four carbon aliphatic glucosinolates; orange, five carbon aliphatic glucosinolates; yellow, glucoalyssin; red, progoitrin; blue, glucobrassicin; dark orange, glucobrassicanapin; light green, total aliphatic glucosinolates; purple, total glucosinolate content; white, gluconapin and the lengths of various shapes specified their 95% of confidence intervals of QTLs.

CHAPTER 2

Quantitative Trait Locus Analyses of Seed Glucosinolates in *Brassica napus*

Jun Liu, Arvind H. Hirani, Zhe Li, Chunren Wu, Peter B. E. McVetty, and Genyi Li

J. Liu. Monsanto Canada Inc., 900 - One Research Road, Winnipeg, MB R3T6E3, Canada
A.H. Hirani. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
Z. Li. Monsanto Canada Inc., 900 - One Research Road, Winnipeg, MB R3T6E3, Canada
C. Wu. Monsanto Canada Inc., 900 - One Research Road, Winnipeg, MB R3T6E3, Canada
P.B.E. McVetty. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
G. Li. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

Corresponding author: Genyi Li (e-mail: genyi.li@umanitoba.ca)

Abstract

Glucosinolates are major anti-nutritional metabolites affecting meal quality in canola (rapeseed). In this research, two *B. napus* doubled haploid (DH) populations were developed using parents with relatively high and low glucosinolate contents respectively. All DH lines and their parents were genotyped with Illumina *Brassica* 60K Infinium® array and sequence-related amplified polymorphism (SRAP) markers, and phenotyped for glucosinolate contents using high-performance liquid chromatography (HPLC). Five major seed glucosinolates progoitrin, gluconapin, glucoalyssin, glucobrassicanapin and glucobrassicin were identified. Aliphatic glucosinolates, more specifically a 4-carbon (4C) aliphatic glucosinolate progoitrin predominated in rapeseed. In contrast, indole glucosinolate glucobrassicin predominated in canola. Transgressive DH lines for low-leveled glucosinolates were observed in the DH populations. QTLs regulating these glucosinolates and their combinations were identified predominately on chromosome A9 in the population with high glucosinolate content and on chromosome C7 in the population with low glucosinolate content. Properties of the QTL include the ability for the same QTL to regulate multiple glucosinolate traits as well as the ability for multiple QTLs on the same or different chromosomes to regulate the same glucosinolate trait. Additive genetic effects of the QTLs played main roles in regulation of seed glucosinolate accumulation in rapeseed (canola). Also, epistatic genetic effects from these QTLs were observed but were minor. The closely linked flanking SNP markers of the QTLs could be used in marker assisted selection for glucosinolate traits.

Key words: *Brassica napus*, rapeseed, canola, glucosinolates, quantitative trait locus mapping.

Introduction

Oilseed rape (*Brassica napus* L.) ranks in the second place for grain and meal production after soybean (*Glycine max*) among oil crops in the world (USDA 2015). However, oilseed rape meal quality is greatly affected by glucosinolate content and composition in seed. Glucosinolates (β-thioglucoside-N-hydroxysulfates) are secondary metabolites produced naturally and almost exclusively in the order *Brassicales*. There have been 135 different glucosinolates identified (Agerbirk and Olsen 2012) and 30 of them are found in *B. napus* (Lou et al. 2008). Glucosinolates are the derivatives of amino acids. There are three types of glucosinolates based on their precursors and side chain modifications: aliphatic glucosinolates derived mainly from methionine (Halkier and Gershenzon 2006); aromatic glucosinolates originating mainly from phenylalanine; and indolyl glucosinolates stemming from tryptophan (Zukalova and Vasak 2002). All glucosinolates share a common structure as defined by Ettlinger and Lundeen (1956): a β-thioglucose, a sulfonated oxime and an aglycone side chain. When plants are under biotic or abiotic stresses, glucosinolates located in vacuoles of cells (Weese et al. 2015) may come into contact with hydrolytic enzymes myrosinases (EC 3.2.1.147) in the idioblast (Zukalova and Vasak 2002) or myrosin cells (Drozdowska et al. 1992) and their cofactors with water as the substrate which triggers hydrolysis. The degraded intermediates are not stable and thus further broken down into sulphate, isothiocyanates, thiocynates, nitriles and epithionitriles (Bones and Rossiter 1996). The hydrolytic products of glucosinolates have defensive effects providing protection for host plants from herbivores. Malfunctions

of the kidney, thyroid and liver, as well as palatability issues arise from livestock consuming glucosinolates were reported (Walker and Booth 2001). However, isothiocynates were also reported to be anticancer agents by Bones and Rossiter (1996) and Ishida et al. (2014). Therefore, glucosinolate modification and optimization remains one of the major goals for *Brassica* breeders in the industry.

Seed glucosinolate content is a quantitative trait controlled by polygenes in *Brassica* species. For *Brassica napus*, Uzunova et al. (1995), Toroser et al. (1995) and Howell et al. (2003) reported 4, 5 or 4 QTLs (quantitative trait loci) regulating total seed glucosinolates and total seed aliphatic glucosinolates using restriction fragment length polymorphism (RFLP) markers from either bi-parental DH or backcrossed populations. Feng et al. (2012) used 8 marker systems and found 205 QTLs spread over 18 linkage groups (LGs) involved with total and individual glucosinolate content for a bi-parental DH population. Li et al. (2014) used Illumina *Brassica* 60K Infinium® array screen 472 accessions and observed QTLs on chromosomes A09, C02, C07 and C09 regulating total glucosinolate content. Also, Liu et al. (2016) used the same technology identify 9 QTLs residing on chromosomes A1, A8, A9, C2, C3 and C9 controlling major aliphatic and indolic glucosinolates. The most significant QTLs were located on chromosome A9 in a bi-parental DH population. Fu et al. (2015) identified 43 minor QTLs distributed on chromosomes A2, A3, A4, A7, A9, C3 and C8 controlling total glucosinolate content using simple sequence repeat (SSR) markers for a low glucosinolate doubled haploid (DH) population. The genetic mechanism controlling glucosinolates had also been reported by other researchers (Liu et al. 2011; Lu et al. 2014; Magrath et al. 1993; Quiroz and Mithen 1996; Zhao and Meng 2003a). For *B. juncea* seed, Gupta et al. (2015) used

SSR markers from a bi-parental recombinant inbred line (RIL) population to report significant QTLs on chromosomes A4, A7 and A9 regulating aromatic, indole and aliphatic glucosinolate content. Mahmood et al. (2003) used RFLP markers in a bi-parental DH population whereas Ramchiary et al. (2007) used amplified fragment length polymorphism (AFLP) markers in a bi-parental backcrossed population to reach the conclusion that multiple QTLs could regulate various glucosinolates. For *B. oleracea* seed, Sotelo et al. (2014) used SSR and RFLP markers and found 13 QTLs on 8 LGs (excluding LG1) regulating total and individual glucosinolate content from a bi-parental DH population. For *B. rapa* seed, Rahman et al. (2014) reported 3 QTLs on chromosomes A2, A7 and A9 regulating total glucosinolate content, with the QTL on chromosome A9 having the most significance in a bi-parental RIL population. For experiments involving seeds of the model plant Arabidopsis, Brader et al. (2006) used inbred lines while Kliebenstein et al. (2001) used a bi-parental RIL population to study locus/loci regulating different glucosinolate components. Although plentiful research has been done to investigate the genomics behind glucosinolate accumulation in *B. napus* seeds orthologously and paralogously, the intrinsic mechanisms controlling this trait are still poorly understood. In the current research, we used the newly developed 60K *Brassica* Infinium® SNP beadchip as well as SRAP (sequence related amplified polymorphism) markers on two different DH populations derived from parents with various glucosinolate contents in order to investigate the mechanisms regulating total and major glucosinolates in *B. napus* seeds.

Materials and Methods

Plants and Planting

Each DH population was derived from F_1 plants using the microspore culture procedure outlined by Weber et al. (2005). A spring-type canola cultivar 'Topas' was pollinated with a high glucosinolate semi-winter cultivar 'Zhongyou821' to produce the 'ZT' population while a canola breeding line 'M69' was crossed to a relatively low glucosinolate semi-winter breeding line 'M29' in the 'M692' population. There were 121 and 83 DH lines in the ZT and M692 populations, respectively.

Six to eight plants per DH line were grown in peat soil along with their parents (Sunshine® Mix#4, Sun Gro Horticulture Canada Ltd, Deba Beach, Canada) in 10-cm pots. One plant was planted in each pot either in greenhouses or growthrooms under 22/16 °C and 16/8 hr day/night environmental conditions. The plants were watered once daily with 100 ppm fertilizer (20-8-20 at 62.74 g/l and $MgSO_4$ at 8.74 g/l). The plants were grown in a completely randomized design.

Plant Genotyping and QTL Analyses

Plant DNA used in SRAP marker analysis for population M692 was extracted according to the CTAB (cetyl trimethyl ammonium bromide) method (Li and Quiros 2001). The forward primers were labeled with FAM (blue), NED (yellow) or VIC (green) (Table S1). The PCR cycles for SRAP and SSR, genetic mapping, chromosome assignment and QTL analyses were all done as described by Liu et al. (2016).

SNP marker analysis was carried out for population M692 using Illumina *Brassica* 60K Infinium® SNP BeadChips at the Monsanto lab (Monsanto Company, St. Louis, USA). The genetic map for population ZT was adapted from Liu et al. (unpublished paper).

Glucosinolate Quantification

Glucosinolate extraction and quantification from seeds of the two DH populations were performed by the method reported by Liu et al. (2016).

Results

Glucosinolate Variation in Two DH Populations

Five major glucosinolate components, progoitrin (PRO, 2-hydroxy-3-butenyl), gluconapin (GNP, 3-butenyl), glucoalyssin (GLS, 5-methylsulfinylpentyl), glucobrassicanapin (GBN, 4-pentenyl) and glucobrassicin (GBC, 4-hydroxy-3-indolylmethyl) were identified in the seed for both DH populations M692 and ZT (Table 1).

Most seed aliphatic glucosinolates in seeds of *B. napus* were 4C aliphatic glucosinolates in which PRO predominated. This phenomenon was more prominent in rapeseed parents. For example, 84.43% and 99.80% of total glucosinolate content were aliphatic glucosinolates; 89.94% and 84.43% of total aliphatic glucosinolates were 4C aliphatic glucosinolates, and 70.81% and 60.27% of 4C aliphatic glucosinolates were PRO for the semi-winter rapeseed parents M29 and Zhongyou 821, respectively. In canola seeds, GBC constituted a large portion of total glucosinolate content. For example, GBC accounted for 57.58% and 53.19% of the total glucosinolate content for the spring canola parents M69 and 'Topas', respectively (Liu et al. 2016).

All of the rapeseed DH lines in the two populations over 81% of their total glucosinolate content comprised of aliphatic glucosinolates. In both DH populations, there were DH lines that had levels of PRO, 4C aliphatic glucosinolates, total aliphatic glucosinolate and

GBC content lower than their respective canola parents. In addition, very low glucosinolate content DH lines, with approximately 24% of the total glucosinolate content of the low glucosinolate parent M69 such as the DH line 95, and very high glucosinolate content lines, with a 3.5-fold increase in total glucosinolate content as compared to the high glucosinolate parent M29 such as the DH line 84, were observed.

Construction of Genetic Maps

Two genetic maps spanning 19 chromosomes in two *Brassica napus* DH populations were developed either using SRAP markers for the ZT population or using both SNP and SRAP markers for the M692 population (Table 2).

The genetic map for the M692 population was assembled from 8,344 SNPs and 61 SRAPs, covering 2,474 cM with an average density of 0.40 bins/cM. Among these SNP markers, 2,999 SNPs (35.94%) were assigned onto the A subgenome while 5,345 SNPs (64.06%), were placed onto the C subgenome. A genetic distance of 1,303 cM in the A subgenome was longer than the C subgenome with a genetic distance of 1,170.9 cM. The A subgenome had densities of 0.39 bins/cM whereas the C subgenome had densities of 0.40 bins/cM.

In the A subgenome of population M692, chromosome A3 had the most SNPs (491) while chromosome A8 had the fewest SNPs In the C subgenome of M692, chromosome C2 had the most SNPs (1,275) while chromosome C9 had the fewest SNPs.

There were some 'hotspots' that harbored large numbers of SNPs on both the A and C subgenomes. For example, 3.7% of the SNPs in the A subgenome were clustered in groups having more than 10 markers per bin with a range from 0.9% on chromosome A3 to 13.6% on chromosome A8. This phenomenon was more prominent in the C

subgenome than the A subgenome. A mean of 5.7% of the SNPs were clustered in groups containing more than 10 markers per bin with a range from 4.2% on chromosome C5 to 16.0% on chromosome C2. In general, 5.9% of SNPs were attributed to clusters containing 10 or more markers across the genome of M692.

Another phenomenon worth mentioning was that some stretches of chromosomes had very few or even entirely lacked SNP markers. For instance, out of the total 19 chromosomes, 15 lacked SNPs on their left ends excluding the 4 chromosomes A1, A6, A7, and C1 which started from their left telomeres. The gaps lacking SNPs on the left ends of these chromosomes measured 0.2 cM on A8, 0.3 cM on C5, 2.0 cM on A10, 7 – 9 cM on A5, A9 and C3, 10.7 – 19.6 cM on C7, C2, C4, C8 and C6, 24.4 cM on A3 and 28.3 cM on A2.

Gaps other than those on the left ends of the chromosomes were also observed. For example, all 19 chromosomes possessed gaps greater than 5 cM, with the number of gaps ranging from 1 on chromosomes A8 and C6 to 9 on chromosome A2, A6 and A9. The longest gap was on chromosome A8 measuring 34.4 cM.

QTLs Regulating Seed Glucosinolates

Population M692. Eighteen QTLs individually regulating PRO, GNP, GLS, GBN, GBC and their combinations were identified on 6 chromosomes: A3, A6, C2, C7, C8 and C9 from population M692 (Table 3; Figure 1). Five of the QTLs were located on the A subgenome. Chromosome A3 possessed three QTLs, one negatively controlling 5C aliphatic glucosinolates at 158.3 cM, one positively controlling glucosinolate at 33.2 cM and the last negatively controlling glucoalyssin GLS at 157.5 cM. Chromosome A6 had two QTLs negatively controlling GBC at both 113.0 cM and 123.7 cM. In addition to the

5 QTLs on the A subgenome, 13 QTLs were identified on the C subgenome. Chromosome C7 alone had 8 QTLs at either 97.9 cM or 100.0 cM in charge of positively controlling PRO, GNP, GLS, GBN (excluding GBC) and their combinations. Chromosome C9 possessed three QTLs: two QTLs at 101.0 cM positively controlling GBN and negatively controlling GBC and one QTL at 104.7 cM positively controlling 5C aliphatic glucosinolates. There was one QTL on each of the chromosomes C2 (16.4 cM) and C8 (114.7 cM) positively controlling GLS and negatively controlling 4C aliphatic glucosinolates. Chromosome C7 harbored not only the most QTLs, but also the most important QTLs. For example, the most influential 8 out of the total 18 QTLs identified were located on chromosome C7. These QTLs were responsible for phenotypic variances ranging from 32.62% to 57.39%.

A subset of the 18 QTLs regulating glucosinolate components, including their combinations, shared 95% of their confidence intervals (CI). This highly suggests that they may refer to the same QTLs. For example, the 8 QTLs *Bna6G1-1* to *Bna6G1-8* on chromosome C7 shared a CI of 1.5 cM from *Bna6G1-4* and *Bna6G1-5* at 96.5 cM to *Bna6G1-6* at 98.0 cM, so they might be the same QTL regulating the 8 glucosinolate traits excluding GBC. The QTLs *Bna6G3-1* and *Bna6G3-2* on chromosome A3 shared a CI of 4.9 cM from 155.4 cM to 160.3 cM that regulated GLS and 5C aliphatic glucosinolates. The QTL *Bna6G4-1* to *Bna6G4-3* on chromosome C9 had no left border for their CI and shared their right borders of CI at 106.5 cM, and regulated GBN, 5C aliphatic glucosinolates and GBC. The QTL *Bna6G5-1* and *Bna6G5-2* on chromosome A6 shared a CI of 21.4 cM and regulated GBC.

The QTLs on chromosome C7 had significant effects on the major aliphatic glucosinolate content in this relatively low glucosinolate population. The QTLs on chromosome C9 and A6 had minor effects on GBC.

Population ZT. Twenty five QTLs were identified on 6 chromosomes: A3, A9, C3, C5, C7 and C9. These QTLs individually regulated PRO, GNP, GBN and GBC, along with their combinations (Table 3; Figure 2). Chromosome A9 harbored a majority of the QTLs (11 out of the 25) as well as the most important QTLs (the top 5 significant QTLs). The QTL at 2.5 cM had a positive effect on GBC while the QTL at 23.2 cM had a negative genetic effect on GBC. The QTLs at 32.0 cM positively regulated 4C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content. The QTL at 40.1 cM positively regulated PRO. The QTLs at 41.8 cM positively regulated GNP, GBN, 4C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content.

There were 5 QTLs on each of chromosomes C3 and C9 which had positive genetic effects at 17.6 cM, 20.0 cM, 22.0 cM and 23.0 cM on C3 controlling PRO, total aliphatic glucosinolates, total glucosinolate content and GNP; at 22.8 cM, 33.3 cM, 38.9 cM and 44.4 cM on C9 controlling GBC, GBN and 5C aliphatic glucosinolates.

There were two QTLs at 9.8 cM on chromosome C5 that negatively regulated total aliphatic glucosinolate and total glucosinolate content, one on each of chromosome A3 and C7 positively controlling PRO.

Likewise, in population ZT, QTLs *BnaZG4-1* to *BnaZG4-9* on chromosome A9 shared a CI spanning 4.2 cM from 39.2 cM (*BnaZG4-8* and *BnaZG4-9*) to 43.4 cM (*BnaZG4-4*) and regulated PRO, GNP, 4C aliphatic glucosinolates, GBN, total aliphatic glucosinolates and total glucosinolate content. The QTLs *BnaZG5-1* to *BnaZG5-5* on chromosome C3

shared a CI of 7.6 cM, originating at 17.4 cM and terminating at 25.0 cM and regulated PRO, GNP, total aliphatic glucosinolates and total glucosinolate content. The QTL *BnaZG6-1* and *BnaZG6-2* shared a CI of 6.6 cM spanning from 8.4 cM to 15.0 cM which regulated total aliphatic glucosinolates and total glucosinolate content. The QTLs *BnaZG10-1* to *BnaZG10-3* shared a CI of size 7.9 cM extending from 35.0 cM to 42.9 cM and regulated GBN, 5C aliphatic glucosinolates and GBC.

Discussion

In *B. napus*, seed is the major sink to store glucosinolates (EFSA 2008; Velasco et al. 2008). In the present research five glucosinolates were identified as major glucosinolate components in *B. napus* seed. Among these five glucosinolates, the predominant forms presented themselves as progoitrin in 4C aliphatic glucosinolates, 4C aliphatic glucosinolates in total aliphatic glucosinolates, and total aliphatic glucosinolates in total glucosinolate content. This confirms the results obtained by Liu et al. (2016) and agrees with the observations of Velasco et al. (2008). However, when total glucosinolate content is low, total aliphatic glucosinolate levels diminish in importance due to indolic glucosinolate glucobrassicin contributed more to total glucosinolate content. For example, when total glucosinolate content was below 3.4 μmol/g seed in population M692 or 4.6 μmol/g seed in population ZT, there were 7 and 6 DH lines having total aliphatic glucosinolate content amounting to below 40% of the total glucosinolate content in the two populations, respectively. The percentages of total aliphatic glucosinolates to total glucosinolate content for the two canola parents in population M692 and ZT were 41.70% and 47.46%, respectively, whereas the canola parent in the population M730 was 48.03%

(Liu et al. 2015). This indicates that selection against seed aliphatic glucosinolates in canola breeding has been quite successfully implemented for decades, glucobrassicin might not be the major target to be selected against in the past or it might be difficult to be decreased as described by Rahman et al. (2014).

In the present study, two out of the 83 DH lines in the M692 population had lower total glucosinolate content and lower glucobrassicin than the canola parent M69. Also, two out of 121 DH lines had lower glucosinolate content than the canola parent in the ZT population. This suggests that the genes in canola can contribute to increased glucosinolate content through epistatic interaction of genes from both parents. The genotype of *Brassica* plants is a critical factor in influencing glucosinolate content (Johansen et al. 2016). Therefore, it is desirable to find recombinant lines containing low aliphatic glucosinolates, low glucobrassicin, or low total glucosinolate content (Brand et al. 2007; Fu et al. 2015; Lu et al. 2014).

QTLs controlling total seed glucosinolate content have been identified in *Brassica* crops. Rahman et al. (2014) reported QTLs controlling glucosinolates on chromosomes A2, A7 and A9 with the QTL located at the left end on A9 having the most significance in *B. rapa*. Ramchiary et al. (2007) identified two major QTLs controlling glucosinolates (Collard et al. 2005) on chromosomes A3 and A9 in *B. juncea*. Howell et al. (2003) and Li et al. (2014) independently located four QTLs regulating glucosinolates on chromosomes A9, C2, C7 and C9 in *B. napus*. Aforementioned studies were conducted either using bi-parental mapping populations or diversified inbred lines. For example, Liu et al. (2016) confirmed the major QTLs controlling total glucosinolate content and other glucosinolate traits at 44.3 cM – 55.4 cM on chromosome A9 from a *B. napus* bi-parental

DH population. In the present study, chromosome A9 in the high glucosinolate population ZT harbored the most significant QTLs, as well as the greatest number when compared to any other chromosomes. At least one major QTL within the interval of 32.0 cM – 41.8 cM on chromosome A9 was responsible in controlling 4C aliphatic glucosinolates, total aliphatic glucosinolates, total glucosinolate content and progoitrin. On chromosome A9 there also existed one major QTL at 2.5 cM that controlled glucobrassicin, one minor QTL (Collard et al. 2005) positioned at 41.8 cM controlled both gluconapin and glucobrassicanapin and one minor QTL at 23.2 cM controlled glucobrassicin. All the results from several previous and current reports suggest QTLs on chromosome A9 have major contributions to high glucosinolate contents, especially seed glucosinolates. Based on the analysis of whole *B. rapa* genome sequence, over a dozen genes have been identified on chromosome A9 and some of them may correspond to the mapped QTLs on A9.

Within population ZT, there was one major QTL located at 22.0 cM controlling gluconapin on chromosome C3, one major QTL at 67.7 cM controlling progoitrin on chromosome C7, at least three major QTLs at 22.8 cM – 44.4 cM controlling glucobrassicanapin, 5C aliphatic glucosinolates and glucobrassicin on chromosome C9, and at least five minor QTLs controlling six glucosinolate traits located on chromosomes A3, A9, C3 and C5. Although the distance between the major QTLs controlling progoitrin, glucobrassicanapin, 4C aliphatic glucosinolates, total aliphatic glucosinolates and the total glucosinolate content was only 2.3cM – 7.3 cM in both populations ZT and M730 (Liu et al. 2016), there were some differences between the QTLs in the two populations. The QTLs controlling glucoalyssin were located on chromosome A1 and A9

in population ZT, but no such QTL controlling this trait was able to be found in population M730. In addition, the number of QTLs controlling most of the glucosinolate traits as well as the chromosomes on which they were located was different. Therefore, different *B. napus* populations may have different QTL profiles controlling glucosinolate traits despite possibly sharing the same major QTLs as reported by Quiroz and Mithen (1996).

Compared to the germplasm used in most glucosinolate studies, M692 employed in the present study is a relatively low glucosinolate population as a result of its two low glucosinolate parents, the canola line M69 containing 6.6 μmol/g seed and the rapeseed line M29 containing 21.2 μmol/g seed of total glucosinolate content. Quiroz and Mithen (1996) reported that differences in total glucosinolate content arose from differential QTL expression. Therefore, any QTLs found on population M692 would be more valuable in practical canola breeding. The QTL profile in population M692 was quite different from those in populations ZT and M730. Chromosome C7 of population M692 harbored all of the major QTLs controlling aliphatic and total glucosinolate content. These QTLs had highly significant genetic effects contributing to 32.62% - 57.39% of the phenotypic variance. In addition, most of these QTLs resided on the C subgenome.

Transgressive DH lines with distinct levels of total and major glucosinolate components from their parental lines were found in the high glucosinolate population ZT and low glucosinolate population M692. This suggests that complementary effects from both additive and epistatic QTL functions controlled glucosinolate accumulation in *B. napus* seed, as reported by Howell et al. (2003) and Rahman et al. (2014).

Pleiotropic effects of the QTLs controlling seed glucosinolates were observed from both populations ZT and M692 in this study. This was also reported in a *B. napus* DH population M730 by Liu et al. (2016). In addition, pleiotropic effects of QTLs controlling glucosinolates have also been reported in *B. oleracea* by Sotelo et al. (2014), in *A. thaliana* by Kliebenstein et al. (2001), in *B. juncea* by Ramchiary et al. (2007), and in *B. napus* by Feng et al. (2012) and Hirani et al. (2012). This indicates the complexity of the genetic mechanism regulating seed glucosinolates in *Brassica* plants.

Acknowledgements

This research was funded by Monsanto Canada Inc. and the National Sciences and Engineering Research Council of Canada.

Table 1. Glucosinolates (μmol/g Seed) of the *Brassica napus* Doubled Haploid Populations M692 and ZT*

Pop	Content	μmol/g seed									%			
		PRO	GNP	4C	GLS	GBN	5C	T-Ali	GBC	TGC	PRO /4C	4C/ T-Ali	T-Ali /TGC	GBC /TGC
M692	Min	0	0	0	0	0	0	0	0.5	1.6	55.70	68.15	0	1.52
	Max	54.9	14.0	62.4	9.9	10.4	19.0	73.0	4.5	75.1	100	100	97.20	100
	#DH lines†	10	8	9	18	23	22	9	82	17	-	-	-	-
	M69‡	1.7	0.6	2.3	0.3	0.2	0.5	2.7	3.8	6.6	73.91	85.19	40.91	57.58
	M29‡	11.4	4.7	16.1	0.5	1.3	1.8	17.9	3.2	21.2	70.81	89.94	84.43	15.09
ZT	Min	0	0	0	0	0	0	0.3	0	0.3	2.98	0	8.11	0
	Max	65.5	56.2	91.8	15.6	24.4	34.9	112.9	4.0	113.7	90.00	100	100	91.89
	#DH lines†	7	7	7	7	5	5	7	90	7	-	-	-	-
	Topas‡	1.5	0.4	1.9	0.2	0.1	0.3	2.2	2.5	4.7	78.95	86.36	46.81	53.19
	Zhongyou821‡	49.3	32.4	81.8	4.4	10.8	15.2	97.0	0.3	97.2	60.27	84.33	99.80	0.31

*: PRO: progoitrin; GNP: gluconapin; 4C: four carbon aliphatic glucosinolates; GLS: glucoalyssin; GBN; glucobrassicanapin; 5C; five carbon aliphatic glucosinolates; T-Ali: total aliphatic glucosinolates; GBC: glucobrassicin; TGC: total glucosinolate content.

†: Number of DH lines having glucosinolate content lower than their canola parent.

‡: Parental lines.

Table 2. Parameters of the Genetic Map of a *Brassica napus* Doubled Haploid Population M692 Using Single Nucleotide Polymorphism (SNP) and Sequence-Related Amplified Polymorphism (SRAP) Markers

Chromosome	#Bin	#SNP	#SRAPs	GD[a]	Bins/cM
A1	64	340	2	130.6	0.49
A2	41	237	8	158.9	0.26
A3	62	491	6	162.5	0.38
A4	60	300	0	99.6	0.60
A5	40	366	3	137.8	0.29
A6	68	230	6	181.6	0.37
A7	54	331	2	124.5	0.43
A8	25	220	0	68.9	0.36
A9	46	258	3	146.0	0.32
A10	39	226	1	92.8	0.42
Subgenome A	499	2,999	31	1303.2	0.39
C1	49	834	5	135.8	0.36
C2	35	1275	0	131.4	0.27
C3	74	807	4	186.6	0.40
C4	71	612	5	166.7	0.43
C5	42	202	1	136.3	0.31
C6	34	180	8	71.4	0.48
C7	68	832	2	144.0	0.47
C8	51	454	5	135.7	0.38
C9	28	149	0	63.0	0.44
Subgenome C	452	5,345	30	1170.9	0.40
Genome AC	951	8,344	61	2474.1	0.40

[a]: Genetic distance in cM

Table 3. Quantitative Trait Loci (QTLs) Regulating Glucosinolates in Seed of the *Brassica napus* Doubled Haploid Populations M692 and ZT Using Sequence-Related Amplified Polymorphic or Single Nucleotide Polymorphic Markers*

Pop	Ch	Mk	QTL	Peak	CI	LB	RB	LM	RM	R^2	GSL
M692	C7	36	*Bna6G1-1*	97.9	6.5	96.0	102.5	94.6	98.4	0.5375	4C
	C8	32	*Bna6G2*	114.7	12.4	104.5	116.9	112.7	115.4	0.0756	4C
	C7	38	*Bna6G1-2*	100.0	6.4	96.3	102.7	98.4	101.5	0.3657	5C
	C9	2	*Bna6G4-1*	104.7	-	-	107.5	101.0	107.7	0.0673	5C
	A3	40	*Bna6G3-1*	158.3	4.9	155.4	160.3	155.5	159.0	0.1047	5C
	A6	50	*Bna6G5-2*	123.7	21.4	112.6	134.0	120.3	127.2	0.1286	GBC
	A6	48	*Bna6G5-1*	113.0	21.4	112.6	134.0	111.3	120.3	0.1252	GBC
	C9	1	*Bna6G4-2*	101.0	-	-	106.8	-	104.7	0.1198	GBC
	C7	36	*Bna6G1-3*	97.9	9.5	92.9	102.4	94.6	98.4	0.3815	GBN
	C9	1	*Bna6G4-3*	101.0	-	-	106.5	-	104.7	0.0714	GBN
	C7	38	*Bna6G1-4*	100.0	6.2	96.5	102.7	98.4	101.5	0.3262	GLS
	C2	1	*Bna6G7*	16.4	-	-	26.6	-	20.2	0.0867	GLS
	A3	4	*Bna6G6*	33.2	4.6	29.9	34.5	28.4	34.6	0.0683	GLS
	A3	39	*Bna6G3-2*	157.5	12.6	147.7	160.3	153.9	158.2	0.1278	GLS
	C7	36	*Bna6G1-5*	97.9	6.2	96.5	102.7	94.6	98.4	0.5625	GNP
	C7	36	*Bna6G1-6*	95.9	2.9	95.1	98.0	94.6	98.4	0.5083	PRO
	C7	36	*Bna6G1-7*	97.9	6.2	96.3	102.5	94.6	98.4	0.5739	T-Ali
	C7	36	*Bna6G1-8*	97.9	6.2	95.6	101.8	94.6	98.4	0.4983	TGC
ZT	A9	8	*BnaZG4-1*	32.0	13.1	31.6	44.7	31.0	32.5	0.1852	4C
	A9	15	*BnaZG4-5*	41.8	6.0	37.8	43.8	40.9	43.3	0.1731	4C
	C9	16	*BnaZG10-1*	38.9	7.9	35.0	42.9	37.9	39.3	0.1343	5C
	C9	12	*BnaZG9*	33.3	4.1	32.5	36.6	32.3	33.9	0.1803	GBC
	C9	6	*BnaZG8*	22.8	2.8	22.5	25.3	18.9	24.1	0.1745	GBC
	A9	2	*BnaZG2*	2.5	5.1	1.3	6.4	0.0	8.5	0.138	GBC
	C9	19	*BnaZG10-3*	44.4	22.2	22.4	44.6	43.4	45.1	0.1228	GBC
	A9	6	*BnaZG3*	23.2	13.0	17.0	30.0	17.0	27.8	0.0886	GBC
	C9	16	*BnaZG10-2*	38.9	15.8	28.8	44.6	37.9	39.3	0.1033	GBN
	A9	15	*BnaZG4-6*	41.8	12.9	31.4	44.3	40.9	43.3	0.0957	GBN
	C3	7	*BnaZG5-4*	22.0	7.6	17.4	25.0	21.0	25.5	0.1167	GNP
	A9	15	*BnaZG4-7*	41.8	10.5	33.1	43.6	40.9	43.3	0.0874	GNP
	A9	13	*BnaZG4-4*	40.1	5.6	37.8	43.4	38.7	40.9	0.1653	PRO
	C7	25	*BnaZG7*	67.7	15.0	56.6	71.6	66.7	73.2	0.1114	PRO
	C3	5	*BnaZG5-1*	17.6	13.6	11.6	25.2	16.6	19.0	0.0952	PRO
	C3	7	*BnaZG5-5*	23.0	13.8	11.4	25.2	21.0	25.5	0.0949	PRO
	A3	1	*BnaZG1*	3.0	-	-	11.1	0.0	4.7	0.0879	PRO
	A9	15	*BnaZG4-8*	41.8	4.5	39.2	43.7	40.9	43.3	0.2011	T-Ali
	A9	8	*BnaZG4-2*	32.0	15.7	31.7	47.4	31.0	32.5	0.1901	T-Ali
	C3	6	*BnaZG5-2*	20.0	19.5	9.8	29.3	19.0	21.0	0.0871	T-Ali
	C5	4	*BnaZG6-1*	9.8	6.7	8.4	15.1	9.7	15.6	0.0722	T-Ali
	A9	15	*BnaZG4-9*	41.8	4.5	39.2	43.7	40.9	43.3	0.1996	TGC
	A9	8	*BnaZG4-3*	32.0	15.4	31.7	47.1	31.0	32.5	0.1917	TGC
	C3	6	*BnaZG5-3*	20.0	20.0	9.8	29.8	19.0	21.0	0.086	TGC
	C5	4	*BnaZG6-2*	9.8	6.6	8.4	15.0	65.0	71.7	0.0721	TGC

*: Ch, chromosome; Mk, marker; Peak, QTL position in cM from left telomere; CI, 95% confidence interval of the QTL; LB, left border of CI, RB, right border of CI; LM, left marker position in cM; RM, right marker position in cM; GSL, glucosinolate; PRO, progoitrin; GNP, gluconapin; 4C, four-carbon aliphatic glucosinolates; GBN, glucobrassicanapin; 5C, five-carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content.

Table S1. Sequence-Related Amplified Polymorphic Primer Pairs in PCR for the *Brassica napus* Doubled Haploid Population M692

Forward Primer	Dye	Reverse Primer	Number of Markers
Odd	FAM	BG4	1
		BG10	1
		BG11	1
		BG23	1
		BG29	1
		BG32	1
		BG33	1
		BG41	1
		BG54	1
		BG56	1
		BG68	1
		BG69	1
EM	VIC	BG4	1
		BG11	1
		BG23	1
		BG41	1
		BG44	1
		BG77	1
		BG80	1
		BG93	1
GA		BG5	1
		BG10	1
		BG11	1
		BG12	1
BG23	NED	BG4	1
		BG11	1
		BG14	1
		BG25	1
		BG31	1
		BG33	1
		BG66	1
		BG67	1
		BG68	1
		BG69	1
		BG70	1
		BG89	1

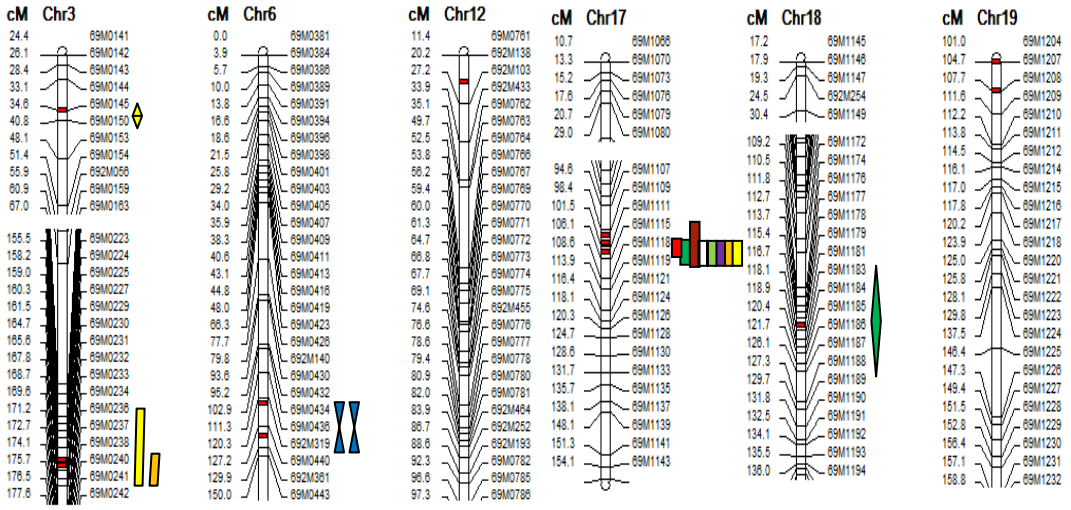


Figure 1. QTLs Regulating Seed Glucosinolates Identified in a *Brassica napus* Doubled Haploid Population M692*

*:Red bars on the chromosomes indicate peak locations of QTLs. Rectangle bar, diamond, parallelogram, double-sided border bar, two triangle, diamond with an across, ellipse are QTL 1-7. Dark green, four-carbon aliphatic glucosinolates; Orange, five-carbon aliphatic glucosinolates; Yellow, glucoalyssin; Red, progoitrin; Blue, glucobrassicin; Dark orange, glucobrassicanapin; Light green, total aliphatic glucosinolates; Purple, total glucosinolate content; and White, gluconapin. The lengths of various shapes specify their 95% of confidence intervals of QTLs.

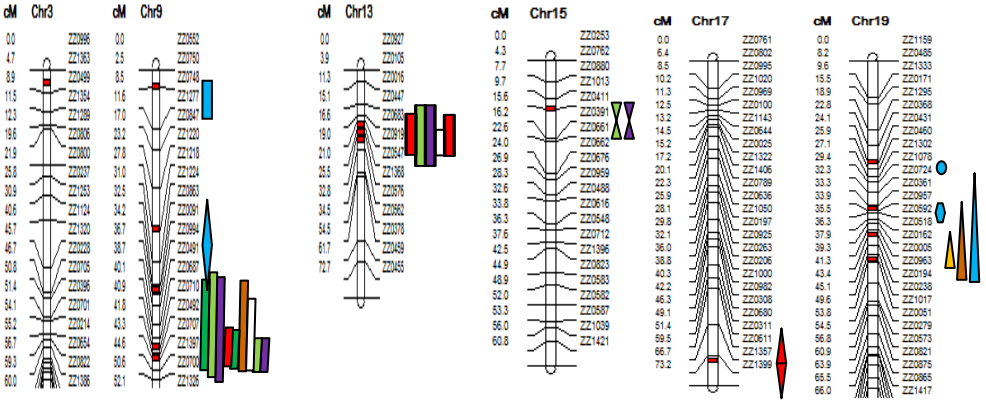


Figure 2. QTLs Regulating Seed Glucosinolates Identified in a *Brassica napus* Doubled Haploid Population ZT*

*:Red bars on the chromosomes indicate peak locations of QTLs. Rectangle bar, diamond, parallelogram, double-sided border bar, two-triangle, diamond with an across, ellipse, hexagon and triangle are QTL 2-10. Dark green, four-carbon aliphatic glucosinolates; Orange, five-carbon aliphatic glucosinolates; Red, progoitrin; Blue, glucobrassicin; Dark orange, glucobrassicanapin; Light green, total aliphatic glucosinolates; Purple, total glucosinolate content; and White, gluconapin. The lengths of various shapes specify their 95% of confidence intervals of QTLs.

CHAPTER 3

Quantitative Trait Loci Controlling Sclerotinia Stem Rot Resistance in *Brassica napus* L.

Jun Liu[2], Xueping Liu[1], Ravneet S. Behla[1], Arvind Hirani[1], Zhe Li[2], Chunren Wu[2], Peter B.E. Mcvetty[1] and Genyi Li[1*]

[1]*University of Manitoba, Plant Science, 66 Dafoe Rd., Winnipeg, MB, Canada R3T 2N2*

[2]*Monsanto Canada Inc., 900-One Research Rd., Winnipeg, MB, Canada R3T 6E3*

*Correspondence to: Genyi Li. E-mail: Genyi.Li@umanitoba.ca

Abstract

Sclerotinia stem rot is one of the major threats for canola/rapeseed production worldwide. The efficient and long-term solution to minimize crop losses from the causal fungus *Sclerotinia sclerotiorum* comes from the built-in resistance in crops which, in turn, is dependent upon the understanding of genetic mechanisms controlling plant defensive reactions. In the present study, we used a parent with partial resistance to *Sclerotinia* stem rot and a relatively susceptible parent to produce doubled haploid lines. These double haploid lines were inoculated with *S. sclerotiorum* mycelium plugs placed on adult plant stems under controlled environmental conditions. Sequence related amplified polymorphism technology was used to assembly a genetic linkage map where all 19 linkage groups were assigned to their corresponding chromosomes using single nucleotide polymorphism (SNP) and microsatellite markers (SSR). The 19 linkage groups contained 778 polymorphic markers covering a genetic distance of 1,731.58 centiMorgans (cM) with marker densities ranging from 0.21 to 0.74 per cM with an average of 0.45 markers per cM. Thirteen quantitative trait loci (QTLs) were identified on chromosomes A3, A7, C3 and C6. Three common QTLs located on A3, A7 and C3 explained about 10% of total phenotypic variances for different genetic effects affecting resistance to *Sclerotinia* stem rot. The flanking markers from the common QTLs identified in this study could be used for marker assisted selection in breeding programs or for fine mapping *Sclerotinia* resistance genes.

Keywords: *Brassica napus*, *Sclerotinia sclerotiorum*, *Sclerotinia* stem rot resistance, genetic map, QTL mapping.

Introduction

Sclerotinia stem rot, caused by the necrotrophic fungus *S. sclerotiorum* (Lib.) de Bary, is a common disease in canola/rapeseed growing areas globally (Saharan and Mehta 2008). Due to the aggressiveness (Boland and Hall 1994), the longer survival ability (Coley-Smith 1979; Coley-Smith and Cooke 1971) and the infectious characteristics of the fungus, chemical, cultural, biological and ecological control measures are either expensive or impractical. Breeding *Sclerotinia* resistant cultivars should be the most effective, efficient and sustainable solution to minimize *Sclerotinia* disease risks. However, the lack of understanding of inheritance of *Sclerotinia* resistance greatly hinders the efforts to breed *Sclerotinia* resistant commercial canola cultivars, especially since there is no complete resistance donor source available in *B. napus* and/or its relatives. In addition to seeking more *Sclerotinia* resistant donors (Ding et al. 2013; Li et al. 2009; Mei et al. 2013) researchers have tried to elucidate plant defensive mechanisms against this pathogen from a genomic perspective. In *B. napus*, Zhao and Meng (2003a) used a combination of restricted fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) SSR and randomly amplified polymorphic DNA (RAPD) markers to identify three QTLs for *Sclerotinia* leaf resistance at the seedling stage and another three QTLs for *Sclerotinia* stem resistance at the adult stage in a bi-parental $F_{2:3}$ population. Each of the two sets of QTLs explained collectively 40.7% and 49.0% of phenotypic variation, respectively. In another study, Zhao and Meng (2003b) reported that two QTLs, one regulating aliphatic glucosinolates and another regulating indolic glucosinolates, were associated with leaf and stem resistance to *Sclerotinia* at the seedling and adult stages, respectively. Zhao et al. (2007) used whole genomic

microarray from Arabidopsis to study gene expression of a partially resistant and a susceptible accession challenged by *Sclerotinia* pathogen and discovered that hundreds of genes were involved in the regulation of *Sclerotinia* infections. Wu et al. (2013) used SSR markers and identified 10 and three QTLs for *Sclerotinia* stem and leaf resistance, respectively, on 9 linkage groups (LGs). One of two major QTLs was related to the gene At1g76790 in Arabidopsis. Other researchers also contributed to the reports of QTLs related to *Sclerotinia* resistance in canola/rapeseeds (*B. napus*) (Yin et al. 2010; Zhao et al. 2004; Zhao et al. 2006), in *Brassica* vegetables (*B. oleracea*) (Disi et al. 2014; Mei et al. 2013), in sunflower (*Helianthus annuus* L.) (Micic et al. 2005; Rönicke et al. 2005; Yue et al. 2008), and in soybean (*Glycine max* L.) (Li et al. 2010). In the present study, a DH population developed from a cross of a semi-winter cultivar 'Zhongyou 821' which has partial resistance to *Sclerotinia* and a *Sclerotinia* susceptible spring canola cultivar 'Topas'. All DH lines were challenged by *S. sclerotiorum* in replicated tests grown in a greenhouse. SRAP markers were used to perform QTL mapping for the objective to identify QTLs controlling *Sclerotinia* stem rot in *B. napus*.

Materials and Methods

Plant Materials

The semi-winter cultivar 'Zhongyou 821', which has partial resistance (also known as field resistance) to *Sclerotinia* (Zhao et al. 2009), was used as the female parent pollinated by the *Sclerotinia* susceptible spring canola cultivar 'Topas' to produce F_1 seeds. DH lines were generated using the microspore culture method of described by

Weber et al. (Weber et al. 2005). A total of 99 DH lines comprised the DH line population used in this study.

Growing Conditions

Individual DH line plants were grown in 10 cm plastic pots filled with #4 peat soil (Sun Gro Horticulture, Canada) supplied with 5 g of slow-released fertilizer (20-20-20, Plant-Prod®, ON, Canada) at the seedling stage. Seven plants per DH line were grown in greenhouses at the University of Manitoba in the fall of 2009 and the spring of 2010. The DH line plants along with parents were grown using a completely randomized design in both 2009 and 2010. The greenhouses were maintained at 22/16 °C with supplementary lighting used to maintain a 16/8 h day/night.

***S. sclerotiorum* Disease Testing**

Sclerotia harvested from canola plants grown at Carman, Manitoba in 2003 were used to propagate inoculum. Potato glucose agar (PGA) 4:20:15 (*g/L*), Fluka™ Analytical, Spain was used as the culturing media. One PGA plug (0.8mm in diameter) per plant, infested with *Sclerotinia* mycelia on the surface of one side, were attached to the internode of the stem 20 to 30 cm above the soil with the mycelium-infested side of each PGA plug facing the stem and wrapped up with Parafilm® (Neenah, WI, USA) at the flowering stage. Ninety-nine lines from the DH population were challenged by the *S. sclerotiorum* pathogen along with a control spring canola cultivar 'Westar' and the two parental lines. Lesion lengths were measured in cm at three weeks post-inoculation. All lesion length data points were screened for statistical outliers due to inoculation failures or extreme infections.

DNA Extraction

Five hundred mg samples from fresh leaves at the 4 or 5-leaf stages were taken from each DH line. DNA was extracted according to a cetyltrimethyl ammonium bromide (CTAB) method (Li and Quiros 2001) with a minor modification whereby a half volume of iso-propanol of supernatant was added to precipitate DNA.

Molecular Marker Analysis and Chromosome Localization

One hundred and sixty-eight SRAP primer pairs selected from 11 fluorescently-labeled forward primers including FAM (blue), NED (yellow), PET (red) and VIC (green), and 89 reverse primers were used (Table 1 and Table S1). The SRAP PCR program was: 94 °C for 4 min, five cycles at 94 °C for 55 s, 35 °C for 55 s, 72 °C for 55 s, followed by 30 cycles at 94 °C for 55 s, 50 °C for 55 s and 72 °C for 55 s. The SSR PCR program was: 94 °C for 4 min, five cycles at 94 °C for 55 s, 60 °C with -0.8 °C after each cycle for 55 s, 72 °C for 55 s, followed by 30 cycles at 94 °C for 55 s, 55 °C for 55 s and 72 °C for 55 s. PCR products were separated on a ABI Genetic Analyzer (3130xl Genetic analyzer, Life Technologies, USA), data were analyzed by the ABI GeneScan 3.7 software (Life Technologies, USA) and scored by Genographer software. A genetic map was assembled using JoinMap® 3.0 (Van Ooijen and Voorrips 2001).

Common SRAP markers as those detected by Sun et al. (2007) and genetic maps with SRAP and SNP markers (unpublished data) were aligned with chromosomes. Some of the linkage groups (LGs) were also assigned onto chromosomes using SSR markers (Table S2).

QTL Mapping

WinQTLCart v2.5_009 (NCSU, USA) was used to locate QTLs. The mapping method used was Composite Interval Mapping with 1,000 permutations at significance level of 0.05. The calculation method used was Model 6 with Kosambi function, walk speed at 1 cM and a window size of 10 cM. Five control markers and backward regression were chosen. A LOD (logarithm of odds) score was set at 2.5 (Yin et al. 2010) for the declaration of putative QTLs.

Statistical Analyses

PROC MIXED from SAS v9.3 (SAS Institute Inc., NC, USA) was used as the analysis method. Two-way ANOVA with two categorical groups, genotype with 99 levels and time with 2 levels was used as the analysis model (Table 2).

Results

Phenotyping

Sclerotinia lesion spread up and down from the inoculation sites. The lesion lengths for the 99 DH lines along with the DH line parents were measured (Table S3). 'Zhongyou 821', the *Sclerotinia* partial resistant parent, had an average lesion length of 2.94 cm. In contrast, the susceptible parent 'Topas' had an average lesion length of 21.20 cm. There were two DH lines which had shorter lesion lengths than 'Zhongyou 821' while 17 DH lines had longer lesion lengths than 'Topas'. Lesion lengths for the 99 DH lines ranged from 1.68 cm to 30.22 cm with a grand mean of 15.87 cm and a median of 15.61 cm.

The ANOVA analysis indicated that genotypic effects and the interaction between genotypic and replicate effects were significant while replicate effects were not statistically significant (Table 2 and Table S4).

Genotyping

Seven hundred and seventy-eight mapped polymorphic SRAP markers were generated from 169 SRAP primer pairs. The fewest number of markers developed from a single primer pair was one, the most 14 and the mean 4.6 (Table S1). All SRAP markers were assembled onto 19 LGs and then assigned onto 19 chromosomes by aligning common SRAP and SSR markers to reference genetic maps. The genetic map covered 1 731.58 cM. Marker densities ranged from 0.21 to 0.74 markers per cM with a mean of 0.45.

QTL Identification

Thirteen QTLs were identified, three on chromosome A3, five on chromosome A7, four on chromosome C3 and one on C6 (Table 3). Three common QTLs were located on chromosomes A3, A7 and C3 explained 10.10%, 9.10% and 8.53% of total phenotypic variance, respectively. The common QTL on A3 had positive additive genetic effects while the other common ones on A7 and C3 had negative additive genetic effects. The closely linked flanking SRAP markers for the common QTLs would be useful for marker assisted selection in *B. napus Sclerotinia* resistant cultivar breeding.

The common QTL on A3 was identified by the marker 43 at 138.6 cM from the left telomere in both 2009 and 2010 with LOD values of 4.21 and 2.73, respectively. This common QTL contributed to the development of *Sclerotinia* lesions by having positive additive effects and explained on average 10.10% of phenotypic variance. Another QTL on A3 identified by the marker 42 at position 128.7 cM was only observed in 2010. This

QTL had negative genetic effect on *Sclerotinia* development even though the effect was minor, explaining only 7.66% of phenotypic variance. A7 harbored more QTLs with one common QTL flanked by the marker 59 at 117.5 cM and three others, by the marker 51 at 101.4 cM in 2009, the marker 53 at 104.7 cM in 2010 and the marker 55 at 106.7 in 2009. All five QTLs had negative additive genetic effects on *Sclerotinia*. There was one common QTL and two other QTLs found on C3. The common QTL was identified by the marker 9 at 32.8 cM. The other two QTLs on C3 were identified by the marker 7 at 22.0 cM and by the marker 10 at 45.5 cM. The common QTL and the QTL identified by the marker 10 had negative additive genetic effects while the QTL identified by the marker 7 had positive genetic effect. There was only one QTL identified on C6 by the marker 4 at 10.8 cM. This QTL had negative additive genetic effect.

Discussion

Diversely and widely distributed SRAP markers were obtained in the present study, similar to the results reported by Sun et al. (2007). This indicates the substantial potential of SRAP markers to explain detailed genetic mechanisms for traits of interest (Aneja et al. 2012). Since the donor parent for the *Sclerotinia* resistance trait was the same cultivar as used by Sun et al. (2007), the previous published ultradense genetic map was used as the reference for anchoring the linkage groups to their corresponding chromosomes. Some of the SSR markers from both Sun et al. (2007) and Piquemal et al. (2005) were also used to assign LGs to their corresponding chromosomes. In addition, the same SRAP markers (the same sized markers generated from the same primer pair) co-segregating with the *Brassica* 60 K Infinium® SNP markers in another study (unpublished data) were used to

localize markers to chromosomes based on the reproducibility of SRAP markers (Aneja et al. 2012).

Additive QTL effects were reported to be important factors involved in resistance to *S. sclerotiorum* in *B. napus* (Zhao and Meng 2003a). Additive genetic effects were observed in our DH population. However, the genetic effects of the QTLs identified in this study were generally low to medium which were similar to the previously reported data (Wei et al. 2014; Yin et al. 2010; Zhao et al. 2004; Zhao et al. 2006).

Three common QTLs on A3, A7 and C3 were consistently identified both in 2009 and 2010 (Figure 1a, 1b, 1c, 1d and Figure 2). These QTLs could be treated as major ones. The three QTLs identified by the markers 51, 53 and 55 on A7 were only 2-3 cM apart. All three QTLs had negative additive effects and followed the trend that R^2 increased when the markers moved from left to right. Therefore, the three might represent a common QTL which showed a peak at position circa 106 cM (Figure 1c).

Acknowledgement

This research was financially supported by The Natural Sciences and Engineering Research Council of Canada (NSERC), Monsanto Canada Inc., Bunge Canada, and DL Seeds Inc.

Table 1. Mapped Sequence-Related Amplified Polymorphism (SRAP) Markers for the Whole Genome from the DH Population of Zhongyou 821 x Topas of *B. napus*

Chromosome	Number of Markers	Genetic Distance (cM)	Markers /cM
A1	31	56.5	0.55
A2	25	45.3	0.55
A3	55	178.6	0.31
A4	55	114.8	0.48
A5	82	139	0.59
A6	42	70.1	0.60
A7	63	128.4	0.49
A8	19	70.1	0.27
A9	43	95.8	0.45
A10	27	57.0	0.47
Subgenome A	442	955.4	0.48
C1	34	102.7	0.33
C2	87	117.1	0.74
C3	15	72.7	0.21
C4	35	91.3	0.38
C5	29	60.8	0.48
C6	40	89.9	0.44
C7	26	73.2	0.35
C8	16	43.9	0.36
C9	54	124.5	0.43
Subgenome C	336	776.2	0.41
Genome AC	778	1731.6	-
μ	-	-	0.45

Table 2. ANOVA for *Sclerotinia* Lesion Length (cm) of 99 DH Lines of the Population of *B. napus* Derived from a Cross between Zhongyou 821 x Topas in Two Replicated Years*

Effect	Num DF	Den DF	F Value	Pr > F
Line	97	739	1.38	0.012
Year	1	739	0.31	0.58
Year*Line	98	739	1.38	0.012

1: numerator degree of freedom
2: denominator degree of freedom

Table 3. Quantitative Trait Loci Identified from the DH Population of Zhongyou 821 x Topas and Their Effects on *Sclerotinia* Stem Rot Disease in *B. napus* *

Chromosome	Marker	Position (cM)	LOD	Additive	R^2	TRT
A3	42	128.7	2.617	-1.8838	0.0766	2009
A3	43	138.6	4.205	2.4212	0.1201	2009
A3	43	138.6	2.7342	1.9705	0.0818	2010
A7	51	101.4	3.8792	-2.3645	0.1152	2009
A7	53	104.7	4.4751	-2.7762	0.1368	2010
A7	55	106.7	5.1725	-2.8672	0.1491	2009
A7	59	117.5	3.253	-2.2602	0.0995	2009
A7	59	117.5	2.5209	-2.0445	0.0824	2010
C3	7	22	3.9402	4.0102	0.3431	2010
C3	9	32.8	2.6377	-1.9791	0.0721	2009
C3	9	32.8	3.2762	-2.2891	0.0985	2010
C3	10	45.5	3.1823	-3.1157	0.1886	2010
C6	4	10.8	2.6512	-3.8928	0.3131	2010
Σ	-	-	-	-17.0712	-	

*: LOD, logarithm of odds; Additive, additive genetic effect; TRT, treatment.

Table S1. Sequence-Related Amplified Polymorphism (SRAP) Markers Developed from Fluorescently-Labeled Forward Primer and Unlabeled Reverse Primer Pairs in the DH Population of *B. napus* Developed from a Cross between Zhongyou 821 x Topas

Forward Primer	Fluorescence	Reverse Primer	Number of Markers
ALK	NED	CE26	3
ALK	NED	FC09	2
ALK	NED	PM29	3
ALK	NED	PM53	3
ALK	NED	PM60	1
BG23	NED	BG14	7
BG23	NED	BG18	2
BG23	NED	BG19	1
BG23	NED	BG32	5
BG23	NED	BG33	10
BG23	NED	BG34	6
BG23	NED	BG35	3
BG23	NED	BG37	8
BG23	NED	BG38	7
BG23	NED	BG39	3
BG23	NED	BG41	8
BG23	NED	BG44	7
BG23	NED	BG48	1
BG23	NED	BG60	5
BG23	NED	BG62	4
BG23	NED	BG66	8
BG23	NED	BG67	9
BG23	NED	BG68	3
BG23	NED	BG69	12
BG23	NED	BG70	9
BG23	NED	BG73	2
BG23	NED	BG80	2
BG23	NED	BG82	7
BG23	NED	BG88	2
BG23	NED	PM01	2
BG23	NED	PM04	2
BG23	NED	PM05	6
BG23	NED	PM118	6
BG23	NED	PM29	9
BG23	NED	PM30	1
BG23	NED	PM47	1
BG23	NED	PM56	3
BG23	NED	PM75	1
DC1	PET	BG01	2
DC1	PET	BG05	3
DC1	PET	BG33	3
DC1	PET	BG44	2
DC1	PET	BG48	8
DC1	PET	BG67	2
DC1	PET	BG70	2
DC1	PET	BG72	8
DC1	PET	BG73	3
DC1	PET	FE06	3
DC1	PET	FE08	1
DC1	PET	FE14	2
DC1	PET	MC01	3

DC1	PET	PM04	2
DC1	PET	PM103	1
DC1	PET	PM114	1
DC1	PET	PM18	2
DC1	PET	PM33	3
DC1	PET	PM34	1
DC1	PET	PM58	1
DC1	PET	PM66	6
DC1	PET	PM75	2
DC1	PET	PM77	3
DC1	PET	PM80	4
ELONG	PET	BG60	4
ELONG	PET	FE03	1
EM1	VIC	BG75	4
EM1	VIC	FE10	6
EM1	VIC	MC01	9
EM1	VIC	ODD13	4
FAD	FAM	BG6	4
FAD	FAM	BG60	5
FAD	FAM	BG68	10
FAD	FAM	BG69	10
FAD	FAM	BG94	5
FC1	FAM	BG10	5
FC1	FAM	BG11	13
FC1	FAM	BG12	5
FC1	FAM	BG13	2
FC1	FAM	BG29	6
FC1	FAM	BG31	11
FC1	FAM	BG32	5
FC1	FAM	BG33	1
FC1	FAM	BG34	2
FC1	FAM	BG35	1
FC1	FAM	BG37	3
FC1	FAM	BG38	6
FC1	FAM	BG39	3
FC1	FAM	BG40	3
FC1	FAM	BG41	9
FC1	FAM	BG43	5
FC1	FAM	BG45	4
FC1	FAM	BG48	6
FC1	FAM	BG53	10
FC1	FAM	BG55	3
FC1	FAM	BG56	4
FC1	FAM	BG59	3
FC1	FAM	BG60	9
FC1	FAM	BG67	5
FC1	FAM	BG68	4
FC1	FAM	BG69	2
FC1	FAM	BG73	7
FC1	FAM	BG76	4
FC1	FAM	BG80	6
FC1	FAM	BG86	8
FC1	FAM	BG93	3
FC1	FAM	FE03	3
FC1	FAM	PM04	3
FC1	FAM	PM05	4

FC1	FAM	PM06	4
FC1	FAM	PM09	5
FC1	FAM	PM103	4
FC1	FAM	PM114	6
FC1	FAM	PM29	9
FC1	FAM	PM47	5
FC1	FAM	PM55	5
FC1	FAM	PM75	5
GA3	VIC	FE01	4
GA3	VIC	FE31	9
GA3	VIC	PM53	5
PM88	PET	BG01	7
PM88	PET	BG02	4
PM88	PET	BG05	4
PM88	PET	BG18	2
PM88	PET	BG23	4
PM88	PET	BG25	1
PM88	PET	BG27	3
PM88	PET	BG31	2
PM88	PET	BG33	2
PM88	PET	BG67	3
PM88	PET	BG73	7
PM88	PET	FE22	2
PM88	PET	PM47	1
PRO	NED	BG45	2
PRO	NED	FC10	3
PRO	NED	FD03	4
PRO	NED	FE03	5
SA7	VIC	BG12	3
SA7	VIC	BG25	9
SA7	VIC	BG29	2
SA7	VIC	BG31	9
SA7	VIC	BG33	4
SA7	VIC	BG34	8
SA7	VIC	BG35	11
SA7	VIC	BG37	4
SA7	VIC	BG38	4
SA7	VIC	BG39	14
SA7	VIC	BG40	6
SA7	VIC	BG45	3
SA7	VIC	BG53	2
SA7	VIC	BG55	7
SA7	VIC	BG56	7
SA7	VIC	BG59	2
SA7	VIC	BG63	5
SA7	VIC	BG66	6
SA7	VIC	BG67	7
SA7	VIC	BG69	2
SA7	VIC	BG70	4
SA7	VIC	BG72	5
SA7	VIC	BG76	6
SA7	VIC	BG80	4
SA7	VIC	BG93	7
SA7	VIC	PM05	7
SA7	VIC	PM06	1
SA7	VIC	PM09	7

SA7	VIC	PM114	7
SA7	VIC	PM17	5
SA7	VIC	PM29	7
SA7	VIC	PM47	5
SA7	VIC	PM55	4
SA7	VIC	PM75	5
Σ	-	-	778
$\overline{x}$	-	-	4.6

Table S2. Sequence-Related Amplified Polymorphism (SRAP) Marker Alignment to Chromosomes of *B. napus* Genetic Map Developed from Sun et al. [26] and Simple sequence repeat markers

Pop	Marker	Mk (bp)	Marker bin position	FP	RP	Sun pairs	same	NT1	NT2	Chrom.	SRAP /SNP
M730	730W050	271		BG23	BG11	BG23-BG11-271	1	1		5	5
Sun	a02826	271	N05-21	BG23	BG11	BG23-BG11-271	1	1		5	5
M730	730W052	370		BG23	BG11	BG23-BG11-370	2	2			1
Sun	a00493	371	N01-35	BG23	BG11	BG23-BG11-371		2			1
M730	730W067	256		BG23	BG14	BG23-BG14-256	6	5		8	9
Sun	a05560	256	N08-10	BG23	BG14	BG23-BG14-256	6	5		8	9
LXP	ZZ0577	362		BG23	BG14	BG23-BG14-362	7				7
M730	730W068	362		BG23	BG14	BG23-BG14-362	7				7
Sun	a04807	187	N07-21	BG23	BG25	BG23-BG25-187		8			7
M730	730W082	188		BG23	BG25	BG23-BG25-188		8			7
M730	730W114	355		BG23	BG33	BG23-BG33-355	14	14			5
Sun	a04498	235	N07-01	BG23	BG4	BG23-BG4-235	20			7	7
Sun	a04500	235	N07-01	BG23	BG4	BG23-BG4-235	20	25		7	7
M730	730W040	236		BG23	BG4	BG23-BG4-236		25			7
LXP	ZZ0464	318		BG23	BG66	BG23-BG66-318	31				3
M730	730W124	318		BG23	BG66	BG23-BG66-318	31				3
LXP	ZZ0499	238		BG23	BG67	BG23-BG67-238	35				3
M730	730W134	238		BG23	BG67	BG23-BG67-238	35				3
Sun	a05817	357	N08-28	BG23	BG68	BG23-BG68-357		45			8
M730	730W150	359		BG23	BG68	BG23-BG68-359		45			8
M730	730W164	257		BG23	BG69	BG23-BG69-257					5
M730	730W185	312		BG23	BG89	BG23-BG89-312		58			7
M730	730W009	252		EM1	BG1	EM1-BG1-252	53			2	2
Sun	a00856	252	N02-21	EM1	BG1	EM1-BG1-252	53			2	2
Sun	a06146	335	n09-11	EM1	BG5	EM1-BG5-335					9
M730	730W037	337		EM1	BG5	EM1-BG5-337					9
M730	730W078	428		EM1	BG80	EM1-BG80-428	55			3	3
Sun	a01877	428	N03-45	EM1	BG80	EM1-BG80-428	55			3	3
M730	730W079	431		EM1	BG80	EM1-BG80-431	56	69		3	3
Sun	a01880	431	N03-45	EM1	BG80	EM1-BG80-431	56	69		3	3
M730	730W245	193		FC1	BG11	FC1-BG11-193					10
LXP	ZZ0546	203		FC1	BG11	FC1-BG11-203	57				4
M730	730W246	203		FC1	BG11	FC1-BG11-203	57				4
M730	730W247	243		FC1	BG11	FC1-BG11-243		73			7
M730	730W269	153		FC1	BG29	FC1-BG29-153		75			4
M730	730W273	431		FC1	BG29	FC1-BG29-431		77			9
M730	730W283	168		FC1	BG33	FC1-BG33-168	63				8
M730	730W293	156		FC1	BG35	FC1-BG35-156		78			10
M730	730W323	302		FC1	BG38	FC1-BG38-302		82			1
LXP	ZZ0293	165		FC1	BG41	FC1-BG41-165	66				4
M730	730W330	165		FC1	BG41	FC1-BG41-165	66				4
M730	730W335	374		FC1	BG41	FC1-BG41-374		84			7
M730	730W338	435		FC1	BG41	FC1-BG41-435	71				1
M730	730W377	107		FC1	BG55	FC1-BG55-107		90			2
M730	730W411	224		FC1	BG66	FC1-BG66-224		92			3
M730	730W412	316		FC1	BG66	FC1-BG66-316	86				10

M730	730W425	328		FC1	BG67	FC1-BG67-328			4
M730	730W426	343		FC1	BG67	FC1-BG67-343		94	7
M730	730W427	356		FC1	BG67	FC1-BG67-356		95	9
M730	730W438	133		FC1	BG68	FC1-BG68-133		96	9
M730	730W439	180		FC1	BG68	FC1-BG68-180		97	7
M730	730W209	414		GA3	BG13	GA3-BG13-414		100	7
M730	730W238	225		GA3	BG33	GA3-BG33-225	92		1
M730	730W060	359		Odd3	BG11	Odd3-BG11-359			7
M730	730W072	297		Odd3	BG23	Odd3-BG23-297		101	10
M730	730W117	360		Odd3	BG33	Odd3-BG33-360	95		4
M730	730W118	363		Odd3	BG33	Odd3-BG33-363	96		4
M730	730W034	245		Odd3	BG4	Odd3-BG4-245	97		2
M730	730W127	140		Odd3	BG41	Odd3-BG41-140	98		7
M730	730W142	333		Odd3	BG54	Odd3-BG54-333	101		2
M730	730W176	274		Odd3	BG69	Odd3-BG69-274		104	5
M730	730W305	248		SA12	BG23	SA12-BG23-248	108		7
M730	730W395	208		SA12	BG68	SA12-BG68-208		113	3
M730	730W267	158		SA7	BG13	SA7-BG13-158	109		3
Sun	a00421	356	N01-35	SA7	BG18	SA7-BG18-356		116	1
M730	730W286	357		SA7	BG18	SA7-BG18-357		116	1
Sun	a00422	465	N01-35	SA7	BG18	SA7-BG18-465		117	1
LXP	ZZ0117	208		SA7	BG32	SA7-BG32-208	120		9
M730	730W354	208		SA7	BG32	SA7-BG32-208	120		9
M730	730W364	233		SA7	BG33	SA7-BG33-233		129	2
M730	730W372	151		SA7	BG34	SA7-BG34-151			1
M730	730W404	273		SA7	BG37	SA7-BG37-273		138	8
Sun	a00033	477	N01-03	SA7	BG40	SA7-BG40-477			1
M730	730W417	479		SA7	BG40	SA7-BG40-479			1
Sun	a04741	167	N07-18	SA7	BG43	SA7-BG43-167			7
M730	730W444	179		SA7	BG43	SA7-BG43-179			7
Sun	a04742	181	N07-18	SA7	BG43	SA7-BG43-181			7
M730	730W251	220		SA7	BG6	SA7-BG6-220	152		9
M730	730W252	335		SA7	BG6	SA7-BG6-335			6

[1]: "Quantitative trait loci controlling glucosinolate content in seeds of *Brassica napus* L." is in preparation for publication

Table S3. Averaged Lesion Lengths (cm) Caused by *Sclerotinia* Stem Rot from Two Replicates in the DH Population of Zhongyou 821 x Topas in *B. napus*

Line	2009	2010	Variance	Mean (cm)
ZT008	1.7	1.6	0.003	1.7
ZT147	1.5	4	3.125	2.8
ZY821	2.3	3.7	0.980	2.9
ZT062	1.5	5.3	7.125	3.4
ZT020	3.9	3.3	0.211	3.6
ZT111	2.3	5.2	3.967	3.7
ZT059	3.6	3.9	0.056	3.8
ZT070	3.6	4.9	0.772	4.3
ZT072	3.6	5.3	1.473	4.4
ZT119	3.6	5.4	1.687	4.5
ZT096	3.3	6.4	4.909	4.8
ZT030	6.3	4.4	1.897	5.4
ZT100	6.5	4.6	1.882	5.5
ZT113	7	4.6	2.92	5.8
ZT098	6	6	0.001	6
ZT136	10.3	4.4	17.503	7.3
ZT080	5.5	10.7	13.594	8.1
ZT082	10.6	9.8	0.361	10.2
ZT001	12.3	8.7	6.346	10.5
ZT031	12.7	8.6	8.532	10.6
ZT021	13.8	8.5	14.222	11.2
ZT011	9	14	12.5	11.5
ZT036	10.7	12.4	1.466	11.5

ZT037	10.5	13.4	4.157	11.9
ZT048	14.3	10	9.389	12.2
ZT039	10.8	13.7	4.438	12.2
ZT127	11.5	13	1.125	12.3
ZT052	13	11.8	0.681	12.4
ZT047	11.3	13.8	3.125	12.6
ZT002	14.4	11.3	4.901	12.9
ZT099	12	14.1	2.205	13.1
ZT007	13.8	12.4	0.995	13.1
ZT104	12	15.8	7.347	13.9
ZT125	11.3	17	16.531	14.1
ZT015	14.8	13.7	0.551	14.2
ZT066	16.1	12.8	5.396	14.5
ZT042	14.7	14.5	0.029	14.6
ZT089	16	13.7	2.722	14.8
ZT124	14.8	15	0.02	14.9
ZT010	16.6	13.4	5.189	15
ZT055	14.4	16.4	2.121	15.4
ZT033	17.2	13.8	5.951	15.5
Grand	15.6	15.4	0.009	15.5
ZT038	16.7	14.4	2.645	15.5
ZT027	18	13.2	11.452	15.6
ZT061	14.2	17.1	4.109	15.6
Grand	15.8	16.0	0.02	15.9
ZT097	16.5	15.2	0.845	15.9
ZT083	13.1	18.6	14.89	15.9
ZT094	17.9	14	7.67	16
ZT068	15.1	17.1	1.883	16.1
ZT041	19.3	13.7	15.587	16.5
ZT032	17.5	15.5	2	16.5
ZT060	16.4	16.8	0.061	16.6
ZT005	17.1	17.3	0.018	17.2
ZT016	17.4	17.1	0.023	17.3
ZT022	18.3	16.2	2.347	17.3
ZT046	18.4	16.9	1.207	17.7
ZT012	15.9	19.7	7.22	17.8
ZT123	15	20.9	17.405	18
ZT088	19	17	2	18
ZT004	16.7	19.8	4.651	18.2
ZT018	18.6	18.3	0.064	18.5
ZT009	16.5	20.7	8.681	18.6
ZT078	20.5	16.9	6.635	18.7
ZT006	18.2	19.3	0.551	18.7
ZT115	20.3	17.1	5.147	18.7
ZT090	21.2	16.8	9.753	19
ZT138	19.5	18.7	0.347	19.1
ZT043	18.3	20.1	1.561	19.2
ZT101	18.7	19.8	0.589	19.3
ZT128	19.8	18.9	0.383	19.3
ZT095	22.9	16	23.92	19.5
ZT075	18.8	20.4	1.32	19.6
ZT117	17.3	22.5	13.52	19.9
ZT120	19.4	20.8	0.873	20.1
ZT077	19.8	20.6	0.313	20.2
ZT134	18.7	21.9	5.281	20.3
ZT058	20	20.7	0.245	20.4
ZT081	19	21.9	4.253	20.5
ZT114	19.5	21.5	2	20.5
ZT092	17.1	24	23.51	20.6

ZT014	19.3	22	3.684	20.6
ZT079	22.5	19.2	5.398	20.9
ZT013	21.2	21	0.02	21.1
Topas	20.0	22.3	2.645	21.2
ZT017	20.9	21.6	0.309	21.3
ZT118	18.2	24.8	21.67	21.5
ZT129	23	20.1	4.133	21.6
ZT023	23.8	20.6	5.281	22.2
ZT110	23.6	22.2	0.987	22.9
ZT122	21.3	24.6	5.243	23
ZT091	22	24	2	23
ZT065	20	26.3	19.845	23.2
ZT029	21.3	25.7	9.596	23.5
ZT140	26.1	22	8.288	24
ZT076	27.3	21.5	16.531	24.4
ZT056	27.7	21.8	17.701	24.7
ZT003	24	29.7	16.056	26.8
ZT057	23.5	31.3	30.031	27.4
ZT063	30.7	25.1	15.309	27.9
ZT024	33	25.4	28.88	29.2
ZT074	28	32.4	9.827	30.2

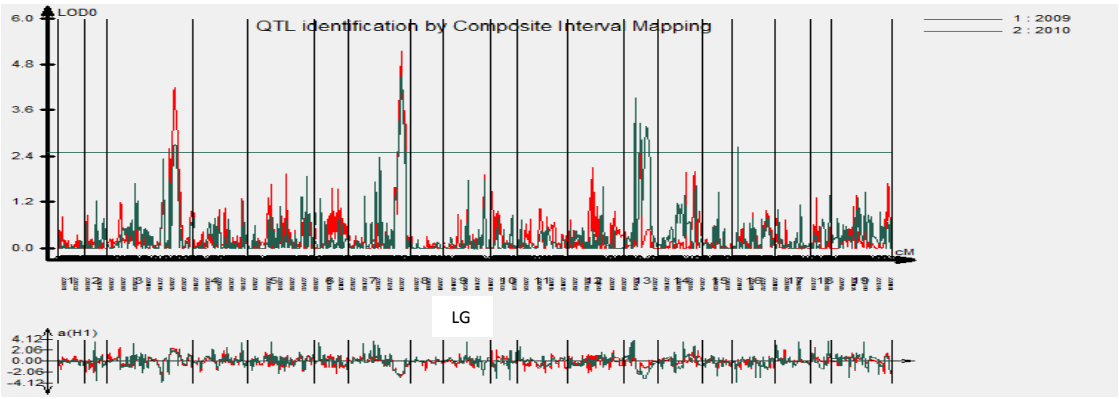
Table S4. The GLM Procedure for *Sclerotinia* Lesion Length (cm) Measured in Two Replicates of 99 DH Lines of *B. napus* Generated Based on a Cross of Zhongyou 821 x Topas

a.

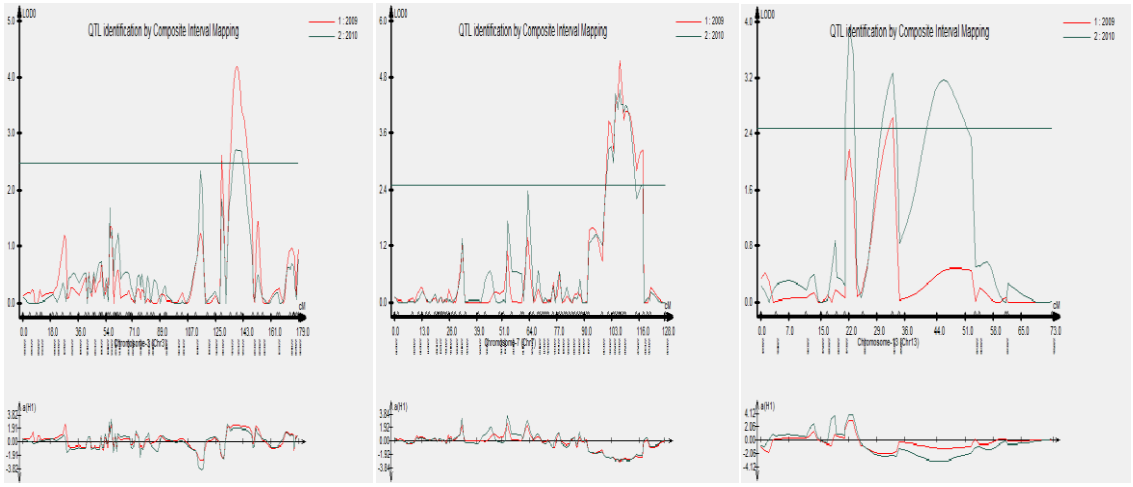
Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	197	42387.62178	215.16559	11.81	<.0001
Error	739	13459.24467	18.21278		
Corrected Total	936	55846.86645			

b.

R-Square	Coeff Var	Root MSE	Lesion Mean
0.758997	27.50232	4.267643	15.5174



a.



b. c. d.

Figure 1. Quantitative Trait Loci Identified from the 19 Chromosomes of the DH Population of *B. napus* Derived from F_1 of a Cross between Zhongyou 821 x Topas from Two Replicates Grown in 2009 and 2010

a. Nineteen chromosomes. **b**. Chromosome A3. **c**. Chromosome A7. **d**. Chromosome C3.

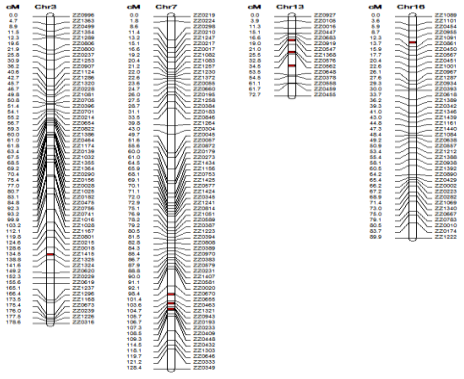


Figure 2. QTLs identified on three chromosomes of the DH population of *B. napus* derived from a cross between Zhongyou 821 x Topas

CHAPTER 4

Genetic Analyses of Sclerotinia Stem Rot Resistance in Canola (*Brassica napus*)

Jun Liu, Arvind H. Hirani, Zhe Li, Chunren Wu, Peter B.E. McVetty and Genyi Li

J. Liu. Monsanto Canada Inc., 900 - One Research Road, Winnipeg, MB R3T6E3, Canada
A.H. Hirani. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
Z. Li. Monsanto Canada Inc., 900 - One Research Road, Winnipeg, MB R3T6E3, Canada
C. Wu. Monsanto Canada Inc., 900 - One Research Road, Winnipeg, MB R3T6E3, Canada
P.B.E. McVetty. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
G. Li. Department of Plant Science, University of Manitoba, Winnipeg, MB R3T 2N2, Canada

Corresponding author: Genyi Li (e-mail: genyi.li@umanitoba.ca)

Abstract

Sclerotinia stem rot is one of the most devastating plant diseases in canola (*B. napus*). Breeding resistant cultivars is a preferred method to control this disease. However, poor understanding of the mechanisms controlling plant defense to Sclerotinia stem rot has hindered efforts to develop promising disease resistant cultivars. In the present study, the two *B. napus* genetic linkage maps of doubled haploid (DH) populations M730 and M692 were previously assembled using the recently developed Illumina Infinium® *Brassica* 60K single nucleotide polymorphism (SNP) microarray BeadChip as well as sequence related amplified polymorphism (SRAP) technologies. These two bi-parental DH populations were artificially inoculated with *S. sclerotiorum* mycelia on stems to evaluate plant resistance. The SNP markers covered the entire AC genome of *B. napus* but resulted in clusters and gaps. Clustering meant more than two SNPs located at the same position and gapping meant no SNP marker found within a region on the genetic maps. The SNP rich clusters offered alternative SNPs which could be validated and used as common markers in wide germplasm while the gaps indicated where more research were needed to identify potential SNPs or investigate the cause. Plant resistance to *S. sclerotiorum* was evident in the populations but sensitive to the environment. Quantitative trait Loci (QTLs) controlling Sclerotinia stem rot were observed to have both positive and negative effects and were most frequently identified on chromosomes A2, A7, A9, C6 and C9. The most significant QTLs identified for population M730 were those on chromosomes A7, A9 and C6. No major QTLs were observed for population M692. These major QTLs could be targeted for the trait of interest and deserved for further investigation. The additive QTL effect was the factor behind plant response to

Sclerotinia stem rot in the two populations. The closely linked flanking SNP markers need to be validated in wide germplasm for efficient marker assisted selection in practical breeding.

Key Words: *Brassica napus*, *Sclerotinia sclerotiorum*, Sclerotinia stem rot, quantitative trait loci, single nucleotide polymorphism, sequence related amplified polymorphism.

Introduction

Sclerotinia stem rot (SR, also called white mould) is a major disease in many crops including *B. napus* (genome AACC) worldwide. It is caused by the homothallic and necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary which infects more than 400 plant species, mainly dicotyledonous plants (Bolton et al. 2006; Sharma et al. 2015). Its wide range of hosts, biological features and current agricultural practices make this phytopathogen difficult to manage using traditional cultural, biological, chemical or other methods. Therefore, breeding SR resistant *B. napus* cultivars has long been emphasized (Li et al. 1999; Wang et al. 2004) as disease-resistant cultivar breeding has been proven to be an economic, effective, efficient and environmentally friendly approach to *Sclerotinia* control in many other crops.

However, there are two major obstacles to the development of SR resistant canola cultivars. Firstly, there is no source for strong SR resistance available despite numerous efforts that have been made to identify trait donors in *Brassicaceae* species including *B. napus*, *Raphanus alboglabra* (RR) and *B. alboglabra* (CC) (Alkooranee et al. 2015), *B. napus*, *B. juncea* (AABB) (Barbetti et al. 2015) and *B. carinata* (BBCC) (Barbetti et al. 2014), *B. oleracea* (CC) (Ding et al. 2013; Mei et al. 2011), *B. incana* (CC) and *B.*

alboglabra (Disi et al. 2014), *B. napus* and *B. juncea* (Li et al. 2009; Sharma et al. 2009) and *Orychophragmus violaceus* (Wu et al. 2009). Sang et al. (2013) attempted transferring a gene for the SR resistance from the frog (*Xenopus laevis*) to *B. napus*. The partially resistant *B. napus* cultivar 'Zhongyou 821' was developed from a genetic mixing of *B. napus*, *B. rapa* (AA) and *R. sativus* (RR) (Li et al. 1999) and used in SR research (Buchwaldt et al. 2012; Sang et al. 2013; Yin et al. 2010; Zhao et al. 2009). Secondly the SR resistance trait is believed to be controlled by polygenes with complex interactions. Zhao et al. (2007) and Zhao et al. (2009) observed that over a thousand genes might be involved in SR resistance in *B. napus* such as those encoding proteins for pathogenesis, oxidative burst, kinases, transportation, cell activities and abiotic stresses. Zhao et al. (2006) used the first-generation molecular marker restriction fragment length polymorphisms (RFLPs) to report instances of one and 8 quantitative trait loci (QTLs) controlling SR resistance from two bi-parental DH (doubled haploid) *B. napus* populations. Zhao and Meng (2003b) used RFLP, amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and random amplified polymorphic DNA (RAPD) markers and found 3 QTLs for leaf resistance on seedlings, another 3 QTLs for stem resistance on matured plants and additive by additive interactions of the QTLs to *S. Sclerotiorum* in a bi-parental F_3 *B. napus* population. Yin et al. (2010) used SSR, RAPD, SRAP, RFLP and expressed sequence tag (EST) markers and identified QTLs for SR resistance on LG A3, A4 and C2 in a bi-parental *B. napus* DH population. Wu et al. (2013) used SSR markers and reported QTLs controlling stem and leaf Sclerotinia resistance numbering 10 and three, respectively, spread over 9 chromosomes with two major QTLs residing on chromosomes A9 and C6 in a *B. napus*

DH population. Mei et al. (2013) used SSR, SRAP and AFLP markers and found 12 QTLs controlling leaf *S. sclerotiorum* and 6 QTLs controlling stem resistance with two major QTLs on chromosome C9 in a *B. oleracea* F_2 population. Li et al. (2015) observed QTLs for leaf and stem *S. sclerotiorum* resistance numbering 8 and 27, respectively, with common QTLs located at 22.5 – 27.5 Mb on chromosome A9 and 29.5 – 36.1 Mb on chromosome C6 from a *B. napus* genome by analyzing the data collected by Mei et al. (2013), Wei et al. (2014), Wu et al. (2013), Zhao and Meng (2003b) and Zhao et al. (2006). In addition, multiple QTLs controlling Sclerotinia disease resistance are also reported in sunflower (*Helianthus annuus* L.) (Micic et al. 2004; Micic et al. 2005; Rönicke et al. 2005; Yue et al. 2008) and soybean (*Glycine max* (L.) Merr.) (Li et al. 2010). However, the limited research results confounded with various molecular marker systems used, increasing the difficulty in finding the fundamental genetic mechanism controlling SR resistance in *B. napus*. In the present study, the genetic linkage maps of two *B. napus* DH populations were assembled using recently developed Illumina Infinium® *Brassica* 60K SNP array technology and supplemented with SRAPs. The plants were phenotyped using a commonly adopted *in vivo* mycelium stem inoculation method. QTLs controlling SR resistance were localized on chromosomes A2, A7, A9, C6 and C9 with the major ones positioned on A7, A9 and C6. The QTL effects and their interactions on SR resistance were discussed.

Materials and Methods

Mapping Populations. Two bi-parental *B. napus* DH populations segregating for SR resistance were adopted from Liu et al. (2016) (population M730 which was derived from inbred lines M23 x M77) and Liu et al. (unpublished paper, population M692 which was

derived from inbred lines M29 x M69). The semi-winter rapeseed parents M23 and M29 have partial resistance while the spring canola parents M77 and M69 were relatively susceptible to SR. In addition to be grown in artificial environments, the population M730 was also grown in summer field nurseries in 2012 and 2013. Approximately 40 plants per DH line, along with their parents, were grown in 2-m-plots with row spacing of 30 cm. The plots were organized into ranges with 2-m alleyway, 2 border spring canola plots on each side of the ranges and one border spring canola range on each side of the field. The experiment was designed according to randomized complete block design (RCBD) with 3 replicates.

Inoculum and Inoculation

The *S. sclerotiorum* sclerotia were harvested from canola plants in Carman, Manitoba, Canada in the year prior to the experiments. The *S. Sclerotiorum* hyphae propagation from the sclerotia and plant stem inoculation with the hypha infested potato dextrose agar discs were referred to Liu et al. (unpublished data). Four to seven plants were inoculated for each DH line and their parents in each replicate. In the field nurseries, plants similar in diameter were selected in the middle of each plot for inoculation. Plant disease lengths on stems were assessed on 2/3, 4/6 and 8/9 DPI (days post inoculation) for population M730 and 6 DPI for M692.

QTL Analyses. The Composite Interval Mapping (CIM) in WinQTLCart v2.5_009 (Wang et al. 2011a; Silva et al. 2012) and calculation environments were referred to Liu et al. (2016). The interactions among the QTLs were analyzed using the Multiple Interval Mapping (MIM) with the model selection according to BIC (Bayesian Information

Criterion)-M0 (c(n) = ln(n)). The MIM forward search was set up with a walking speed of 1 cM. The minimum LOD (logarithm of odds) value to declare putative QTLs was 2.5 based on the research done by Rönicke et al. (2005) and Yin et al. (2010).

Results

Genetic Maps. The genetic maps of population M730 and M692 were reported by Liu et al. (2016) and Liu et al. (unpublished paper).

Similar to those in population M692, the SNPs individually located at their unique SNP positions (UPs) on the genetic linkage map accounted for the largest proportion of the total SNPs in the entire genome of M730 (Figure 1). The percentage of two SNPs at the same UP followed on almost all of the chromosomes except A8 and C6. The general trend portrayed that the percentage of the UPs decreased with the number of SNPs per UP increasing.

Sclerotinia Stem Rot Resistance. The mean lesion lengths (cm) on plant stems caused by *S. sclerotiorum* in the replicates are presented in Table S1 for population M730 and in Table S2 for population M692. Both the semi-winter rapeseed parents M23 and M29 were among the most resistant lines, whereas both the canola parent M77 and M69 were among the most susceptible lines to *S. sclerotiorum* in the two populations. The effects of the genotype, replication and G x E interactions were all highly significant ($P<0.001$) (Table S3 and S4), indicating that the phenotypic variation of disease progression among the DH lines in the two populations were highly affected by environmental conditions.

QTLs Controlling Sclerotinia Stem Rot Resistance. In total, 22 QTLs controlling SR were identified on chromosomes A2, A3, A7, A9, C3, C4, C6 and C9 from 10 replicates

of population M730 (Table 1; Figure 2). Five QTLs for SR were repeatedly observed in 3 replicates including *Bna7S9* which had a positive genetic effect and was identified at approximately 81.3 cM on chromosome A7 and 4 other QTLs *Bna7S11*, *Bna7S13*, *Bna7S17* and *Bna7S18* which had negative genetic effects and were identified at approximately 101.2 cM on chromosome A7, 138.8 cM on chromosome A9, and 8.6 cM and 25.0 cM on chromosome C6. The LOD values specifying these QTLs ranged from 3.3 to 10.4. Six QTLs at 94.1 cM on chromosome A7 including *Bna7S10* which had a positive genetic effect were repeatedly observed in 2 replicates and five other QTLs *Bna7S1*, *Bna7S2*, *Bna7S12*, *Bna7S16* and *Bna7S20* which had negative genetic effects were identified at 26.7 cM and 49.2 cM on chromosome A2, 124.6 cM on chromosome A9, 42.9 cM on chromosome C4 and 53.9 cM on chromosome C9, respectively. The LOD values specifying these 6 QTLs ranged from 2.8 to 9.1. The mean phenotypic variance explained by the 11 QTLs ranged from 10.9% to 21.5%. Each of the other 11 QTLs, three on chromosome A2, three on chromosome A3, two on chromosome C3, one on chromosome C6 and two on chromosome C9 having LOD values ranging from 2.6 to 5.0 and both positive and negative genetic effects, was observed only once from the replicates.

In population M692, only six QTLs with LOD values encompassing 3.7 to 6.7 were identified on chromosome A1, A8, C2, C6 and C7 in 4 replicates (Table 2; Figure 3). All other QTLs had negative genetic effects excluding the one located on chromosome C7 which had a positive genetic effect. The phenotypic variation explained by these QTLs ranged from 12.1% to 23.9%. However, each of the QTLs was only observed once from the 4 replicates.

Discussion

SNP markers were distributed well throughout the *B. napus* genome despite the presence of 'gaps' or 'hotspots' characterized by either a lack or clustering of SNPs as shown in the present study. Li et al. (2015) also reported gaps on most chromosomes of their integrated physical map from *B. napus* and *B. oleracea* populations. Therefore, it is beneficial to have other molecular markers such as SRAPs in the present study to fill the gaps to fine map QTLs when the genetic region associated to a trait needs to be pinpointed. It was noticed that chromosome C2 had the greatest number of SNP markers in the AC genome of the two *B. napus* populations largely due to dense SNPs at 80.5 cM where over 42% of the SNPs on chromosome C2 resided. The high dissimilarity of the spring canola and semi-winter rapeseed parents might be one reason explaining the existing diverse SNP markers. Dense SNPs are the marker of choice to be used to generate integrated linkage or physical maps to study genetic and phenotypic associations across populations. It is advantageous to use SNP markers in canola breeding considering their high polymorphic rate (Durstewitz et al. 2010; Trick et al. 2009), high colinearity of the genetic background (Delourme et al. 2013) and other benefits.

It is critical to accurately phenotype plant traits for QTL analyses. So far there is still no standardized phenotyping method to evaluate Sclerotinia resistance on crops. Stem infection on canola plants with *S. sclerotiorum* mycelia at the adult plant stage is the major explanation for crop yield and quality losses. Although stem and leaf inoculation was observed by Yin et al. (2010) having low reproducibility within and among different methods under various environments, stem inoculation was adopted in the present study because it has been extensively used in *B. napus* (Barbetti et al. 2015, Barbetti et al. 2014;

Fan et al. 2008; Li et al. 2009; Sharma et al. 2009; Taylor et al. 2015; Wei et al. 2014; Zhao and Meng 2003b; Zhao et al. 2009), in soybean (Li et al. 2010) and in tomato (*Solanum lycopersicum* L.) (Gerlagh et al. 1996). It is also reliable compared to other known inoculation methods (Behla 2009). However, it could be seen from this study that although stem inoculation has advantages over other methods to evaluate SR disease, it has limitations due to the degree of reproducibility of results from replicated experiments conducted under various conditions. The highly significant replication and the genotype by replication effects explained the low reproducibility of the disease resistance.

In this study, the QTLs for Sclerotinia stem rot resistance in *B. napus* repeatedly identified from multiple replicates were defined as major QTLs which were located on chromosomes A2, A7, A9, C4, C6 and C9 with the QTLs at approximately 81.3 cM and 101.2 cM on chromosome A7, 138.8 cM on chromosome A9 and 8.6 cM and 25.0 cM on chromosome C6 were more significant in population M730. Likewise, the QTLs observed only once in the replicates were treated as weak QTLs which resided on chromosomes A2, A3, C3, C6 and C9.

Li et al. (2015) reported 27 Sclerotinia stem rot resistance QTLs distributing almost the entire genome except for chromosomes A4, A7 and A10 and the conserved QTLs were located on chromosomes A9 and C6 from five studies in both *B. napus* and *B. oleracea* by Mei et al. (2013), Wei et al. (2014), Wu et al. (2013), Zhao and Meng (2003b) and Zhao et al. (2006). There was no QTL related to Sclerotinia stem rot resistance found on chromosomes A4 and A10 from both populations M730 and M692 and a DH population ZT (unpublished data). But a QTL on chromosome A4 was observed by Yin et al. (2010) in addition to those QTLs on chromosome A3 and C2. The QTLs identified in M730

showed that the A subgenome harbored more major QTLs than the C subgenome which contradicted the findings by Li et al. (2015) and Taylor et al. (2015). All of the other major QTLs identified in populations M730 had negative genetic effects except for the two QTLs at approximately 81.3 cM and 94.1 cM on chromosome A7 which had positive effects on Sclerotinia stem rot.

Six QTLs were identified on five chromosomes in population M692. Each of the QTLs was only observed once out of the four replicates. In *B. napus*, chromosome A2 was homologous to chromosome C2, chromosome A3 partially homologous to chromosomes C3 and C7, and chromosome A7 partially homologous to chromosomes C6 and C7 (Parkin et al. 2005). In the present study, the two QTLs identified at approximately 50 cM (*Bna7S2-1* and *Bna7S2-2*) on chromosome A2 from population M730 might be related to the QTL *Bna6S3* at 49.7 cM on chromosome C2 in population M692. Likewise, *Bna7S6* at 100.9 cM on chromosome A3 from M730 was proximal to *Bna6S6* at 98.4 cM on chromosome C7 in replicate FC from M692. A common QTL was identified on the lower half on chromosome A3 with SRAP markers in two replicates from population ZT. QTLs were observed at approximate 100 cM (*Bna7S11-1*, *Bna7S11-2* and *Bna7S11-3*) on chromosome A7 in three replicates from M730 which were proximal to *Bna6S6* on chromosome C7 from M692 and the common QTL (*BnaZS2-1* and *BnaZS2-2*) on chromosome A7 with SRAP markers from ZT. QTLs at approximately 25 cM (*Bna7S18-1*, *Bna7S18-2* and *Bna7S18-3*) from M730 were proximal to *Bna6S4* at 21.4 cM from M692 on chromosome C6. Further investigations is required to align QTLs identified with SNPs and SRAPs among different genetic backgrounds to pinpoint major and heritable genomic regions contributing to Sclerotinia stem rot resistance trait in *B. napus.*

Sclerotinia stem rot resistance is very complex and easily affected by environmental conditions. This is obviously demonstrated with QTL mapping data from various reports where a lot of QTLs for Sclerotinia stem rot resistance are identified once in multiple replicates and various inoculation methods identify different QTLs. However, Sclerotinia stem rot resistance is controlled by genetic factors since phenotypic variations in a mapping population showed significant differences in most reports. Also, in the current and previous reports, some QTLs were repeatedly identified. Thus it is highly possible that some QTLs identified in different experiments might be the same. For example, QTLs on chromosomes A2, A3, A7, A9, C2, C6 and C9 were identified in several reports. This claim needs to be confirmed in the future since various molecular markers and populations were used in different reports.

Additive major QTL effects are important factors controlling *B. napus* resistance to Sclerotinia stem rot (Zhao et al. 2003b). Additive effects of QTLs/genes are the primary explanation of plant resistance to *S. sclerotiorum* (Fusari et al. 2012). Dominant and additive by additive interactions of QTLs controlling Sclerotinia stem rot resistance in *B. oleracea* were observed by Ding et al. (2013) and Disi et al. (2014). The additive genetic effect of the QTLs on Sclerotinia stem rot was observed in the two DH populations in the present study. There was no significant additive by additive genetic effect by the QTLs found in either DH populations. Either positive or negative genetic effects of the QTLs on Sclerotinia stem rot were observed in populations M730 and M692 as well. In general, genetic effects of the QTLs on phenotypic variance detected in this study were relatively low based on the means of 14.13% in M730, 16.96% in M692, and 8.81%, respectively. All data in this study are consistent to other previous reports where QTLs for SR

resistance generally explain small portion of phenotypic variance (Mei et al. 2013; Wei et al. 2014; Wu et al. 2013; Zhao and Meng, 2003b and Zhao et al. 2006). Weak QTL effects in sunflower controlling resistance to *S. sclerotiorum* disease were reported by Micic et al. (2004) who claimed the usage of marker assisted selection (MAS) in practical breeding for the crop would be challenging. Weak QTL effects were also reported by Li et al. (2010) for white mold in soybean. Therefore, identification of *S. Sclerotinia* resistance in crops as a horizontal trait requires more resistance sources as well as the combined efforts from both traditional and molecular methods in the practical breeding practice.

Acknowledgements

This research was funded by Monsanto Canada Inc. and the National Sciences and Engineering Research Council of Canada.

Table 1. QTLs Controlling Sclerotinia Stem Rot Resistance in a *Brassica napus* Doubled Haploid Population M730 Identified Using SNP and SRAP Markers*

Ch	Mk	QTL	Peak	CI	LB	RB	LM	RM	LOD	Add.	R^2	Rep
A2	1	*Bna7S1-1*	22.8	-	-	34.6	-	28.3	3.4	-0.50	0.123	F813S3
A2	3	*Bna7S1-2*	30.5	-	-	34.8	28.3	32.7	2.9	-0.30	0.116	D3SE12
A2	8	*Bna7S2-1*	42.5	27.2	28.1	55.3	39.3	45.9	3.7	-0.22	0.142	D3OC13
A2	11	*Bna7S2-2*	55.8	16.9	42.9	59.8	48.1	60.2	2.9	-0.39	0.096	D4AP12
A2	17	*Bna7S3*	117.1	21.4	108.9	130.3	74.4	118.1	2.6	0.76	0.072	D6MA12
A2	21	*Bna7S4*	126.4	6.7	120.9	127.6	123.3	127.7	5.0	-0.92	0.127	F813S3
A2	40	*Bna7S5*	171.1	13.1	166.2	179.3	169.0	171.6	4.8	0.53	0.124	F813S3
A3	48	*Bna7S6*	100.9	16.9	89.1	106.0	99.9	101.6	2.8	-0.23	0.102	F412
A3	80	*Bna7S7*	153.9	13.8	143.0	156.8	152.1	155.0	3.7	0.15	0.184	D2JU12
A3	91	*Bna7S8*	167.4	20.2	157.0	177.2	166.3	168.7	2.6	0.28	0.105	F812
A7	56	*Bna7S9-1*	80.2	19.6	70.1	89.7	78.9	81.2	3.3	0.19	0.091	D3OC13
A7	57	*Bna7S9-2*	81.3	15.7	75.6	91.3	80.2	82.1	7.3	0.55	0.178	D4AP12
A7	60	*Bna7S9-3*	84.0	6.6	80.0	86.6	83.0	85.5	6.3	0.47	0.184	D3SE12
A7	68	*Bna7S10-1*	94.1	12.0	83.9	95.9	92.0	95.6	8.7	0.88	0.242	D6MA12
A7	68	*Bna7S10-2*	94.1	4.2	91.4	95.6	92.0	95.6	6.3	0.47	0.187	JUN-93
A7	70	*Bna7S11-1*	97.4	5.4	96.4	101.8	95.6	100.2	4.3	-0.39	0.162	D3SE12
A7	71	*Bna7S11-2*	101.2	6.5	98.0	104.5	97.4	101.7	4.2	-0.30	0.168	F412
A7	74	*Bna7S11-3*	104.7	10.3	99.0	109.3	103.4	108.0	3.8	-0.16	0.203	D2JU12

(Continued)

Ch	Mk	QTL	Peak	CI	LB	RB	LM	RM	LOD	Add.	R^2	Rep
A9	59	*Bna7S12-1*	120.0	9.2	119.6	128.8	119.0	121.0	5.0	-0.79	0.157	SE93
A9	64	*Bna7S12-2*	129.1	13.8	119.7	133.5	128.5	133.4	3.9	-0.29	0.108	D3SE12
A9	68	*Bna7S13-1*	137.3	8.9	136.1	145.0	135.9	138.8	4.5	-0.58	0.111	D6MA12
A9	69	*Bna7S13-2*	138.8	20.7	119.3	140.0	137.3	140.5	3.4	-0.27	0.094	D3SE12
A9	69	*Bna7S13-3*	138.8	9.3	136.1	145.4	137.3	140.5	4.4	-0.37	0.123	Jun-93
C3	4	*Bna7S14*	16.6	9.4	13.2	22.6	12.9	17.3	3.5	0.41	0.094	SE93
C3	88	*Bna7S15*	186.1	15.4	174.4	189.8	185.2	188.1	3.8	-0.35	0.085	D4AP12
C4	14	*Bna7S16-1*	42.0	6.4	37.9	44.3	37.7	42.6	4.2	-0.38	0.184	F812
C4	15	*Bna7S16-2*	43.7	10.9	34.5	45.4	41.0	44.4	2.8	-0.24	0.103	F412
C6	1	*Bna7S17-1*	8.6	-	-	18.7	-	19.2	10.4	-0.66	0.280	D4AP12
C6	1	*Bna7S17-2*	8.6	-	-	18.9	-	19.2	3.3	-0.38	0.137	F812
C6	1	*Bna7S17-3*	13.6	-	-	18.8	-	19.2	5.2	-0.66	0.131	D6MA12
C6	4	*Bna7S18-1*	25.0	-	-	27.8	22.8	27.4	7.5	-0.54	0.228	JUN-93
C6	4	*Bna7S18-2*	25.0	5.0	22.9	27.9	22.8	27.4	4.5	-0.49	0.113	F813S3
C6	5	*Bna7S18-3*	27.4	-	-	29.6	25.0	30.0	4.8	-0.23	0.139	D3OC13
C6	6	*Bna7S19*	34.0	-	-	41.0	27.4	41.6	3.6	-0.21	0.127	D3OC13
C9	1	*Bna7S20-1*	53.9	-	-	62.8	-	56.5	9.1	-0.69	0.284	SE93
C9	1	*Bna7S20-2*	53.9	-	-	65.9	-	56.5	2.9	-0.38	0.071	F813S3
C9	6	*Bna7S21*	71.5	12.5	62.8	75.3	70.4	75.9	4.8	-0.59	0.118	D6MA12
C9	29	*Bna7S22*	159.5	-	149.5	-	148.2	163.3	2.9	0.40	0.079	SE93

Table 2. QTLs Controlling Sclerotinia Stem Rot Resistance in a *Brassica napus* Doubled Haploid Population M692 Identified Using SNP and SRAP Markers*

Ch	Mk	QTL	Peak	CI	LB	RB	LM	RM	R^2	Rep
A1	58	*Bna6S1*	115.3	11.6	106.5	118.1	108.5	118.3	0.1206	J31G
A8	11	*Bna6S2-*	14.8	6.0	9.8	15.8	11.7	16.2	0.1978	J31C
C2	6	*Bna6S3*	49.7	17.1	35.3	52.4	35.1	52.5	0.2390	J31C
C6	2	*Bna6S4*	21.4	-	-	26.0	19.6	25.2	0.1332	O3C
C6	21	*Bna6S5*	54.3	10.1	51.3	61.4	52.3	56.3	0.1524	J31G
C7	37	*Bna6S6*	98.4	7.5	95.8	103.3	95.9	99.9	0.1869	FC6

*: SNP, single nucleotide polymorphism; SRAP, sequence related amplified polymorphism; Ch, chromosome; Mk, marker; Peak, QTL position in cM from left telomere; CI, 95% confidence interval of the QTL; LB, left border of CI, RB, right border of CI; LM, left marker position in cM; RM, right marker position in cM.

Table S1. Mean Stem Lesion Lengths (cm) Caused by *Sclerotinia sclerotiorum* in Different Replicates for a *Brassica napus* Doubled Haploid Population M730

Line	D4AP12	D6MA12	D2JU12	F412	F812	D3SE12	SE93	D3OC13	JUN93	F813S3
M77*	5.3	8.3	1.5	5.2	8.4	4.4	6.3	3.4	6.0	5.7
M23*	3.4	3.9	1.1	2.0	4.1	2.2	3.4	1.1	3.4	1.7
1	4.9	5.8	1.3	-	-	3.3	5.2	3.1	4.5	3.8
2	5.3	6.6	1.2	3.3	6.3	3.7	5.3	2.9	4.4	5.4
3	5.9	6.5	0.9	3.2	6.6	6.1	7.0	2.9	5.3	7.5
4	6.5	8.0	0.8	4.0	6.4	4.1	4.7	3.2	5.5	5.2
5	5.4	5.2	1.3	3.0	5.7	4.2	6.3	2.5	5.3	4.8
6	4.1	5.6	1.2	3.3	5.0	4.5	4.0	3.1	3.8	5.5
7	4.4	5.2	1.0	1.9	6.2	3.7	7.4	3.2	5.1	7.0
8	4.5	5.7	1.0	3.8	6.5	4.0	5.4	2.5	3.6	5.0
10	5.3	7.6	-	4.6	6.9	3.0	6.3	2.1	4.4	5.9
11	6.9	5.9	1.0	3.1	6.5	4.0	7.1	3.1	5.2	4.7
12	6.1	7.9	1.3	4.3	7.7	4.8	6.7	2.8	5.3	4.6
13	4.0	7.1	0.9	4.4	6.7	3.1	6.1	2.3	3.2	4.7
14	4.4	6.3	1.7	3.0	5.6	3.1	5.0	2.1	3.5	3.4
15	6.8	3.5	0.9	3.2	6.6	4.0	6.3	3.4	4.1	5.2
16	5.2	4.2	0.7	3.8	5.9	3.5	5.0	2.3	3.5	4.2
17	4.7	5.2	1.1	2.5	5.0	3.3	6.3	2.5	3.8	3.3
18	3.8	4.8	1.0	2.4	5.6	3.8	4.2	2.8	4.0	3.9
19	6.1	6.3	1.4	3.3	5.8	3.6	6.4	3.0	4.1	5.0
20	6.5	8.3	1.4	2.8	5.9	3.4	6.3	2.5	5.4	6.0
21	7.6	9.7	1.3	3.0	6.2	3.7	6.0	3.2	6.1	3.3
22	5.0	5.1	1.6	3.8	7.7	4.8	5.0	3.1	3.7	3.4
23	5.0	8.5	0.9	-	-	3.4	4.7	3.1	6.7	6.3
24	5.9	6.8	1.4	4.7	7.6	4.9	6.6	3.2	6.2	5.1
25	4.3	4.1	1.4	3.3	6.7	4.3	4.4	3.1	3.6	5.2
26	6.0	5.9	1.2	4.2	7.3	3.8	4.8	2.4	4.6	5.1
27	3.7	5.0	1.4	-	-	-	-	1.7	4.6	2.7
28	4.6	7.1	1.7	3.2	8.1	3.0	4.5	3.4	5.1	4.4
29	7.8	9.6	1.2	4.5	8.3	5.1	7.4	3.9	6.1	5.3
30	8.1	8.7	2.4	4.8	7.1	5.0	5.7	4.4	6.6	7.2
31	5.6	5.4	1.1	3.3	5.9	3.7	4.8	2.3	5.4	3.3
33	4.8	5.5	0.7	3.6	6.4	4.2	7.0	3.7	4.7	5.2
34	2.9	5.6	0.9	2.6	5.9	4.0	9.3	2.2	4.5	4.1
35	3.9	3.9	0.7	3.3	6.4	4.0	3.4	2.5	2.8	5.1
36	5.2	7.3	1.2	4.2	6.3	5.5	7.9	3.3	7.1	5.8

37	5.5	6.4	1.4	2.7	7.7	3.2	5.4	2.6	4.5	4.8
39	5.6	7.0	1.8	3.1	6.5	4.2	5.2	2.0	4.3	3.3
40	5.3	5.2	1.1	3.8	6.7	4.8	6.8	2.9	5.5	5.3
41	6.4	8.2	0.9	3.7	7.3	5.5	9.5	2.7	5.0	3.8
42	4.8	9.3	0.5	4.6	7.9	5.7	6.7	3.2	6.5	6.4
43	6.8	8.8	1.1	4.5	6.6	5.3	6.4	3.7	6.6	6.7
44	4.7	5.9	0.7	3.2	6.7	3.4	5.7	2.1	3.7	4.8
45	6.3	9.1	1.4	3.6	7.7	4.5	6.4	2.5	5.4	5.0
46	6.5	10.0	1.3	4.2	6.5	4.7	6.6	2.8	7.0	7.0
48	5.3	7.0	1.4	2.9	5.2	3.7	4.4	2.3	6.3	4.7
49	5.7	8.1	1.0	3.2	5.7	3.4	6.1	2.3	6.2	6.7
50	4.6	7.0	1.2	3.4	5.5	3.8	7.5	2.3	5.3	5.7
51	5.0	5.9	0.7	2.8	4.7	4.5	6.7	2.5	3.7	2.8
52	5.4	4.0	1.0	2.3	5.8	3.4	5.4	2.8	3.5	3.2
53	7.5	9.4	1.3	4.8	7.1	5.9	9.4	3.7	5.9	6.0
54	6.3	6.5	1.0	3.9	6.1	4.1	6.0	3.5	4.9	6.0
55	4.3	4.5	1.2	3.1	6.3	2.9	7.1	2.5	3.6	3.8
56	7.9	6.5	1.2	3.9	6.3	4.6	7.4	3.1	6.0	5.9
57	6.5	7.7	1.1	2.6	5.6	3.4	6.1	1.9	4.8	2.8
59	3.3	3.1	0.8	-	-	2.9	4.9	2.3	3.2	4.9
61	5.5	5.9	-	4.0	6.6	4.0	4.7	2.7	4.4	5.5
62	6.4	8.6	-	3.6	7.6	4.8	7.3	2.4	5.8	6.5
63	5.3	5.3	-	4.3	7.9	4.1	7.5	2.9	4.7	3.4
64	6.5	3.8	-	-	-	4.4	7.3	2.6	4.8	4.5
66	4.6	5.3	-	-	-	2.0	2.9	2.9	5.4	2.2
67	6.0	6.2	-	3.2	7.2	4.0	5.4	2.8	4.9	-
68	4.8	8.1	-	4.8	7.3	5.3	7.0	2.6	4.5	4.4
69	5.2	8.6	-	-	-	5.5	6.2	2.1	7.0	8.0
70	4.6	5.5	-	2.8	6.3	3.7	4.8	2.2	3.9	2.6
71	4.6	7.0	-	3.2	6.3	3.7	6.2	1.8	4.8	7.7
72	5.0	5.9	-	-	-	4.0	5.0	3.3	4.7	4.3
73	3.8	5.8	-	2.7	5.6	2.9	4.4	2.5	4.2	2.0
75	6.1	4.3	-	3.3	6.9	3.9	6.8	2.4	5.3	3.4
76	4.8	5.7	-	-	-	3.8	5.4	2.4	3.1	4.1
77	5.1	6.5	-	3.6	6.5	3.7	6.0	3.3	5.4	7.4
78	5.1	6.1	-	3.3	5.6	4.6	5.6	2.8	4.4	5.7
79	3.7	4.0	-	2.9	6.6	3.7	5.1	2.6	4.5	2.7
80	7.2	5.7	-	4.3	7.0	4.6	7.9	3.1	5.4	5.4
81	5.5	3.4	-	-	-	5.0	6.0	2.4	5.4	5.5
82	5.1	6.0	-	-	-	3.7	6.3	2.6	5.0	3.8
83	5.0	4.7	-	-	-	3.9	5.8	2.0	5.5	3.1
86	4.1	6.7	-	1.7	6.0	3.2	8.6	1.8	5.2	2.5
87	2.8	7.1	-	-	-	2.7	5.7	1.2	4.6	1.8
90	6.5	7.7	-	3.1	5.9	4.3	6.4	2.8	5.5	4.9
91	6.9	9.1	-	3.3	6.7	5.4	8.7	3.6	5.9	6.2
92	7.1	10.0	-	-	-	4.6	7.1	2.7	6.0	5.8
94	4.2	4.5	-	-	-	4.0	5.9	3.1	4.7	5.1
96	6.5	6.7	-	-	-	4.3	5.3	3.2	6.5	4.2
97	5.1	8.4	-	3.9	6.6	3.3	5.6	2.6	6.0	4.3
98	6.9	8.5	-	-	-	5.0	6.3	2.9	6.3	5.5
99	6.8	6.7	-	4.1	7.1	3.2	5.0	2.1	4.7	3.7
100	5.7	6.3	-	-	-	3.3	6.2	2.5	6.0	5.9
101	-	5.7	-	-	-	4.4	5.0	2.6	3.9	4.2
102	-	5.0	-	-	-	4.8	5.4	4.0	4.9	6.0
105	-	9.1	-	-	-	6.2	7.6	3.6	5.7	6.2
106	-	5.8	-	-	-	-	-	2.1	-	2.2
107	-	3.8	-	-	-	2.7	5.0	2.3	3.6	4.3

*: Parental lines

Table S2. Mean Stem Lesion Lengths (cm) at 6 DPI (Days Post Inoculation) Caused by *Sclerotinia sclerotiorum* in a *Brassica napus* Double Haploid Population M692

Line	J31C6	J31G6	O3C6	FC6	Line	J31C6	J31G6	O3C6	FC6
1	5	11.9	6.9	7.9	**45**	5.5	7	5.9	6.4
2	4.8	7.5	7.1	9.4	**46**	6.2	5.5	6.7	6.5
3	4.7	5.5	3.6	7.6	**47**	6.3	8.4	5.5	5.5
4	3.1	8.5	4.7	7.2	**48**	5.7	7.8	9.2	8.2
5	5.3	8.7	5.8	9.6	**50**	5.4	7.2	7	4.8
6	6.3	8.5	5.7	8.5	**51**	5.8	6.9	7.3	6.2
7	4.1	7.6	4.2	6.7	**52**	5.8	7.9	4.6	7.1
8	5.4	5.9	7.1	6.2	**53**	6.2	8.1	6.7	5.9
9	3.4	4.3	2.2	6.2	**56**	6.2	6.4	6	6.7
10	4.4	7.3	8.1	8.2	**57**	5.2	6.1	5	4.5
11	3.2	4.8	5.4	8.8	**58**	5.6	6.5	6.5	6.4
12	4.7	6.8	6.2	9.3	**59**	4.4	9.9	6.8	9
13	5	6.1	6.2	5.9	**60**	5.6	6.5	6.4	6.3
14	7.1	9.3	6.9	8.6	**61**	3.2	6.5	6.2	7.2
15	6.1	9.6	8.3	9.8	**62**	4.8	7	7	5.5
16	6	6.9	7.5	7.2	**63**	4.9	7.8	5.5	4.5
17	6.8	10.2	8.8	6.6	**64**	4.6	7	7.6	7.5
18	4	7.6	6.5	5.3	**65**	5.3	7.2	5.4	5.9
19	5.7	7.7	5.5	6.6	**66**	5.7	6.5	7.1	6.3
20	5.4	6.3	5.9	8.2	**67**	4.6	7.2	5.2	-
21	5.1	8.6	5.2	5.9	**68**	5.2	8.9	4.8	7.6
22	5.9	10	4.1	8	**69**	4	9.1	7	-
23	6	6	3.2	5.8	**70**	-	-	8.1	5.3
24	4.8	6.2	5.6	6.4	**71**	5.2	5.4	4.9	7.4
25	6.1	5.9	6.7	8	**73**	-	4.9	5.9	8.1
26	3.7	5.3	5.8	7.8	**75**	-	5.7	7.3	8.4
27	6.5	6.2	7.6	5.9	**77**	5.3	7.4	4.5	6.8
28	4.9	6.3	6.2	5.5	**78**	5.5	8.9	6.8	8.7
29	5.2	5.1	5.1	5.4	**79**	-	8.4	8.1	6.5
30	4.9	8.7	7.3	7.2	**81**	4.8	4.5	3.9	6.4
31	6.5	8.3	6.2	7.3	**82**	5.1	9.1	5	6.4
32	5.2	6.3	6.3	4.6	**83**	5	5.9	6.1	5.3
33	5.6	5.9	7.2	6.6	**84**	5	6.2	6.4	9.7
34	6.3	8.5	5.9	6.8	**86**	5.2	7.3	5.8	4.3
35	5.6	8.8	6.1	6.3	**88**	5.8	10.9	5.4	6.2
36	5.7	7.8	6.4	6.8	**89**	5.7	5.7	8.3	5.9
37	3.6	4.2	3.6	5.8	**90**	6.7	8.1	8.3	5
38	5.8	7.2	7.2	8.1	**93**	4.3	8.4	6.5	5.7
39	5.1	8.5	5	9.5	**94**	-	-	8.3	-
40	4.3	3.7	4.8	6.8	**95**	-	8.8	8.4	5.6

41	5.7	7.9	7.2	7.7	**96**	-	-	7	6.1
42	4.2	5.5	6.1	5.7	**97**	4.5	7	6.2	-
43	5.7	10	8.7	7.2	**M69***	6.3	9.7	7.6	6.5
44	5.9	10.2	7.6	7.8	**M29***	3.3	5.2	4.7	4.8

Table S3. ANOVA for Mean Stem Lesion Lengths (cm) Caused by *Sclerotinia sclerotiorum* in a Doubled Haploid *Brassica napus* Population M730

Effect	Num DF	Den DF	F Value	Pr > F
Line	90	2998	12.79	<.0001
Rep	7	2998	674.52	<.0001
Line*Rep	563	2998	2.65	<.0001

Table S4. ANOVA for Mean Stem Lesion Lengths (cm) Caused by *Sclerotinia sclerotiorum* in a Doubled Haploid *Brassica napus* Population M692

Effect	Num DF	Den DF	F Value	Pr > F
Line	90	1160	6.73	<.0001
Rep	3	1160	159.06	<.0001
Line*Rep	254	1160	3.06	<.0001

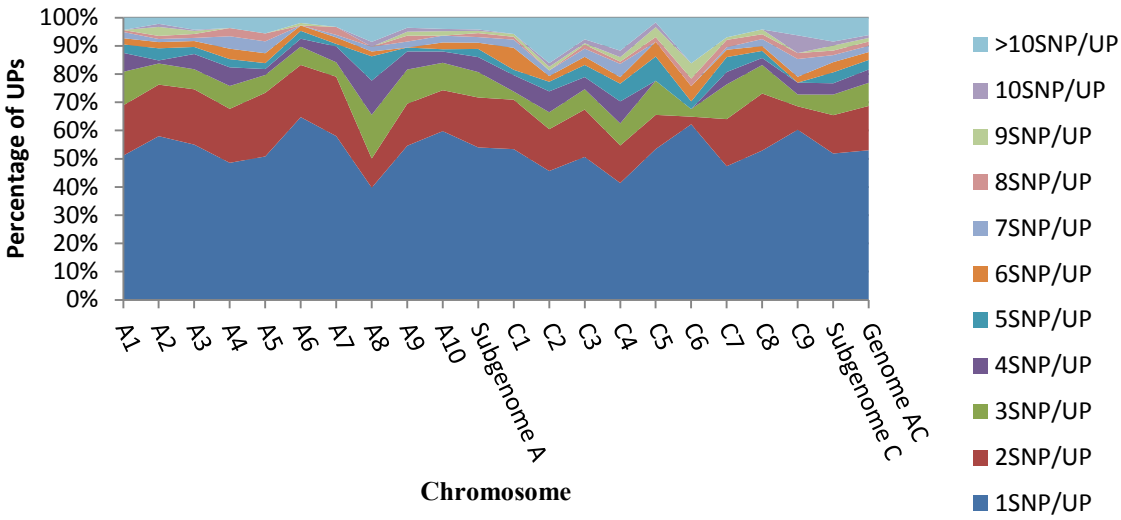


Figure 1. The Percentage of Number of SNPs per Unique SNP Position (UP) on the Genetic Map of a *Brassica napus* Doubled Haploid Population M730

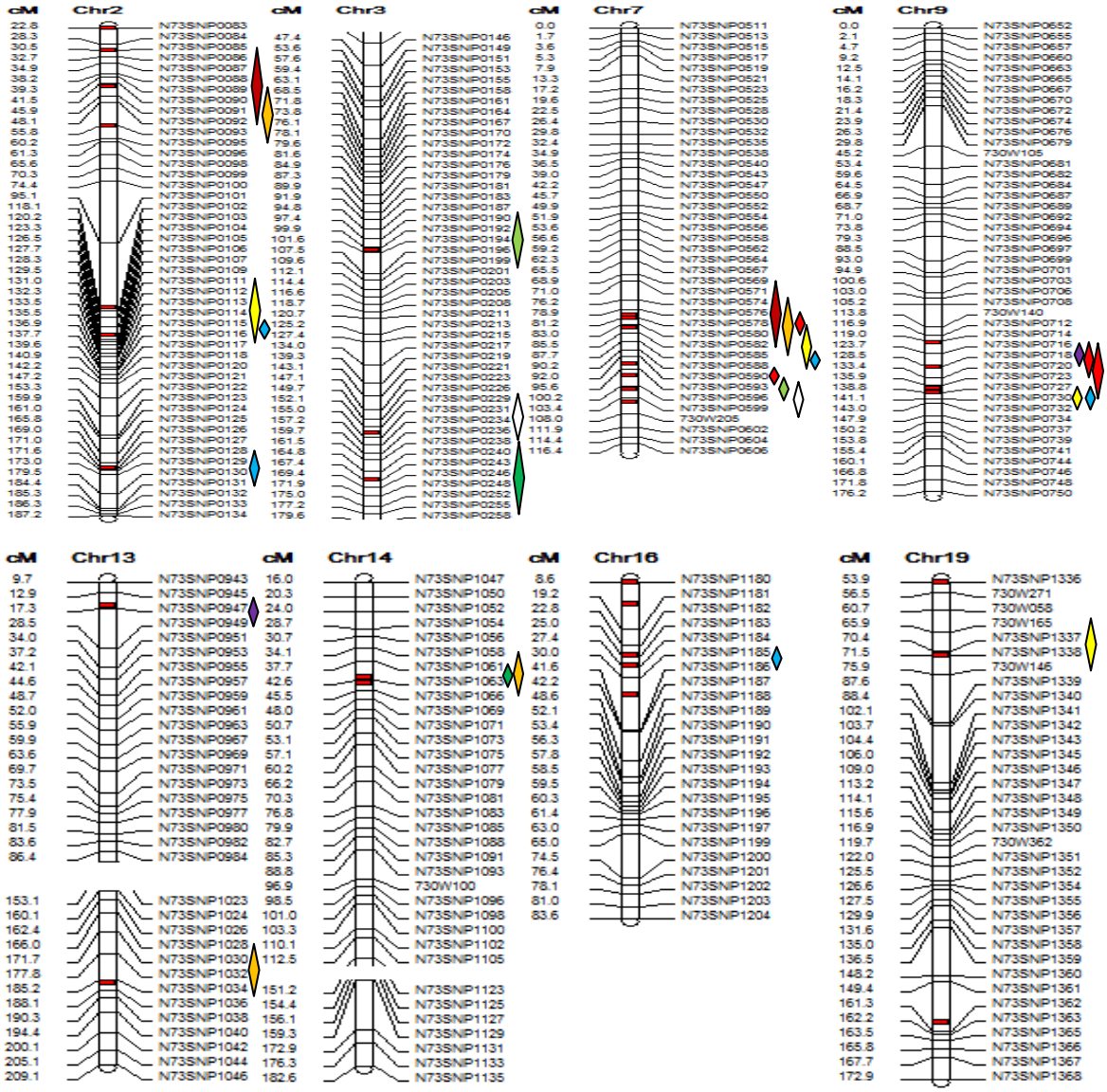


Figure 2. The Genetic Map for QTLs for Sclerotinia Stem Rot Resistance in a Doubled Haploid *Brassica napus* Population M730*

*: Red bars on the chromosomes indicated peaks located by logarithm of odds for the QTLs. Colors in diamonds: No color, replicate D2JU12; Dark red, D3OC13; Red, D3SE12; Orange, D4AP12; Yellow, D6MA12; Light green, F412; Dark green, F812; Blue, F813S3;
Dark blue, JUN93; and Purple, SE93. Lengths of the diamonds specified their 95% of confidence intervals of the QTLs.

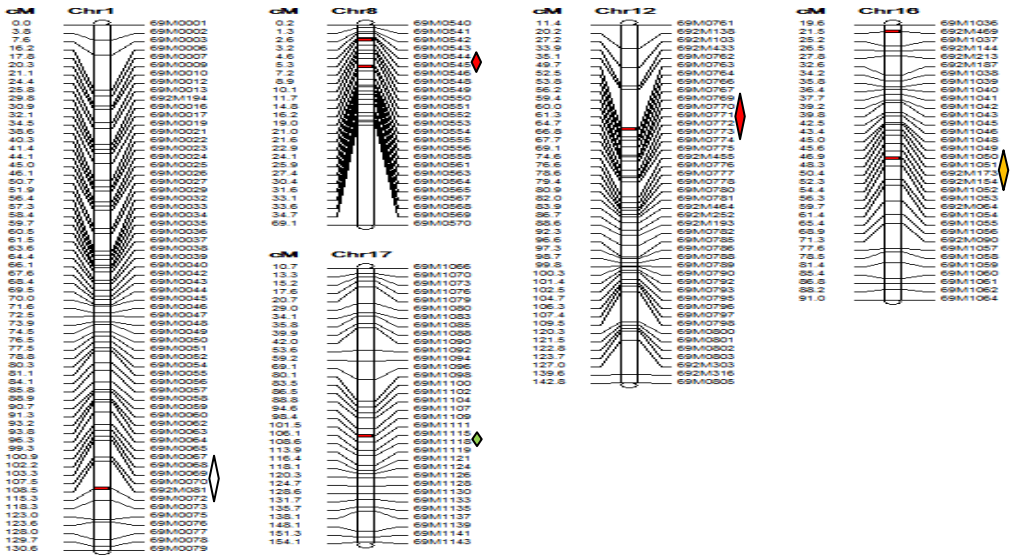


Figure 3. The Genetic Map for QTLs for Sclerotinia Stem Rot Resistance in a Doubled Haploid *Brassica napus* Population M692*

*: Red bars on the chromosomes indicated peaks located by logarithm of odds for the QTLs. Colors in diamonds: No color, J31G; Red, J31C; Orange, O3C, and Light green, FC. Lengths of the diamonds specified their 95% of confidence intervals of the QTLs.

GENERAL DISCUSSION

Plant Materials

Additive, dominant and epistatic genetic effects determine in large part, plant phenotypic traits. Epistatic effects are nonallelic gene interactions and directly related to quantitative traits (Viana 2005). It is important though difficult to correctly and accurately measure gene action and interaction, especially when all three factors controlling quantitative traits are present and confound each other. Compared to their counterparts such as RILs, backcrosses or early filial generations, segregating DH populations have marked advantages such as short development time versus RILs, homozygous state for all genetic loci versus F_2 and backcrossed populations or exposure of recessive alleles so attract more attention in genetic or genomic studies. Homozygous lines such as DH or RIL are especially useful for the study of quantitative traits replicated in various conditions to remove experimental and sampling errors and to identify intrinsic genetic causes. Although RILs developed from multiple meiotic recombination processes, their prolonged generation time makes them less attractive even for self-compatible plants such as *B. napus*. Instead, DH lines have been extensively developed in 22 major crops (Maluszynski et al. 2003) including maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), barley (*Hordeum vulgare* L.), *Brassica* spp. and pepper (*Capsicum annuum* L.). DH populations used for genetic linkage mapping were included in experiments involving *B. napus* by Delourme et al. (2013), Ferreira et al. (1994), Sun et al. (2007), Uzunova et al. (1995), Wu et al. (2013), and Yin et al. (2010), in *B. rapa* by Lou et al. (2008), in *B. juncea* by Rout et al. (2015), and in Arabidopsis by Seymour et al. (2012). Segregating F_1 populations were recommended for mapping studies by Ming and Wai (2015). In this research, three DH populations with contrasting phenotypic traits in

their parental lines were chosen in order to simplify and more accurately evaluate QTL effects. The parental lines in the three DH populations from spring type and semi-winter germplasm pools were quite dissimilar in their genetic composition, which helped generalize the findings in the research.

Plant Genotyping

There are many technologies available in modern times to genotype plants. These technologies include hybridization-based RFLP, PCR-based RAPD, SRAP, CAPs, diversity array technology (DArT) (Jaccoud et al. 2001), AFLP, SSR, target region amplification polymorphism (TRAP) (Hu and Vick 2003), and genotyping by sequencing (GBS). SNPs are the markers of choice to be used in genotyping and mapping studies due to their abundance and wide distribution in the genome. *Brassica* 6K SNP Infinium® array is highly multiplexing compared to previous SNP platforms and was used by Cai et al. (2014), Körber et al. (2015) and Raman et al. (2014) to construct genetic maps in *B. napus*. In the present study, over 8,000 SNP markers from more powerful *Brassica* 60K Infinium® array were identified covering all 19 chromosomes from the DH populations M730 and M692 demonstrating the effectiveness of SNP markers in mapping allotetraploid *B. napus* plants. More SNP markers available not only help fine map QTLs but also have more common markers to be used in wide germplasm. Despite these advantages, these SNP markers were not distributed uniformly. Most SNP markers were more evenly distributed in the A subgenome in populations M730 and M692 since fewer markers were clustered in some "hot spots". The finding that the A subgenome had more bins but fewer SNP markers than those on the C subgenome in both populations contradicted with what Raman et al. (2014) observed. This may be attributed

to either the usage of different SNP marker techniques or different germplasm. Cai et al. (2014) observed that marker density was higher in the A subgenome than that in the C subgenome. In the present study, the A subgenome harbored fewer SNP markers and had longer genetic distance than that on the C subgenome in both populations M730 and M692 causing a lower mean marker density in the A subgenome as compared to the C subgenome. However, the densities of bins and unique marker positions were higher in the A subgenome than the C subgenome in both M730 and M692.

Another component of the uneven distribution of SNP markers was that there were gaps where SNP markers were absent or sparse in the subgenomes. For instance, 12 out of the 19 chromosomes in population M730 and 15 out of the 19 chromosomes in M692 had no SNP marker observed on their left ends. The shortest undetected left ends in M730 and M692 were 1.3 cM and 0.3 cM on chromosome C2 and C5 while the longest were 80.4 cM and 101.0 cM on chromosome A4 and C9, respectively. There were also multiple gaps greater than 10 cM between SNP markers in both M730 and M692. The causes for these SNP gaps might be no matching probes and bad marker calls on the chips, or near centromeric or telomeric regions. No matter what reason it is, the identification and localization of QTLs in these regions will be compromised. Gaps over 20 cM on the genetic map developed from AFLP and SSR were also reported by Lou et al. (2008) in *B. rapa*.

Clustering of molecular markers was observed by Urunova et al. (1995) using RFLP markers in their linkage mapping. SNP marker clustering (unique marker positions) was common in both M730 and M692. Marker clustering was reported by Lou et al. (2008) with AFLP and SSR markers and Uzunova et al. (1995) with RFLP markers. Over 47%

and 44% of the SNP markers in M730 and M692 clustered in more than one marker per position. The clusters containing two and three markers per position accounted for 17.2% and 8.0% in M730, and 15.4% and 7.3% in M692, respectively. The curve for numbers of markers in clusters plateaued after the numbers of markers per cluster reached 4 or more. There were fewer chromosomes having more than 10 markers per cluster in the A subgenome than that in the C subgenome. For example, two chromosomes A5 and A8 in both M730 and M692 had over 5% of clusters containing more than 10 SNP markers per cluster. However, 7 chromosomes C1, C2, C3, C4, C6, C7 and C9 in M730 and 6 chromosomes C1, C2, C3, C4, C7 and C9 had over 5% of clusters containing more than 10 SNP markers per cluster.

In both populations M730 and M692, chromosome C2 had the most SNP markers while chromosome C9 had the fewest SNP markers in their 19 chromosomes. This finding was at odds with the results reported by Wang et al. (2014) who also used *Brassica* 60K Illumina® Infinium SNP array to genotype a panel of 472 *B. napus* inbred lines and observed that chromosome C3 harbored the most markers whereas chromosome A2 had the fewest SNP markers. Chromosome C3 was observed to have the longest and chromosome A8 the shortest genetic distances on the genetic linkage maps developed from the SNPs in both population M730 and M692. The longest genetic distance, however, was located on chromosome A3 and the shortest distance on chromosome C8 on the SRAP map from population ZT. Lou et al. (2008) observed the longest genetic distance to be on chromosome A9, while A1 and A4 were the shortest LGs.

Sun et al. (2007) developed a high density genetic linkage map for *B. napus* by using over 11,000 SRAP markers. This reported clearly the diversity and wide distribution of

SRAP markers in *B. napus*. In population ZT, one pair of SRAP primers developed an average of 4.6 markers distributed among 19 chromosomes of the *B. napus* population with narrower and fewer gaps longer than 10 cM than both populations M730 and M692 with SNP markers. This exemplified the usefulness of SRAP markers to dissect the detailed genetic foundation controlling Sclerotinia resistance traits in rapeseed/canola plants as observed by Aneja et al. (2012). Polymorphic SSR markers were also used in this study by referring to research on these *B. napus* populations done by Sun et al. (2007) and Piquemal et al. (2005) to assign the observed LGs to their corresponding chromosomes. In addition, some consensus SRAP markers were determined to be co-segregating *Brassica* 60K Infinium® SNP markers in the three populations and had high coincidence in the assignments of these LGs among SRAPs, SSRs and SNPs.

Glucosinolates in Canola/Rapeseed Seed

Approximately 30 GSLs were found in *B. napus* (Lou et al. 2008). Fu et al. (2015) reported transgressive segregation in the progeny from two low glucosinolate content parental lines (canola quality for glucosinolates) which caused dramatic segregation of total glucosinolate content with a range of 10.6 – 88.6 μmol g^{-1} meal in seed for a *B. napus* DH population. Five common major GSLs were detected in the seed of three bi-parental rapeseed/canola DH populations and their corresponding parents in the present research. The threshold value of 16.5 μmol/g seed (45% oil content) was used to divide the DH lines into canola quality or rapeseed quality based on the sum of progoitrin, gluconapin and glucobrassicanapin content according to the definition of canola (Canola Council of Canada).

Canola Seed. Progoitrin was reported to be a major glucosinolate in canola (Brand et al. 2007). In all three *B. napus* DH populations, progoitrin was the most prevalent glucosinolate among 4C and 5C aliphatic glucosinolates. The minimum percentages of progoitrin in total aliphatic and total glucosinolate were from population ZT and accounted for 56.91% and 30.90% of the contents, respectively. Four carbon aliphatic glucosinolates were predominant over 5C aliphatic glucosinolates with minimum percentages of 82.19% and 44.34% in total aliphatic and total glucosinolate content from population ZT as well. Five carbon aliphatic glucosinolates, glucoalyssin or glucobrassicanapin, played minor roles and accounted for less than 10% of total aliphatic and total glucosinolate content. Although aliphatic glucosinolates as a whole were predominant over indolic glucobrassicin in total glucosinolate content, glucobrassicin had a similar importance to progoitrin, and was more important than the other three glucosinolates gluconapin, glucoalyssin and glucobrassicanapin. For example, progoitrin accounted for 5% or more than glucobrassicin in total glucosinolate contents in both M730 and M692, but 10% less than the latter in population ZT (Table S6).

There were trends in the three DH populations suggesting that the proportion of total aliphatic glucosinolate content in total glucosinolate content decreased and the total content of progoitrin, gluconapin and glucobrassicanapin in canola also decreased, while glucobrassicin in total glucosinolate content increased. These findings were reflected by their correlation coefficients r_{M730} = 0.90 (total three glucosinolates vs. total aliphatic glucosinolate) and -0.90 (total three glucosinolates vs. glucobrassicin); r_{ZT} = 0.80, -0.80 and r_{M692} = 0.79, -0.79. The total content of the three glucosinolate components in canola

could be reflected by their correlation coefficients with total glucosinolate content from the three populations, or if the minimum r was greater than 98%.

Rapeseed Seed. Sang and Salisbury (1988) reported that aliphatic GSLs gluconapin (3-butenyl), progoitrin (2-hydroxy-3-butenyl), glucobrassicanapin (4-pentenyl), napoleiferin (2-hydroxy-4-pentenyl) and indolyl GSL 4-hydroxyglucobrassicin (4-hydroxy-3-indolylmethyl) were major GSLs in rapeseed. The present study showed that progoitrin was predominant in 4C aliphatic glucosinolates with a minimum percentage of 67.59% from population M730, and in total aliphatic and total glucosinolate content with minimum percentages of 56.43% and 54.63% respectively from population ZT. Gluconapin was the second most important glucosinolate component after progoitrin among the five glucosinolates with maximum percentages of 28.94% and 27.89% of total aliphatic and total glucosinolate content from population M730. Both glucoalyssin and glucobrassicanapin accounted for less than 11% of total aliphatic and total glucosinolate content from the three populations. Compared to that in canola, indolic glucobrassicin in rapeseed played minor roles in total glucosinolate content with a maximum percentage of 6.60% from population M692, the population derived from the lower total glucosinolate content rapeseed parent.

Similar to those in canola, the total glucosinolate content, progoitrin, gluconapin and glucobrassicanapin were all positively related to total aliphatic glucosinolates and negatively related to glucobrassicin with the correlation coefficients ranging from ±0.73 to ±0.78. The correlation coefficients for the total content of the three glucosinolate components with total glucosinolate content were approximately 99% from the three populations.

Feng et al. (2012) found that aliphatic and indolic GSLs were positively correlated. They also observed that complex epistatic interactions were involved in single and total GSLs in *B. napus*, though epistatic effects were major in leaves but only minor in seeds. The present study showed that total aliphatic glucosinolate content and indolic glucobrassicin content were negatively correlated while the correlation was weak in high GSL content populations M730 and ZT with r = -0.57 and -0.46. There was almost no correlation in the low GSL content population M692 with r = -0.06.

Sclerotinia Stem Rot Evaluation

It is critical to accurately phenotype plant traits for any QTL analyses. In the present study, stem inoculation was used to assess *B. napus* plant resistance to Sclerotinia stem rot. Stems are major and vulnerable organs for *S. sclerotiorum* infection in *B. napus*. Infected plants rarely avoid Sclerotinia stem rot symptoms under suitable environmental conditions unless host resistance is strong enough to deter infection. Therefore, canola Sclerotinia stem rot is a major disease to rapeseed/canola (Disi et al. 2014; Wei et al. 2014). Stem mycelial inoculation is advantageous over leaf, petiole or root inoculation, and folial ascospore spray. It is the method recommended for disease evaluation currently by the Western Canada Canola/Rapeseed Recommending Committee (WCC/RRC) and is used extensively in canola/rapeseed (Barbetti et al. 2014; Fan et al. 2008; Li et al. 2009; Sharma et al. 2009; Taylor et al. 2015; Wei et al. 2014; Yin et al. 2010; Zhao and Meng 2003b; Zhao et al. 2009). Stem inoculation with *S. sclerotiorum* was additionally used in soybean experiments (Li et al. 2010). However, it could be seen from this study that stem inoculation had its limitation in evaluating plant resistance to *S. sclerotiorum* due to the degree of reproducibility of results from replicated experiments under various conditions.

One of the explanations for this inconsistency may be a flaw in simulating the natural infection processes. Lesion areas on the surface, inside plants and depths into plant piths were all involved in the *S. sclerotiorum* infection processes in *B. napus* plants, even though lesion lengths on stem surfaces was the only factor measured.

Three spring type canola parents of the three DH populations were susceptible to canola Sclerotinia stem rot and among the most susceptible lines in each population, whereas three semi-winter rapeseed parents had certain levels of resistance to canola Sclerotinia stem rot and were similar to the most Sclerotinia resistant lines in each of the three populations. All DH lines in each population were sorted by levels of canola Sclerotinia stem rot resistance and had continuous distributions of stem lesion lengths which indicated usefulness of the three bi-parental populations.

Genotype by replication effects for canola Sclerotinia stem rot resistance in all the three populations were significant for population ZT ($P = 0.012$) and highly significant for both populations M730 and M692 ($P < 0.001$), indicating that resistant genotypes could only be effectively selected based on their general performance over a range of growing conditions.

QTLs Controlling Glucosinolates

Chromosomes Harboring QTLs

Li et al. (2014) identified QTLs for total glucosinolate content on chromosomes A9, C2, C7 and C9 from *B. napus*. Rahman (2014) found QTLs for total glucosinolate content on chromosomes A2, A7 and A9 from *B. rapa*. The C genome contains at least three major loci controlling glucosinolates in *B. napus* (Rahman et al. 2015). In the present study QTLs regulating different GSL traits were identified on six chromosomes A1, A8, A9,

C2, C3 and C9 in M730, and six chromosomes A3, A9, C3, C5, C7 and C9 in ZT, the two high glucosinolate populations, respectively. Chromosome A9 harbored much more and more significant QTLs than other chromosomes. For instance, eight QTLs regulating 8 out of the 9 GSL traits except for gluconapin were located on chromosome A9 in M730. These QTLs explained 14.18% to 40.10% of the phenotypic variances. These QTLs explained higher percentage of the phenotypic variances for progoitrin, 4C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content ranging from 28.05% to 40.10% than those for glucoalyssin, glucobrassicanapin and 5C aliphatic glucosinolates ranging from 14.18% to 18.78%. On the other hand, 11 QTLs regulating 7 out of the 9 GSL traits excluding both glucoalyssin and 5C aliphatic glucosinolates were located on chromosome A9 in population ZT, explaining 16.53% to 20.11% of the phenotypic variances for progoitrin, 4C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content, and less than 10% of the phenotypic variances for glucobrassicanapin, glucobrassicin and gluconapin. The 8 QTLs in M730 were located at 42.5 to 55.4 cM on chromosome A9. Nine out of 11 QTLs in ZT were located at 32.0 to 41.8 cM on chromosome A9. Both Li et al. (2014) and Rahman et al. (2014) detected significant QTLs on the left end of chromosome A9 regulating total glucosinolate content.

Population M692 was quite different from the aforementioned two populations in respect to the rapeseed donor parent M69 having much less total glucosinolate content than either M23 or ‘Zhongyou 821’. Instead of chromosome A9, chromosome C7 harbored all the major QTLs that regulated 8 out of the 9 GSL traits except for glucobrassicin. These QTLs explained 50% or more percentage of phenotypic variances for progoitrin,

gluconapin, 4C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content, and less than 39% of their phenotypic variances for glucoalyssin, glucobrassicanapin and 5C aliphatic glucosinolates.

Numbers of QTLs

Seventeen individual QTLs regulating 9 GSL traits were identified from population M730. Some of the QTLs either shared the same peak, were identified by the same markers, overlapped CI, or had similar genetic effects, suggesting that they might be the same QTL with multiple functions. For example, *Bna7G2-1* and *Bna7G2-2* were located at 102.0 cM on chromosome A1 and specified by the same marker 68 and the flanking left and right markers at 100.2 and 107.6 cM, and had overlapped CI from 94.7 to 106.0 cM and positive genetic effects. *Bna7G2-1* regulated 5C aliphatic glucosinolates and *Bna7G2-2* regulated glucoalyssin. The latter was part of the former. Therefore, the two QTLs might be one and the same. Likewise, two QTLs *Bna7G4-1* and *Bna7G4-2* on chromosome A9 might be the same QTL regulating both progoitrin and glucobrassicin. Four QTLs *Bna7G5-1* to *-4* on chromosome A9 regulating 4C aliphatic glucosinolates, glucobrassicanapin, total aliphatic glucosinolates and total glucosinolate content might be the same QTL. *Bna7G6-1* and *-2* on chromosome A9 regulating 5C aliphatic glucosinolates and glucoalyssin might be the same QTL. Three QTLs, *Bna7S8-1* to *-3* on chromosome C3 regulating 4C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content might be identical as well.

Twenty five QTLs individually identified from population ZT could be treated as 10 common QTLs. Nine QTLs *BnaZG4-1* to *BnaZG4-9* regulating progoitrin, gluconapin, 4C aliphatic glucosinolates, glucobrassicanapin, total aliphatic glucosinolates and total

glucosinolate content on chromosome A9 might be the same. Five QTLs *BnaZG5-1* to *BnaZG5-5* on chromosome C3 regulating progoitrin, gluconapin, total aliphatic glucosinolates and total glucosinolate content might be identical. Three QTLs *BnaZG10-1* to *BnaZG10-3* on chromosome C9 regulating 5C aliphatic glucosinolates, glucobrassicanapin and glucobrassicin might be the same QTL.

Eighteen QTLs identified from population M692 could be treated as 7 common QTLs. *Bna6G3-1* and *Bna6G3-2* on chromosome A3 regulating 5C aliphatic glucosinolates and glucoalyssin might be the same QTL. *Bna6G5-1* and *Bna6G5-2* on chromosome A6 regulating glucobrassicin might be the same QTL. Eight QTLs *Bna6G1-1* to *Bna6G1-8* on chromosome C7 regulating progoitrin, gluconapin, 4C aliphatic glucosinolates, glucoalyssin, glucobrassicanapin, 5C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content might be the same QTL. Three QTLs *Bna6G4-1* to *Bna6G4-3* on chromosome C9 regulating glucobrassicanapin, 5C aliphatic glucosinolates and glucobrassicin might be the same QTL.

Therefore, one QTL could have multiple functions on related or different GSL traits. This observation agrees with the studies done by Sotelo et al. (2014). One GSL trait could be regulated by multiple QTLs. Kliebenstein et al. (2001) observed 6, 6, 3 QTLs controlling total aliphatic, total indolic and aromatic glucosinolates in both seeds and leaves of Arabidopsis, respectively. Sotelo et al. (2014) found 13 QTLs controlling more than one GSL in *B. oleracea*. Lou et al. (2008) observed that the QTLs controlling total aliphatic glucosinolates also influenced the accumulation of gluconapin in *B. rapa* leaves. QTLs having negative genetic effects were much fewer in number than those having positive genetic effects. For instance, 5 out of the 17 individual QTLs in M730 had negative

genetic effects. Four of them regulated 4C aliphatic glucosinolates, total aliphatic glucosinolates and total glucosinolate content. Six out of the 25 individual QTLs in ZT had negative genetic effects. Two of them regulated total aliphatic glucosinolates and total glucosinolate content. Six out of the 18 individual QTLs in M692 had negative genetic effects. Three of them regulated 4C aliphatic glucosinolates, glucoalyssin and 5C aliphatic glucosinolates. All the QTLs having negative genetic effects on aliphatic or total glucosinolate content were located on other chromosomes except for A9 and C7, had minor genetic effects and explained less than 13% of their phenotypic variances.

Almost all of the QTLs controlling glucobrassicin had negative genetic effects except for the one at 2.5 cM on chromosome A9 in ZT.

QTLs Controlling Sclerotinia Stem Rot Resistance in *B. napus*

QTLs controlling canola Sclerotinia stem rot resistance in *B. napus* were distributed unevenly among the A and C subgenomes and the 19 chromosomes. Li et al. (2015) analyzed the QTLs identified from five studies by Mei et al. (2013), Wei et al. (2014), Wu et al. (2013), Zhao and Meng (2003b) and Zhao et al. (2006) and found that the C subgenome with chromosome C9 and C6 possessed the most QTLs whereas chromosome A4, A7 and A10 had no QTLs. The markers on the physical map from Li et al. (2015) had large gaps on most of the 19 chromosomes.

Population M730

Thirty eight QTLs were identified from 10 replicates. Based on their peak positions, CI and genetic effects, these QTLs could be grouped into 22 QTLs. For example, QTLs *Bna7S1-1* and *Bna7S1-2* might be the same QTL detected from replicates F813S3 and

D3SE12. These two QTLs were close to each other on the same chromosome A2, might share CI, and had similar negative genetic effects. QTLs *Bna7S2-1* and *Bna7S2-2* on chromosome A2 from D3OC13 and D4AP12 shared CI from 42.9 to 55.3 cM and both had negative genetic effects, suggesting they might be the same QTL. Likewise, three QTLs *Bna7S9-1* to *Bna7S9-3* on chromosome A7 from replicates D3OC13, D4AP12 and D3SE12, two QTLs *Bna7S10-1* and *Bna7S10-2* on chromosome A7 from replicates D6MA12 and JUN93, three QTLs *Bna7S11-1* to *Bna7S11-3* on chromosome A7 from replicates D3SE12, F412 and D2JU12, two QTLs *Bna7S12-1* and *Bna7S12-2* on chromosome A9 from replicates SE93 and D3SE12, three QTLs *Bna7S13-1* to *Bna7S13-3* on chromosome A9 from replicates D6MA12, D3SE12 and JUN93, two QTLs *Bna7S16-1* and *Bna7S16-2* on chromosome C4 from replicates F812 and F412, three QTLs *Bna7S17-1* to *Bna7S17-3* on chromosome C6 from replicates D4AP12, F812 and D6MA12, three QTLs *Bna7S18-1* to *Bna7S18-3* on chromosome C6 from replicates JUN93, F813S3 and D3OC13, and two QTLs *Bna7S20-1* and *Bna7S20-2* on chromosome C9 from replicates SE93 and F813S3, each group might be a common QTL detected from different replicates.

Population M692 and ZT

In population M692, 6 QTLs controlling Sclerotinia stem rot resistance were detected on 5 chromosomes individually from 4 replicates. There were no common QTLs detected from different replicates.

In population ZT, four QTLs controlling Sclerotinia stem rot resistance were detected from Year 2009 and 2010 could be grouped into two common ones, or the common QTLs *BnaZS1* on chromosome A3 and *BnaZS2* on chromosome A7.

QTLs across the Three DH populations

In *B. napus*, chromosome A2 was homologous to C2, chromosome A3 partially homologous to C3 and C7, and chromosome A7 partially homologous to C6 and C7 (Parkin et al. 2005). In the present study, QTLs were identified around 50 cM (*Bna7S2-1* at 42.5 cM and *Bna7S2-2* at 55.8 cM) from the left telomere on chromosome A2 in replicates D3OC13 and D4AP12 from population M730. One QTL – *Bna6S3* at 49.7 cM on chromosome C2, was observed in J31C from population M692. These markers might be closely related. Likewise, *Bna7S6* at 100.9 cM on chromosome A3 in replicate F412 from M730 was proximal to *Bna6S6* at 98.4 cM on chromosome C7 in replicate FC6 from M692. A common QTL was identified on the lower half on chromosome A3 with SRAP markers in two replicates from population ZT. QTLs were observed around 100 cM (*Bna7S11-1* at 97.4 cM, *Bna7S11-2* at 101.2 cM and *Bna7S11-3* at 104.7 cM) on chromosome A7 in three replicates from M730 which were proximal to *Bna6S6* on chromosome C7 from M692 and the common QTL (*BnaZS2-1* and *BnaZS2-2* at 117.5 cM) on chromosome A7 with SRAP markers from ZT. QTLs at around 25 cM (*Bna7S18-1* and *Bna7S18-2* at 25.0 cM, and *Bna7S18-3* at 27.4 cM) on chromosome C6 from M730 were proximal to *Bna6S4* at 21.4 cM from M692 on chromosome C6. It is possible that homoeologs from both the A and C subgenomes contribute to Sclerotinia stem rot resistance since several QTLs were mapped to homoeologous chromosome regions. However, further investigations would be needed to align QTLs identified with SNPs and SRAPs to pinpoint major and heritable genomic regions explaining Sclerotinia stem rot resistance trait in *B. napus* and identify the genes underlying the traits through gene mapping and gene functional analysis.

Genetic Control of Sclerotinia Stem Rot Resistance

Additive, epistatic and major QTL effects were important factors in *B. napus* resistance to canola Sclerotinia stem rot (Zhao et al. 2003a). The additive effects of QTLs/genes are the primary explanation of plant resistance to *S. sclerotiorum* (Fusari et al. 2012). Both positive and negative genetic effects of the QTLs on canola Sclerotinia stem rot resistance were observed in populations M730 and M692 while positive genetic effects were solely observed in ZT. In general, genetic effects of the QTLs detected in the study were relatively low based on the means of 14.13% in M730, 16.96% in M692, and 8.81% in ZT that explained phenotypic variances by these QTLs, respectively. Weak QTL effects in sunflower controlling resistance to *S. sclerotiorum* disease were reported by Micic et al. (2004), who claimed the usage of marker assisted selection (MAS) in practical breeding for the crop would be difficult. Weak QTL effects were also reported by Li et al. (2010) on white mold in soybean.

The Relationship between Seed Glucosinolates and Sclerotinia Stem Rot

Glucosinolates are recommended as biocides to control plant diseases in *Brassica* (Sotelo et al. 2015). In *B. napus,* glucosinolates function to suppress insect pests and *S. sclerotiorum*, and seed and leaf glucosinolate contents are associated (Lu et al. 2014). It means that plant resistance to Sclerotinia stem rot can be compromised when low seed glucosinolate content canola cultivars are developed. Therefore, it is necessary to determine the relationship between seed glucosinolate content and Sclerotinia stem rot resistance in canola. Zhao and Meng (2003a) observed that one QTL regulated aliphatic glucosinolates and another regulated indolic glucosinolates in seed contributed to canola Sclerotinia stem rot resistance. Fan et al. (2008) reported that the glucosinolate 2-

hydroxy-4-pentenyl increased canola Sclerotinia stem rot resistance, 1-methoxy-3-indole-methyl suppressed canola Sclerotinia stem rot resistance, but total glucosinolate content had no effect on plant resistance to canola Sclerotinia stem rot. Zhao et al. (2006) reported that there was no relationship between QTLs for seed total glucosinolate content and plant Sclerotinia stem rot resistance in *B. napus*. Sotelo et al. (2015) tested *S. sclerotiorum* under different dosages of several glucosinolates and hydrolytic glucosinolate products *in vitro* and found that 2-phenylethyl, phenetyl, allyl, gluconapin, sulforaphane, glucoiberin and glucobrassicanapin had different levels of resistance with indole-3-ylmethyl being emphasized.

In the present study, there was only one case where the QTLs regulating progoitrin and suppressing canola Sclerotinia stem rot were located at the same position, *Bna7G9* for progoitrin and *Bna7S20-1* and *Bna7S20-2* at 53.9 cM on chromosome C9 in M730. All other QTLs for GSL traits were far away from those involved with canola Sclerotinia stem rot resistance on the same or on different chromosomes. Therefore, the two traits involved in contributing to seed GSLs and canola Sclerotinia stem rot resistance did not share common genetic components and therefore must have different genetic mechanisms. In addition, there was no association determined between phenotypic performances of the two traits, glucosinolate contents from the five components progoitrin, gluconapin, glucoalyssin, glucobrassicanapin and 4-hydroxy-3-indolylmethyl and their summations and stem lesion lengths caused by artificial inoculation of *S. sclerotiorum* mycelia on adult plants found in the three populations.

GENERAL CONCLUSIONS

1. SNP markers are well suited for genetic linkage mapping, genotyping, and QTL analyses. There are abundant numbers of SNP markers in the whole genome of *B. napus*. SNP markers tend to be widely scattered among the 19 chromosomes, though there are instances of large clusters of SNP markers. This trend highlights that the number of clusters decreases as the number of markers per cluster increases. There are "hotspots" in *B. napus* genome which harbor large numbers of SNP markers. On the other hand, there are also "coldspots" where SNP markers are lacking either on the ends or at other locations in the chromosomes. SNPs can be used together with SRAPs to facilitate QTL mapping.

2. In *B. napus*, 2-hydroxy-3-butenyl, 3-butenyl, 5-methylsulfinylpentyl, 4-pentenyl and 4-hydroxy-3-indolylmethyl are major glucosinolate components in seeds. The glucosinolate 2-hydroxy-3-butenyl generally predominates in 4C aliphatic glucosinolates which are more prevalent in aliphatic glucosinolates. Rapeseed seeds contain predominantly aliphatic glucosinolates. There is a tendency that the proportion of aliphatic glucosinolate content decreases or that the proportion of indolic 4-hydroxy-3-indolylmethyl increases with the decrease of total glucosinolate content. In spring canola, 4-hydroxy-3-indolylmethyl predominates in total glucosinolate content.

3. Major glucosinolates and their combinations in seeds and involvement in Sclerotinia stem rot resistance in *B. napus* are quantitative traits and controlled by QTLs. These QTLs can have major or minor, positive or negative, additive or both additive and epistatic genetic effects on the two traits in segregating populations with homozygous individual lines.

4. The left half of chromosome A9 harbors major QTLs regulating 2-hydroxy-3-butenyl, major 4C aliphatic glucosinolate combinations, 4-pentenyl, major aliphatic glucosinolate combinations, 4-hydroxy-3-indolylmethyl and major glucosinolate combinations in seeds of high glucosinolate content *B. napus* populations. These QTLs are the major ones contributing to the accumulation of major glucosinolate components and their combinations in *B. napus* seed. However, these QTLs are ready to be eliminated, and can cause a great reduction of total glucosinolate content and changes in glucosinolates. There is a major QTL approximately 40 cM from the left telomere on chromosome A9 which regulates 2-hydroxy-3-butenyl in high glucosinolate content rapeseed.

5. In *B. napus*, a single QTL can regulate multiple glucosinolate components. Likewise, multiple QTLs can work in a concerted effort to regulate a single glucosinolate component in seed.

6. In *B. napus*, Sclerotinia stem rot resistance in populations derived from 'Zhongyou 821' and similar trait donors is weak and strongly influenced by environmental factors.

7. There is no direct genetic and phenotypic relationship between adult plant Sclerotinia stem rot resistance and its major glucosinolate content and combinations in *B. napus* seed.

FUTURE STUDIES

New resistant sources to Canola Sclerotinia Stem Rot Resistance. Sources of strong resistance to canola Sclerotinia stem rot in *B. napus* are necessary to efficiently and effectively transfer the trait to different germplasm. Wild *B. incana* in the C genome was reported to have strong resistance to *S. sclerotiorum* while *B. rapa* lacked (Ding et al. 2013; Mei et al. 2013; Mei et al. 2015; Taylor et al. 2015). In addition, other sources with strong resistance to canola Sclerotinia stem rot in *B. napus* (Li et al. 2009) and *B. juncea* (Sharma et al. 2009) were reported. Major QTLs controlling canola Sclerotinia stem rot resistance in the A, B, C genomes and other close relatives of *B. napus* which can be readily introgressed should be prioritized in future genetic and genomic studies and molecular breeding practices.

Accurately phenotyping methods to assess canola Sclerotinia stem rot resistance. Reproducible, quick, easy-to-operate and cheap inoculation methods are critical for scientific researches and practical breeding with quantitative traits including canola Sclerotinia stem rot resistance. Although stem inoculation with *Sclerotinia* mycelia has been widely adopted in *Brassica* ssp. and other economically important crops, more accurate and easier inoculation, disease assessment and analytical methods to truly examine the entirety of the infection are necessary for canola Sclerotinia stem rot resistance cultivar breeding.

Gene analysis and trait transfer. Trait improvements are in constant demand for crops, and involve gene annotation, transfer and pyramiding. Sclerotinia stem rot resistance and glucosinolate reduction and optimization in seeds are two important traits prioritized for oilseed rape cultivar development, especially in canola. There is more work needed to

decipher genetic and genomic mechanisms controlling the two traits in wide range of germplasm, especially in wild species of Brassicaceae. Due to the colinearity of species in Brassicaceae, gene and sequence information from Arabidopsis, *B. rapa, B. oleracea, B. nigra* and *B. napus* can be used to annotate functional genes/major QTLs controlling the two traits to facilitate localizing transferrable genes/QTLs in practical breeding.

Compared to bi-parental linkage mapping, association mapping will be another powerful tool to study quantitative traits including Sclerotinia stem rot resistance and glucosinolate regulation as long as accurate phenotypic data collected from a wide range of germplasm are available. Also, association mapping can be used to anchor QTLs and haplotypes associated with traits of interest at high resolution.

NGS as a new technology characterized by high throughput, multiplexing, fast and low cost and accuracy, has become more and more popular, and has been used to assemble *de novo* sequence scaffolds of *B. rapa* "Chiifu-401", *B. oleracea* "TO1000" and *B. napus* "Darmor-*bzh*". NGS is useful for genotyping, gene localization and annotation, and marker development. An important function of NGS is to be used in RNA sequencing (RNA-Seq) to study levels of gene expression under biotic and abiotic stresses. With the aid of newly assembled *B. rapa, B. oleracea* and *B. napus* reference genomic maps, NGS will be a valuable tool to be used for anchoring genes regulating Sclerotinia stem rot resistance and glucosinolate contents within the aforementioned QTL regions in diverse germplasm for cultivar development.

Trait transfer is another consideration for selected traits. Mei et al. (2015) developed a hexaploid breeding population as a bridge to transfer canola Sclerotinia stem rot resistance from the C genome into *B. napus*. The resistance to canola Sclerotinia stem rot

resistance was expressed well in their BC_1 plants but no further backcrosses were done to confirm the transferability of the trait in higher backcrossed generations which were required to eliminate drag from unfavorable genes. More work is needed to improve the transgression of quantitative traits.

The relationship between seed glucosinolates and Sclerotinia stem rot. The present study concluded that there was no association between seed glucosinolate content and plant Sclerotinia stem rot resistance in *B. napus*. This relationship will be deemed appropriate for study if combinations of major glucosinolate components in plant organs, especially in stems, are proven to have relationships to plant Sclerotinia stem rot resistance.

REFERENCES

Adams, P.B. and Ayers, W.A. 1979. Ecology of *Sclerotinia* species. Phytopathol. 69:896-898.

Aeron, A., Dubey, R.C., Maheshwari, D.K., Pandey, P., Bajpai, V.K. and Kang, S.C. 2011, Multifarious activity of bioformulated *Pseudomonas fluorescens* PS1 and biocontrol of *Sclerotinia sclerotiorum* in Indian rapeseed (*Brassica campestris* L*.).* Eur. J. Pl. Pathol. 131:81-93.

Agerbirk, N. and Olsen, C.E. 2012. Glucosinolate structures in evolution. Phytochem. 77:16-45.

Alkooranee, J.T., Aledan, T.R., Xiang, J., Lu, G. and Li, M. 2015. Induced systemic resistance in two genotypes of *Brassica napus* (AACC) and *Raphanus oleracea* (RRCC) by *Trichoderma* isolates against *Sclerotinia sclerotiorum*. Amer. J. Pl. Sci. 6:1662-1674.

Allender, C.J. and King, G.J. 2010. Origins of the amphiploid species *Brassica napus* L. investigated by chloroplast and nuclear molecular markers. BMC Pl. Biol. 10:54.

Anas, O. and Reeleder, R.D. 1988. Feeding habits of larvae of *Bradysia coprophila* on fungi and plant tissue. Phytoprot. 69:73-78.

Aneja, B., Yadav, N.R., Chawla, V. and Yadav, R.C. 2012. Sequence-related amplified polymorphism (SRAP) molecular marker system and its applications in crop improvement. Mol. Breed. 30:1635-1648.

Arumuganathan, K. and Earle, E.D. 1991. Nuclear DNA content of some important plant species. Pl. Mol. Biol. Reporter, 9(3):211-215.

Auclair, J., Roland, G.J., Cober, E., Grael, G.L., Steadman, J.R., Zilka, J. and Rajcan, I. 2004. Development of a new field inoculation technique to assess partial resistance in soybean to *Sclerotinia sclerotiorum*. Can. J. Pl. Sci. 84:57-64.

Babula, D., Kaczmarek, M., Barakat, A., Delseny, M., Quiros, C.F. and Sadowski, J. 2003. Chromosomal mapping of *Brassica oleracea* based on ESTs from *Arabidopsis thaliana*: Complexity of the comparative map. Mol. Genet. Genomics, 268:656-665.

Barari, H., Alavi, V., Yasari, E. and Badalyan, S.M. 2011. Study of genetic variations based on the morphological characteristics, within the population of *Sclerotinia sclerotiorum* from the major oilseed planting areas in Iran. Int. J. Bio. 3:61-66.

Bardin, S.D. and Huang, H.C. 2001. Research on biology and control of *Sclerotinia* diseases in Canada. Can. J. Pl. Pathol. 23(1):88-98.

Barbetti, M.J., Banga, S.K., Fu, T.D., Li, Y.C., Singh, D., Liu, S.Y., Ge, X.T. and Banga, S.S. 2014. Comparative genotype reactions to *Sclerotinia sclerotiorum* within breeding populations of *Brassica napus* and *B. juncea* from India and China. Euphytica, 197:47-59.

Barbetti, M.J., Li, C.X., Banga, S.S., Banga, S.K., Singh, D., Sandhu, P.S., Singh, R., Liu, S.Y. and You, M.P. 2015. New host resistances in *Brassica napus* and *Brassica juncea* from Australia, China and India: Key to managing Sclerotinia stem rot (*Sclerotinia sclerotiorum*) without fungicides. Crop Prot. 78:127-130.

Baskar, V., Gururani, M.A., Yu, J.W. and Park, S.W. 2012. Engineering glucosinolates in plants: current knowledge and potential uses. Appl. Biochem. Biotechnol. 168(6):1694-717.

Bateman, D.F. 1972. The polygalacturonase complex produced by *Sclerotium rolfsii*. Physio. Pl. Pathol. 2(2):175-184.

Becelaere, G.V. and Miller, J.F. 2004. Methods of inoculation of sunflower heads with *Sclerotinia sclerotiorum*. Helia. 27(41):137-142.

Bednarek, P., Piślewska-Bednarek, M., Svatoš, A., Schneider, B., Doubský, J., Mansurova, M., Humphry, M., Consonni, C., Panstruga, R., Sanchez-Vallet, A., Molina, A. and Schulze-Lefert, P. 2009. A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. Sci. 323:101-106.

Behla, R.S., Fernando, W.G.D. and Li, G. 2009. Identification of quantitative trait loci for resistance against Sclerotinia stem rot in *Brassica napus*. Can. J. Pl. Pathol. 31:477-478.

Boland, G.J. and Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. Can. J. Pl. Pathol. 16:93-108.

Bolton, M.D., Thomma, B.P.H.J. and Nelson, B.D. 2006. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. Mol. Pl. Pathol. 7:1-16.

Bom, M. and Boland, G.J. 2000. Evaluation of disease forecasting variables for Sclerotinia stem rot (*Sclerotinia sclerotiorum*) of canola. Can. J. Pl. Sci. 80:889-898.

Bones, A.M. and Rossiter, J.T. 1996. The myrosinase-glucosinolate system, its organization and biochemistry. Physiologia Plantarum, 97(1):194-208.

Botstein, D., White, R.L., Skolnick, M. and Davis, R.V. 1980. Construction of a genetic map in man using restriction fragment length polymorphisms. Amer. J. Hum. Genet. 32:314-331.

Brader, G., Mikkelsen, M.D., Halkier, B.A. and Palva, E.T. 2006. Altering glucosinolate profiles modulates disease resistance in plants. Pl. J. 46:758-767.

Bradley, C.A., Henson, R.A., Porter, P.M., LeGare, D.G., del Rio, L.E. and Khot, S.D. 2006a. Response of canola cultivars to *Sclerotinia sclerotiorum* in controlled and field environments. Pl. Dis. 90:215-219.

Bradley, C.A., Lamey, H.A., Endres, G.J., Henson, R.A., Hanson, B.K., McKay, K.R., Halvorson, M., LeGare, D.G. and Porter, P.M. 2006b. Efficacy of fungicides for control of Sclerotinia stem rot of canola. Pl. Dis. 90:1129-1134.

Brand, T.S., Smith, N. and Hoffman, L.C. 2007. Anti-nutritional factors in canola produced in the Western and Southern Cape areas of South Africa. S. Afr. J. Anim. Sci. 37(1):45-50.

Bressan, M., Roncato, M.A., Bellvert, F., Comte, G., Haichar, F.Z., Achouak, W. and Berge, O. 2009. Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. ISME J. (3):1243-1257.

Brown, A.F., Yousef, G.G., Chebrolu, K.K., Byrd, R.W., Everhart, K.W., Thomas, A., Reid, R.W., Parkin, I.A., Sharpe, A.G., Oliver, R., Guzman, I. and Jackson, E.W. 2014. High-density single nucleotide polymorphism (SNP) array mapping in *Brassica oleracea*: identification of QTL associated with carotenoid variation in broccoli florets. Theor. Appl. Genet. 127(9):2051-2064.

Buchwaldt, L., Hegedus, D., Lydiate, D., Parkin, I., Rimmer, R., Fu, F., Garg, H. and Gyawali. 2012. Advances in Sclerotinia resistance research. Agriculture and Agri-Food Canada, Canola Industry Meeting, Saskatoon, Canada. URL: http://www.agwest.sk.ca/kaizen/CIM-AGCI/CIM2012/Buchwaldt_CIM2012.pdf (4 November 2015).

Cai, G., Yang, Q., Yi, B., Fan, C., Edwards, D., Batley, J. and Zhou, Y. 2014. A complex recombination pattern in the genome of allotetraploid *Brassica napus* as revealed by a high-density genetic map. PLoS ONE, 9(10): e109910. doi:10.1371/journal.pone.0109910.

Campbell, L.D. and Schöne, F. 1998. Effects of antinutritional factors in rapeseed. p. 185-198. In: Proc 3rd Int. Workshop on 'Antinutritional factors in legume seeds and rapeseed'. Eur. Asso. Anim. Prod. Publication, No. 93.

Cavell, A.C., Lydiate, D.J., Parkin, I.A.P., Dean, C. and Trick, M. 1998. Colinearity between a 30-centiMorgan segment of *Arabidopsis thaliana* chromosome 4 and duplicated regions within the *Brassica napus* genome. Genome, (41):62-69.

Cessna, S.G., Sears, V.E., Dickman, M.B. and Low, P.S. 2000. Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. Pl. Cell. 12:2191-2199.

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I.A.P., Tang, H., Wang, X., Chiquet, J., Belcram, H., Tong, C., Samans, B., Corréa, M., Silva, C.D., Just, J., Falentin, C., Koh, C.S., Clainche, I.L., Bernard, M., Bento, P., Noel, B., Labadie, K., Alberti, A., Charles, M., Arnaud, D., Guo, H., Daviaud, C., Alamery, S., Jabbari, K., Zhao, M., Edger, P.P., Chelaifa, H., Tack, D., Lassalle, G., Mestiri, I., Schnel, N., Paslier, M.C.L., Fan, G., Renault, V., Bayer, P.E., Golicz, A.A., Manoli, S., Lee, T.H., Thi, V.H.D., Chalabi, S., Hu, Q., Fan, C., Tollenaere, R., Lu, Y., Battail, C., Shen, J., Sidebottom, C.H.D., Wang, X., Canaguier, A., Chauveau, A., Bérard, A., Deniot, G., Guan, M., Liu, Z., Sun, F., Lim, Y.P., Lyons, E., Town, C.D., Bancroft, I., Wang, X., Meng, J., Ma, J., Pires, J,C., King, G.J., Brunel, D., Delourme, R., Renard, M., Aury, J.M., Adams, K.L., Batley, J., Snowdon, R.J., Tost, J., Edwards, D., Zhou, Y., Hua, W., Sharpe, A.G., Paterson, A.H., Guan, C. and Wincker, P. 2014. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Sci. 345(6199):950-953.

Chen, Y. and Wang, D. 2005. Two convenient methods to evaluate soybean for resistance to *Sclerotinia sclerotiorum*. Pl. Dis. 89:1268-1272.

Cheung, F., Trick, M., Drou, N., Lim, Y.P., Park, J.Y., Kwon, S.J., Kim, J.A., Scott, R., Pires, C., Paterson, A.H., Town, C. and Bancroft, I. 2009. Comparative analysis between homoeologous genome segments of *Brassica napus* and its progenitor species reveals extensive sequence-level divergence. Pl. Cell. 21:1912-1928.

Cheung, W.Y., Landry, B.S., Raney, P. and Rakow, G.F.W. 1998. Molecular mapping of seed quality traits in *Brassica juncea* L. Czern. and Coss. Acta Hort. 459:139-148.

Coley-Smith, J.R. 1979. Survival of plant-pathogenic fungi in soil in the absence of host plants. p. 39-57. In: Soil-Borne Plant Pathogens. Schippers, B. and Gams, W. eds. Academic Press, London.

Coley-Smith, J.R. and Cooke, R.C. 1971. Survival and germination of fungal sclerotia. Annu. Rev. Phytopathol. 9:65-92.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B. and Pang, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica, 142:169-196.

Daxenbichler, M.E., Spencer, G.F., Carison, D.G., Rose, G.B., Brinker, A.M. and Rowell, R.G. 1991. Glucosinolate composition of seeds from 297 species of wild plants. Phytochem. 30(8):2623-2638.

de Bary, A. 1886. Ueber einige *Sclerotinien* und *Sclerotien krankheiten.* Botanische Zeitimg, 44:374-474.

de Quiroz, H. and Mithen, R. 1996. Molecular markers for low-glucosinolate alleles in oilseed rape (*Brassica napus* L.). Mol Breed. 2(3):277-281.

del Río, L.E., Bradley, C.A., Henson, R.A., Endres, G.J., Hanson, B.K., McKay, K., Halvorson, M., Porter, P.M., Le Gare, D.G. and Lamey, H.A. 2007. Impact of Sclerotinia stem rot on yield of canola. Pl. Dis. 91:191-194.

Delourme, R., Falentin, C., Fomeju, B.F., Boillot, M., Lassalle, G., André, I., Duart, J., Gauthier, V., Lucante, N., Marty, A., Pauchon, M., Pichon, J.P., Ribière, N., Trotoux, G., Blanchard, P., Rivière, N., Martinant, J.P. and Pauquet, J. 2013. High-density SNP-based genetic map development and linkage disequilibrium assessment in *Brassica napus* L. BMC Genome, 14:120.

Ding,Y., Mei, J., Li, Q., Liu, Y., Wan, H., Wang, L., Becker, H.C. and Qian, W. 2013. Improvement of *Sclerotinia sclerotiorum* resistance in *Brassica napus* by using *B. oleracea*. Genet. Resour. Crop Evol. 60:1615-1619.

Disi, J.O., Mei, J., Wei, D., Ding, Y. and Qian, W. 2014. Inheritance of leaf and stem resistance to *Sclerotinia sclerotiorum* in a cross between *Brassica incana* and *Brassica oleracea* var. alboglabra. J. Agric. Sci. 152:146-152.

Dong, X., Ji, R., Guo, X., Foster, S.J., Chen, H., Dong, C., Liu, Y., Hu, Q. and Liu, S. 2008. Expressing a gene encoding wheat oxalate oxidase enhances resistance to *Sclerotinia sclerotiorum* in oilseed rape (*Brassica napus*). Planta, 228:331-340.

Drozdowska, L., Thangstad, O.P., Beisvaag, T., Evjen, K., Bones, A. and Iversen, T.H. 1992. Myrosinase and myrosin cell development during embryogenesis and seed maturation. Israel J. Bot. 41:213-223.

Duncan, R.W. 2003. Evaluation of host tolerance, biological, chemical, and cultural control of *Sclerotinia sclerotiorum* in sunflower (*Helianthus annuus* L.). p.7-40, 88-114. MSc. Thesis. University of Manitoba. Winnipeg, MB Canada.

Durstewitz, G., Polley, A., Plieske, J., Luerssen, H., Graner, E.M., Wieseke, R. and Ganal, M.W. 2010. SNP discovery by amplicon sequencing and multiplex SNP genotyping in the allopolyploid species *Brassica napus*. Genome, 53:948-956.

Edwards, D., Batley, J., Cogan, N.O.I., Forster, J.W. and Chagné, D. 2007. Single nucleotide polymorphism discovery. p. 53-76. In: Association mapping in plants. Oraguzie, et al. eds. Springer, New York.

EFSA. 2008. Opinion of the scientific panel on contaminants in the food chain on a request from the European Commission on glucosinolates as undesirable substances in animal feed. The European Food Safety Authority. The EFSA J. 590:1-76.

Eijk, M.V., Hogers, R. and Heijnen, L. 2003. Method for generating oligonucleotides, in particular for the detection of amplified restriction fragments obtained using AFLP. Patent: US 20030175729 A1.

Ettlinger, M.G. and Lundeen, A.J. 1956. The structures of sinigrin and sinalbin: an enzymatic rearrangement. J. Amer. Chem. Soc. 78:4172-4173.

Fahey, J.W., Zalcmann, A.T. and Talalay, P. 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochem. 56:5-51.

Fan, Z.X., Lei, W.X., Sun, X.L., Yu, B., Wang, Y.Z. and Yang, G.S. 2008. The association of *Sclerotinia sclerotiorum* resistance with glucosinolates in *Brassica napus* double-low DH population. J. Pl. Pathol. 90(1):43-48.

Feng, J., Long, Y., Shi, L., Shi, J., Barker, G. and Meng, J. 2012. Characterization of metabolite quantitative trait loci and metabolic networks that control glucosinolate concentration in the seeds and leaves of *Brassica napus*. New Phytologist, 193:96–108.

Fernando, W.G.D., Nakkeeran, S., Zhang, Y. and Savchuk, S. 2007. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary by *Pseudomonas* and *Bacillus* species on canola petals. Crop Prot. 26(2):100-107.

Ferreira, M.E., Williams, P.H. and Osborn, T.C. 1994. RFLP mapping of *Brassica napus* using doubled haploid lines. Theor. Appl. Genet. 89(5):615-21.

Finlayson, J.E., Rimmer, S.R. and Pritchard, M.K. 1989. Infection of carrots by *Sclerotinia sclerotiorum*. Can. J. Pl. Pathol. 11(3):242-246.

Fourmann, M., Barret, P., Froger, N., Baron, C., Charlot, F., Delourme, R. and Brunel, D. 2002. From *Arabidopsis thaliana* to *Brassica napus*: development of amplified consensus genetic markers (ACGM) for construction of a gene map. Theor. Appl, Genet. 105:1196-1206.

Fu, Y., Lu, K., Qian, L., Mei, J., Wei, D., Peng, X., Xu, X., Li, J., Frauen, M., Dreyer, F., Snowdon, R.J. and Qian, W. 2015. Development of genic cleavage markers in association with seed glucosinolate content in canola. Theor. Appl. Genet. 128(6):1029-37.

Fuckel, L. 1870. Symbolic mycologicae. Beitrage zur Kenntniss der Rheinischen Pilze. Jhrb. Nassauischen Vercins Naturk, 23:1-459.

Fusari, C.M., Rienzo, J.A.D., Troglia, C, Nishinakamasu, V., Moreno, M.V., Maringolo, C., Quiroz, F., Alvarez, D., Escande, A., Hopp, E., Heinz, R., Lia, V.V. and Paniego, N.B. 2012. Association mapping in sunflower for Sclerotinia head rot resistance. BMC Pl. Biol. 12:93.

Garg, H., Li, H., Sivasithamparam, K., Kuo, J. and Barbetti, M.J. 2010. The infection processes of *Sclerotinia sclerotiorum* in cotyledon tissue of a resistant and a susceptible genotype of *Brassica napus*. Annu. Bot. doi:10.1093/aob/mcq196. URL: www.aob.oxfordjournals.org (8 November 2015).

Gautam, M., Ge, X. and Li, Z. 2014. *Brassica*. p. 207-229. In: Alien gene transfer in crop plants; Vol. II: Achievements and impacts. Pratap, A. and Kumar, J. Springer, New York.

Gerlagh, M., Whipps, J.M., Budge, S.P. and Goossen-van de Geijn, H.M. 1996. Efficiency of isolates of *Coniothyrium minitans* as mycoparasites of *Sclerotinia sclerotiorum*, *Sclerotium cepivorum* and *Botrytis cinerea* on tomato stem pieces. Eur. J. Pl. Pathol. 102:787-793.

Gilbert, R.G. 1991. Burning to reduce sclerotia of *Sclerotinia sclerotiorum* in alfalfa seed fields of southeastern Washington. Pl. Dis. 75:141-142.

Gulya, T., Rashid, K.Y. and Masirevic, S.M. 1997. Sunflower diseases. p.834. In: Sunflower Technology and Production. Schneiter, A.A. eds. Amer. Soc. Agr. Crop Sci. Soc. Amer. Soil Soc. Amer. Madison.

Guo, X. and Stotz, H.U. 2010. ABA signaling inhibits oxalate-induced production of reactive oxygen species and protects against *Sclerotinia sclerotiorum* in *Arabidopsis thaliana*. Eur. J. Pl. Pathol. 128:7-19.

Gupta, S., Sangha, M.K., Kaur, G., Banga, S., Gupta, M., Kumar, H. and Banga, S.S. 2015. QTL analysis for phytonutrient compounds and the antioxidant molecule in mustard (*Brassica juncea* L.). Euphytica, 201:345-356.

Halkier, B.A. and Gershenzon, J. 2006. Biology and biochemistry of glucosinolates. Annu. Rev. Pl. Biol. 57:303-333.

Harper, A.L., Trick, M., Higgins, J., Fraser, F., Clissold, L., Wells, R., Hattori, C., Werner, P. and Bancroft, I. 2012. Associative transcriptomics of traits in the polyploid crop species *Brassica napus*. Nat. Biotechnol. 30(8):798-802.

Hasan, M., Seyis, F., Badani, A.G., Pons-Kuhnemann, J., Friedt, W., Luhs, W. and Snowdon, R.J. 2006. Surveying genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet. Resour. Crop Evol. 53:793-802.

Heran, A., McCartney, H.A., and Li, Q. 1999. The effect of petal characteristics, inoculum density and environmental factors on infection of oilseed rape by *Sclerotinia sclerotiorum*. In: Proc10th Int. Rapeseed Congress: New Horizons for an Old Crop. Wratten, N. and Salisbury, P.A. eds. 26-29 September 1999, Canberra, Australia. URL. http://www.regional.org.au/au/gcirc/3/428.htm (8 November 2015).

Hirani, A.H., Li, G., Zelmer, C.D., McVetty, P.B.E., Asif, M. and Goyal, A. 2012. Molecular genetics of glucosinolate biosynthesis in *Brassicas*. p. 189-216. In: Genetic Manipulation and Application Aspects, Crop Pl. Aakash Goyal, A. InTech, URL: http://www.intechopen.com/books/crop-plant/molecular-genetics-of-glucosinolate-biosynthesis-in-brassicas (9 November 2015).

Hirani, A.H., Zelmer, C.D., McVetty, P.B.E., Daayf, F. and Li, G. 2013. Homoeologous *GSL-ELONG* gene replacement for manipulation of aliphatic glucosinolates in *Brassica rapa* L. by marker assisted selection. Front Pl. Sci. 4:55.

Howell, P.M., Sharpe, A.G. and Lydiate, D.J. 2003. Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome, 46(3):454-460.

Hu, J. and Vick, B.A. 2003. Target region amplification polymorphism: A novel marker technique for plant genotyping. Pl. Mol. Bio. Rep. 21:289-294.

Hu, X., Roberts, D.P., Jiang, M. and Zhang, Y. 2005. Decreased incidence of disease caused by *Sclerotinia sclerotiorum* and improved plant vigor of oilseed rape with *Bacillus subtilis* Tu-100. Appl. Microbiol. Biotech. 68:802-807.

Hu, X., Roberts, D.P., Maul, J.E., Emche, S.E., Liao, X., Guo, X., Liu, Y., McKenna, L.F., Buyer, J.S. and Liu, S. 2011. Formulations of the endophytic bacterium *Bacillus subtilis* Tu-100 suppress *Sclerotinia sclerotiorum* on oilseed rape and improve plant vigor in field trials conducted at separate locations. Can. J. Microbio. 57(7):539-546.

Huang, H.C. 1980. Control of Sclerotinia wilt of sunflower by hyperparasites. Can. J. Pl. Pathol. 2:26-32.

Huang, H.C. and Blackshaw, R.E. 1995. Influence of herbicides on the carpogenic germination of *Sclerotinia sclerotiorum* sclerotia. Bot. Bull. Acad. Sin. (Taipei) 36:59-64.

Huang, H.C. and Erickson, R.S. 2000. Biocontrol of apothecial production of *Sclerotinia sclerotiorum* in pulse and oilseed crops. Annu. Rep. Bean. Improv. Coop. 43:90-91.

Huang, H.C. and Kozub, G.C. 1991. Temperature requirements for carpogenic germination of sclerotia of *Sclerotinia sclerotiorum* isolates of different geographic origin. Bot. Bull. Academia Sinica, 32:279-286.

Huang, H.C. and Sun, S.K. 1991. Effects of S-H mixture or Perlka^{TM} on carpogenic germination and survival of sclerotia of *Sclerotinia sclerotiorum*. Soil Biol. Biochem. 23:809-813.

Huang, H.C. Chang, C. and Kozub, G.C. 1998. Effect of temperature during sclerotial formation, sclerotial dryness, and relative humidity on myceliogenic germination of sclerotia of *Sclerotinia sclerotiorum*. Can. J. Bot. 76:494-499.

Inaba, R. and Nishio, T. 2002. Phylogenetic analysis of *Brassiceae* based on the nucleotide sequences of the S-locus related gene, *SLR1*. Theor. Appl. Genet. 105:1159-1165.

Ishida, M., Hara, M., Fukino, N., Kakizaki, T. and Morimitsu, Y. 2014. Glucosinolate metabolism, functionality and breeding for the improvement of Brassicaceae vegetables. Breed. Sci. 64:48-59.

Jaccoud, D., Peng, K., Feinstein, D. and Kilian, A. 2001. Diversity Arrays: a solid state technology for sequence information independent genotyping. Nuc. Acids Res. 29(4):e25. Oxford University Press.

Jamaux, I., Gelie, B. and Lamarque, C. 1995. Early stages of infection of rapeseed petals and leaves by *Sclerotinia sclerotiorum* revealed by scanning electron microscopy. Pl. Pathol. 44(1):22-30.

Johansen, T.J., Sidsel Fiskaa, H., S.F., Gunnar, B. and Jørgen M. 2016. Growth temperature affects sensory quality and contents of glucosinolates, vitamin C and sugars in swede roots (*Brassica napus* L. ssp. Rapifera Metzg.). Food Chem. 196:228-235.

Jurke, C.J. and Fernando, W.G.D. 2008. Comparison of growthroom screening techniques for the determination of physiological resistance to Sclerotinia stem rot in *Brassica napus*. Phytopathol. Pl. Prot. 41(3):157-174.

Kirkegaard, J.A., Robertson, M.J., Hamblin, P. and Sprague, S.J. 2006. Effect of blackleg and Sclerotinia stem rot on canola yield in the high rainfall zone of southern New South Wales, Australia. Aus. J. Agr. Res. 57(2):201-212.

Kliebenstein, D.J., Gershenzon, J. and Mitchell-Olds, T. 2001. Comparative quantitative trait loci mapping of aliphatic, indolic and benzylic glucosinolate production in *Arabidopsis thaliana* leaves and seeds. Genet. 159:359-370.

Koch, S., Dunker, S., Kleinhenz, B., Röhrig, M. and von Tiedemann, A. 2007. A crop loss-related forecasting model for *Sclerotinia* stem rot in winter oilseed rape. Phytopathol. 97:1186-1194.

Kohli, Y. and Kohn, L.M. 1998. Random association among alleles in clonal populations of *Sclerotinia sclerotiorum*. Fungal Gen. Biol. 23:139-149.

Kohli, Y., Brunner, L.J., Yoell, H., Milgroom, M.G., Anderson, J.B., Morralls, R.A.A. and Kohn, L.M. 1995. Clonal dispersal and spatial mixing in populations of the plant pathogenic fungus, *Sclerotinia sclerotiorum*. Mol. Eco. 4:69-77.

Kohn, L.M. 1995. The clonal dynamic in wild and agricultural plant-pathogen populations. Can. J. Bot. 73(Suppl. 1), Sect. E-H:S1231-S1240.

Kohn, L.M., Stasovski, E., Carbone, I., Royer, J. and Anderson, J.B. 1991. Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum*. Phytophthol. 81:480-485.

Körber, N., Bus, A., Li, J., Higgins, J., Bancroft, I., Higgins, E.E., Parkin, I.A.P., Salazar-Colqui, B., Snowdon, R.J. and Stich, B. 2015. Seedling development traits in *Brassica napus* examined by gene expression analysis and association mapping. BMC Pl. Biol. 15:136.

Kowalski, S.D., Lan, T.H., Feldmann, K.A. and Paterson, A.H. 1994. Comparative mapping of *Arabidopsis thaliana* and *Brassica oleracea* chromosomes reveals islands of conserved gene order. Genet. 138:499-510.

Kurle, J.E., Grau, C.R., Oplinger, E.S. and Mengistu, A. 2001. Tillage, crop sequence, and cultivar effects on Sclerotinia stem rot incidence and yield in soybean. Agron. J. 93:973-982.

Lagercrantz, U. and Lydiate, D. 1996. Comparative genome mapping in *Brassica*. Genet. 144:1903-1910.

Lamey, H.A. 1995. Survey of blackleg and Sclerotinia stem rot of canola in North Dakota in 1991 and 1993. Pl. Dis. 79(3):322-324.

Lan, T.H., DelMonte, T.A., Reischmann, K.P., Hyman, J., Kowalski, S., McFerson, J., Kresovich, S. and Paterson, A.H. 2000. An EST enriched comparative map of *Brassica oleracea* and *Arabidopsis thaliana*. Genom. Res. 10:776-788.

Landry, B.S., Hubert, N., Etoh, T., Harada, J.J. and Lincoln, S.E. 1991. A genetic map for *Brassica napus* based on restriction fragment length polymorphisms detected with expressed DNA sequences. Genome, 34(4):543-552.

Lanoiselet, H.T.L., Lanoiselet, V.M., Lewington, F.K., Ash, G.J. and Murray, G.M. 2005. Survival of *Sclerotinia* sclerotia under fire. Aus. Pl. Pathol. 34:311-317.

Lelivelt, C.L.C., Lange, W. and Dolstra, O. 1993. Intergeneric crosses for the transfer of resistance to the beet cyst nematode from *Raphanus sativus* to *Brassica napus*. Euphytica, 68:111-120.

Li, G. and Quiros, C.F. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103:455-461.

Li, Y., Chen, J., Bennett, R., Kiddle, G., Wallsgrove, R., Huang, Y. and He, Y. 1999. Breeding, inheritance, and biochemical studies on *Brassica napus* cv. Zhongyou 821: tolerance to *Sclerotinia sclerotiorum* (stem rot). p.61. In: Proc 10th Int. Rapeseed Congress, 26-29 September 1999, Canberra, Australia..

Li, F., Chen, B., Xu, K., Wu, J., Song, W., Bancroft, I., Harper, A.L., Trick, M., Liu, S., Gao, G., Wang, N., Yan, G., Qiao, J., Li, J., Li, H., Xiao, X., Zhang, T. and Wu, X. 2014. Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.). DNA Res. 21(4):355-367.

Li, G.Q., Huang, H.C., Miao, H.J., Erickson, R.S., Jiang, D.H. and Xiao, Y.N. 2006. Biological control of *Sclerotinia* diseases of rapeseed by aerial applications of the mycoparasite *Coniothyrium minitans*. Eur. J. Pl. Pathol. 114(4):345-355.

Li, C.X., Li, H., Siddique, A.B., Sivasithamparam, K., Salisbury, P., Banga, S.S., Banga, S., Chattopadhyay, C., Kumar, A., Singh, R., Singh, D., Agnihotri, A., Liu, S.Y., Li, Y.C., Tu, J., Fu, T.D., Wang, Y.F. and Barbetti, M.J. 2007. The importance of the type and time of inoculation and assessment in the determination of resistance in *Brassica napus* and *B. juncea* to *Sclerotinia sclerotiorum.* Aust. J. Agric. Res. 58:1198-1203.

Li, C.X., Liu, S.Y., Sivasithamparam, K. and Barbetti, M.J. 2009. New sources of resistance to Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* in Chinese and Australian *Brassica napus* and *B. juncea* germplasm screened under western Australian conditions. Aus. Pl. Pathol. 38(2):149-152.

Li, R., Rimmer, R., Buchwaldt, L., Sharpe, A.G., Seguin-Swartz, G., Coutu, C. and Hegedus, D.D. 2004. Interaction of *Sclerotinia sclerotiorum* with a resistant *Brassica napus* cultivar: expressed sequence tag analysis identifies genes associated with fungal pathogenesis. Fungal Genet. Biol. 41:735-753.

Li, D., Sun, M., Han, Y., Teng, W. and Li, W. 2010. Identification of QTL underlying soluble pigment content in soybean stems related to resistance to soybean white mold (*Sclerotinia sclerotiorum*). Euphytica, 172:49-57.

Li, J., Zhao, Z., Hayward, A., Cheng, H. and Fu, D. 2015. Integration analysis of quantitative trait loci for resistance to *Sclerotinia sclerotiorum* in *Brassica napus.* Euphytica, 205(2):483-489.

Libert, M.A. 1837. Plante crytogamicae arduennae (Exsiccati). no. 326.

Litholdo, C.G.J., Gomes, E.V., Lobo, M.J., Nasser, L.C.B. and Petrofeza, S. 2011. Genetic diversity and mycelial compatibility groups of the plant-pathogenic fungus *Sclerotinia sclerotiorum* in Brazil. Genet. Mol. Res. 10(2):868-877.

Liu, Z., Hammerlindl, J., Keller, W., McVetty, P.B.E., Daayf, F., Quiros, C.F. and Li, G. 2011. *MAM* gene silencing leads to the induction of C3 and reduction of C4 and C5 side-chain aliphatic glucosinolates in *Brassica napus*. Mol. Breed. 27(4):467-478.

Liu, J., Hirani, A.H., Li, Z., Wu, C., McVetty, P.B.E. and Li, G. 2016 . QTLs controlling glucosinolate content in seeds of *Brassica napus* L. Aus. J. Pl. Sci. 10(2):152-160.

Liu, Z., Hirani, A.H., McVetty, P.B., Daayf, F., Quiros, C.F. and Li, G. 2012. Reducing progoitrin and enriching glucoraphanin in *Brassica napus* seeds through silencing of the *GSL-ALK* gene family. Pl. Mol. Biol. 79(1-2):179-89.

Liu, F., Wang, M., Wen, J., Yi, B., Shen, J., Ma, C., Tu, J. and Fu, T. 2015. Overexpression of barley oxalate oxidase gene induces partial leaf resistance to *Sclerotinia sclerotiorum* in transgenic oilseed rape. URL: http://onlinelibrary.wiley.com/doi/10.1111/ppa.12374/pdf (9 November 2015).

Liu, S., Wang, H., Zhang, J., Fitt, B.D.L., Xu, Z., Evans., N., Liu., Y., Yang, W. and Guo, X. 2005. In vitro mutation and selection of doubled-haploid *Brassica napus* lines with improved resistance to *Sclerotinia sclerotiorum*. Pl. Cell Reports, 24(3):133-144.

Liu, S., Zhou, B., Yu, Q. and Zhou, L. 1998, Application of oxalic acid for screening resistance oilseed rape to *Sclerotinia sclerotiorum* and its affecting factors, Acta Phytophylacica Sinica, 25(1):56-60.

LMC International. 2013. The economic impact of canola on the Canadian economy. URL: http://www.canolacouncil.org/media/545722/lmc_economic_impact_of_canola_on_the_canadian_economy_october_2013.pdf (7 November 2015).

Lou, P., Zhao, J., He, H., Hanhart, C., Del Carpio, D.P., Verkerk, R., Custers, J., Koornneef, M. and Bonnema, G. 2008. Quantitative trait loci for glucosinolate accumulation in *Brassica rapa* leaves. New Phytol. 179:1017-1032.

Lu, G., Harper, A.L., Trick, M., Morgan, C., Fraser, F., O'Neill, C. and Bancroft, I. 2014. Associative transcriptomics study dissects the genetic architecture of seed glucosinolate content in *Brassica napus.* DNA Res.1-13.

Lukens, L., Zou, F., Lydiate, D., Parkin, I. and Osborn, T. 2003. Comparison of a *Brassica oleracea* genetic map with the genome of *Arabidopsis thaliana*. Genet. 164:359-372.

Lysak, M.A., Koch, M.A., Pecinka, A. and Schubert, I. 2005. Chromosome triplication found across the tribe *Brassiceae*. Genom. Res. 15:516-525.

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

Mahmood, T., Ekuere, U., Yeh, F., Good, A.G. and Stringam, G.R. 2003. Molecular mapping of seed aliphatic glucosinolates in *Brassica juncea*. Genome, 46(5):753-60.

Makowskia, D., Taverneb, M., Bolomierb, J. and Ducarne, M. 2005. Comparison of risk indicators for *Sclerotinia* control in oilseed rape. Crop Prot. 24:527-531.

Maluszynski, M., Kasha, K., Forster, B.P. and Szarejko, I. 2003. Doubled Haploid Production in Crop Plants: A Manual. Springer, Science+Business Media, New York.

Marciano, P., Lenna, P.D. and Magro, P. 1983. Oxalic acid, cell wall-degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. Physiol. Pl. Pathol. 22:339-345.

McCartney, H.A., Doughty, K.J., Norton, G., Booth, E.J., Kightley, S.P.J., Landon, G., West, G., Walker, K.C., and Thomas, J.E. 1999. A study of the effect of disease on seed quality parameters of oilseed rape. In: Proc10th Int. Rapeseed Congress: New Horizons

for an Old Crop. Wratten, N. and Salisbury, P.A. eds. 26-29 September 1999, TRI Publ. Canberra, Australia.

McInnis, A. 2004. The transformation of rapeseed into canola: A Cinderella story. The Winning the Prairie Gamble: The Saskatchewan Story exhibit, North Battleford WDM. URL: http://www.wdm.ca/skteacherguide/WDMResearch/CanolaResearchPaper.pdf (7 November 2015).

McVetty, P.B.E., Cuthbert, J.L., Marwede, V., Paulmann, W., Sass, O., Duncan, R.W., Fernando, W.G.D., Li, G. and Zelmer, C.D. 2014. HYHEAR 1 hybrid Roundup Ready™ high erucic acid, low glucosinolate summer rape. Can. J. Pl. Sci. 94(2):453-455.

Mei, J., Ding, Y., Lu, K., Wei, D., Liu, Y., Disi, J.O., Li, J., Liu, L., Liu, S., Mckay, J. and Qian, W. 2013. Identification of genomic regions involved in resistance against *Sclerotinia sclerotiorum* from wild *Brassica oleracea*. Theor. Appl. Genet.126:549-556.

Mei, J., Liu, Y., Wei, D., Wittkop, B., Ding, Y., Li, Q., Li, J., Wan, H., Li, Z., Ge, X., Frauen, M., Snowdon, R.J., Qian, W. and Friedt, W. 2015. Transfer of *Sclerotinia* resistance from wild relative of *Brassica oleracea* into *Brassica napus* using a hexaploidy step. Theor. Appl. Genet. 128(4):639-44.

Mei, J., Qian, L., Disi, J.O., Yang, X., Li, Q., Li, J., Frauen, M., Cai, D. and Qian, W. 2011. Identification of resistant sources against *Sclerotinia sclerotiorum* in *Brassica* species with emphasis on *B. oleracea*. Euphytica, 177:393-399.

Merriman, P.R. 1976. Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. Soil Biol. Biochem. 8:385-389.

Miah, G., Rafii, M.Y., Ismail, M.R., Puteh, A.B., Rahim, H.A., Islam, K.N. and Latif, M.A. 2013. A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. Int. J. Mol. Sci. 14(11) 22499-22528.

Michael, T.P. and Robert VanBuren, R. 2015. Progress, challenges and the future of crop genomes. Cur. Opin. Pl. Biol. 24:71-81.

Micic, Z., Hahn, V., Bauer, E., Melchinger, A.E., Knapp, S.J., Tang, S. and Schön, C. 2005. Identification and validation of QTL for Sclerotinia midstalk rot resistance in sunflower by selective genotyping. Theor. Appl. Genet. 111:233-242.

Micic, Z., Hahn, V., Bauer, E., Schön, C.C., Knapp, S.J., Tang, S. and Melchinger, A.E. 2004. QTL mapping of Sclerotinia midstalk-rot resistance in sunflower. Theor. Appl. Genet. 109:1474-1484.

Ming, R. and Wai, C.M. 2015. Assembling allopolyploid genomes: no longer formidable. Genome Biol.16:27.

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

Mithen, R.F., Lewis, B.G. and Fenwick, G.R. 1987. Glucosinolates in wild and cultivated *Brassica species*. Phytochem. 26:1969-1973.

Mithen, R.F. and Magrath, R. 1992. Glucosinolates and resistance to *Leptosphaeria maculans* in wild and cultivated *Brassica Species*. Pl. Breed. 108(1):60-68.

Morinaga, T. 1934. Interspecific hybridisation in *Brassica* VI. The cytology of F_1 hybrids of *B. juncea* and *B. nigra*. Cytologia, 6:62-67.

Morral, R.A.A. and Dueck, J. 1982. Epidemiology of Sclerotinia stem rot of rapeseed in Saskatchewan *Sclerotinia sclerotiorum, Brassica napus, Brassica campestris.* Can. J. Pl. Pathol. 4:161-168.

Muellenborn, C., Krause, J.H. and Cerboncini, C. 2011. Analysis of differential transcript expression reveals time-dependent leaf responses to *Sclerotinia sclerotiorum* in wild and cultivated sunflower. Pl. Mol. Biol. Rep. 29:597-608.

Mullins, E., Quinlan, C. and Jones, P. 1995. Analysis of mechanisms of partial physiological resistance to *Sclerotinia sclerotiorum* using induced mutants of *Brassica napus*. Aspects Appl. Biol. 42:307-314.

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

O'Neill, C.M. and Bancroft, I. 2000. Comparative physical mapping Comparative Genomics of *B. napus* 1927 of segments of the genome of *Brassica oleracea* var ssp. alboglabra that are homoeologous to sequenced regions of the chromosomes 4 and 5 of *Arabidopsis thaliana*. Pl. J. 23:233-243.

Park, J.Y., Koo, D.H., Hong, C.P., Lee, S.J., Jeon, J.W., Lee, S.H., Yun, P.Y., Park, B.S., Kim, H.R., Bang, J.W., Plaha, P., Bancroft, I. and Lim, Y.P. 2005. Physical mapping and microsynteny of *Brassica rapa* ssp. pekinensis genome corresponding to a 222 kb gene-rich region of Arabidopsis chromosome 4 and partially duplicated on chromosome 5. Mol. Genet. Genomics, 274:579-588.

Parkin, I.A.P., Gulden, S.M., Sharpe, A.G., Lukens, L., Trick, M., Osborn, T.C. and Lydiate, D.J. 2005. Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genet. 171:765-781.

Parkin, I.A.P., Sharpe, A.G., Keith, D.J. and Lydiate, D.J. 1995. Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome, 38:1122-1131.

Parkin, I.A.P., Sharpe, A.G. and Lydiate, D.J. 2003. Patterns of genome duplication within the *Brassica napus* genome. Genome, 46:291-303.

Phillips, D.V., Carbone, I., Gold, S.E. and Kohn, L.M. 2002. Phylogeography and genotype-symptom associations in early and late season infections of canola by *Sclerotinia sclerotiorum*. Phytopathol. 92:785-793.

Piquemal, J., Cinquin, E., Couton, F., Rondeau, C., Seignoret, E., Doucet, I., Perret, D., Villeger, M.J., Vincourt, P. and Blanchard, P. 2005. Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor. Appl. Genet. 111:1514-1523.

Prakash, S., Wu, X.M. and Bhat, S.R. 2011. History, evolution, and domestication of *Brassica* crops. p. 19-84. In: Pl. Breed. Rev., vol. 35. Janick, J. eds. John Wiley and Sons Inc., New Jersey.

Purdy, L.H. 1979. *Sclerotinia sclerotiorum*: history, diseases and symptomatology, host range, geographic distribution, and impact. Phytopathol. 69:875-880.

Qian, W., Meng, J., Li, M., Frauen, M., Sass, O., Noack, J. and Jung, C. 2006. Introgression of genomic components from Chinese *Brassica rapa* contributes to widening the genetic diversity in rapeseed (*B. napus* L.), with emphasis on the evolution of Chinese rapeseed. Theor. Appl. Genet. 113:49-54.

Quiroz, H.C. and Mithen, R. 1996. Molecular markers for low-glucosinolate alleles in oilseed rape (*Brassica napus* L.). Mol Breed. 2(3):277-281.

Rahman, M.H., Joersbo, M. and Poulsen, M.H. 2001. Development of yellow-seeded *Brassica napus* of double low quality. Pl. Breed. 120:473-478.

Rahman, H., Bennett, R.A. and Se´guin-Swartz, G. 2015. Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* x *B. oleracea* var. alboglabra interspecific crosses. Can. J. Pl. Sci. 95:29-41.

Rahman, H., Kebede, B., Zimmerli, C. and Yang, R. 2014. Genetic study and QTL mapping of seed glucosinolate content in *Brassica rapa* L. Crop Sci. 54:537-543.

Rakow, G. 2004. Species origin and economic importance of *Brassica*. p. 3-7. In: Biotechnology in agriculture and forestry; Vol: 54: *Brassica*. Pua, E.C. and Douglas, C.J. eds. Springer-Verlag, Berlin.

Raman, H., Dalton-Morgan, J., Diffey, S., Raman, R., Alamery, S., Edwards, D. and Batley, J. 2014. SNP markers-based map construction and genome-wide linkage analysis in *Brassica napus*. Pl. Biotech. J. 12:851-860.

Ramchiary, N., Bisht, N.C., Gupta, V., Mukhopadhyay, A., Arumugam, N., Sodhi, Y.S., Pental, D. and Pradhan, A.K. 2007. QTL analysis reveals context-dependent loci for seed

glucosinolate trait in the oilseed *Brassica juncea*: importance of recurrent selection backcross scheme for the identification of 'true' QTL. Theor. Appl. Genet. 116(1):77-85.

Rana, D., van den Boogaart, T., O'Neill, C.M., Hynes, L., Bent, E., Macpherson, L., Park, J.Y., Lim, Y.P. and Bancroft, I. 2004. Conservation of the microstructure of genome segments *in Brassica napus* and its diploid relatives. Pl. J. 40:725-733.

Rich, T.C.G. 1991. Crucifers of Great Britain and Ireland. p. 336. Bot. Soc. British Isles, London.

Riou, C., Freyssinet, G. and Fevre, M. 1991. Production of cell wall-degrading enzymes by the phytopathogenic fungus Sclerotinia sclerotiorum. Appl. Environ. Microbiol. 57(5):1478-1484.

Rollins, J.A. and Dickman, M.B. 2001. pH signaling in *Sclerotinia sclerotiorum*: identification of a pacC/RIM1 homolog. Appl. Environ. Microbiol. 67:75-81.

Rönicke, S., Hahn, V., Vogler, A. and Friedt, W. 2005. Quantitative trait loci analysis of resistance to *Sclerotinia sclerotiorum* in sunflower. Phytopathol. 95:834-839. [19]

Rout, K., Sharma, M., Gupta, V., Mukhopadhyay, A., Sodhi, Y.S., Pental, D. and Pradhan, A.K. 2015. Deciphering allelic variations for seed glucosinolate traits in oilseed mustard (*Brassica juncea*) using two bi‐parental mapping populations. Theor. Appl. Genet. 128(4):657-666.

Saharan, G.S. and Mehta, N. 2008. *Sclerotinia* diseases of crop plants: biology, ecology and disease management. p.14-15. Springer, Science+Business Media B.V.

Sang, J.P. and Salisbury, P.A. 1988. Glucosinolate profiles of international rapeseed lines (*Brassica napus* and *Brassica campestris*). J. Sci. Food Agric. 45:255-261.

Sang, X., Jue, D., Yang, L., Bai, X., Chen, M. and Yang, Q. 2013. Genetic transformation of *Brassica napus* with *MSI-99m* gene increases resistance in transgenic plants to *Sclerotinia sclerotiorum*. Mol. Pl. Breed. 4(30):247-253.

Schatzki, J., Ecke, W., Becker, H.C. and Möllers, C. 2014. Mapping of QTL for the seed storage proteins cruciferin and napin in a winter oilseed rape doubled haploid population and their inheritance in relation to other seed traits. Theo. Appl. Genet. 127(5):1213-1222.

Schmidt, R., Acarkan, A. and Boivin, K. 2001. Comparative structural genomics in the *Brassicaceae* family. Pl. Physiol. Biochem. 39:253-262.

Schranz, M.E., Lysak, M.A. and Mitchell-Olds, T. 2006. The ABC's of comparative genomics in the *Brassicaceae*: Building blocks of *Crucifer* genomes. Trends Pl. Sci. 11:535-542.

Sexton, A.C. and Howlett, B.J. 2004. Microsatellite markers reveal genetic

differentiation among populations of *Sclerotinia sclerotiorum* from Australian canola fields. Cur. Genet. 46(6):357-365.

Seymour, D.K., Filiault, D.L., Henry, I.M., Monson-Miller, J., Ravi, M., Pang, A., Comai, L., Chan, S.W.L. and Maloof, J.N. 2012. Rapid creation of Arabidopsis doubled haploid lines for quantitative trait locus mapping. PNAS. 109(11):4227-4232.

Sharma, P., Kumar, A., Meena, P.D., Goyal, P., Salisbury, P., Gurung, A., Fu, T.D., Wang, Y.F., Barbetti, M.J. and Chattopadhyay, C. 2009. Search for resistance to *Sclerotinia sclerotiorum* in exotic and indigenous *Brassica* germplasm. In: Proc16th Aus. Res. Assem. on Brassicas. Ballarat Victoria. URL: http://www.australianoilseeds.com/__data/assets/pdf_file/0007/6883/57_Search_for_resistance_to_Sclerotinia_sclerotiorum.pdf (3 November 2015).

Sharma, P., Meena, P.D., Verma, P.R., Saharan, G.S., Mehta, N., Singh, D. and Kumar, A. 2015. *Sclerotinia sclerotiorum* (Lib.) de Bary causing Sclerotinia rot in oilseed *Brassicas*: A review. J. Oilseed Brassica, 6:1-44.

Shokouhifar, F., Zamani, M.R. and Motallebi, M. 2011. Expression pattern of the synthetic pathogen-inducible promoter (SynP-FF) in the transgenic canola in response to *Sclerotinia sclerotiorum*. Iranian J. Biotech. 9(1):1-10.

Silva, L.D.C.E., Wang, S. and Zeng, Z.B. 2012. Composite interval mapping and multiple interval mapping: procedures and guidelines for using Windows QTL Cartographer. p. 75-119. In: Methods Mol. Biol. V871. Rifkin, S.A. eds. University of California, San Diego, USA.

Snowdon, R.J. 2007. Cytogenetics and genome analysis in *Brassica* crops. Chromosome Res. 15:85-95.

Snowdon, R.J., Friedrich, T., Friedt, W. and Köhler, W. 2002. Identifying the chromosomes of the A- and C-genome diploid *Brassica* species *B. rapa* (syn. *campestris*) and *B. oleracea* in their amphidiploid *B. napus*. Theor. Appl. Genet. 104(4):533-538.

Snowdon, R.J., Kohler, W., Friedrich, T. and Friedt, W. 2003. Fishing for physical genome information-*Brassica* cytogenetics past, present and future. p. 116-119. In: Proc 11th int. rapeseed congress, vol. I. Copenhagen, Denmark.

Sosulski, F.W. 1979. Organoleptic and nutritional effects of phenolic compounds on oilseed protein products: a review. J. Amer. Oil Chem. Soc. 56:711-714.

Sotelo, T., Soengas, P., Velasco, P., Rodrı´guez, V.M. and Cartea, M.E. 2014. Identification of metabolic QTLs and candidate genes for glucosinolate synthesis in *Brassica oleracea* leaves, seeds and flower buds. PLoS ONE, 9(3):e91428. doi:10.1371/journal.pone.0091428.

Sotelo, T., Lema, M., Soengas, P., Cartea, M.E. and Velasco, P. 2015. *In vitro* activity of glucosinolates and their degradation products against *Brassica*-pathogenic bacteria and fungi. Appl. Environ. Microbiol. 81:432-440.

Sun, Z., Wang, Z., Tu, J., Zhang, J., Yu, F., McVetty, P.B.E. and Li, G. 2007. An ultradense genetic recombination map for *Brassica napus*, consisting of 13551 SRAP markers. Theor. Appl. Genet. 114:1305-1317.

Taylor, A., Coventry, E., Jones, J.E. and Clarkson, J.P. 2015. Resistance to a highly aggressive isolate of *Sclerotinia sclerotiorum* in a *Brassica napus* diversity set. Pl. Pathol. 64(4):932-940.

Teo, B.K., Morrall, R.A.A. and Verma, P.R. 1989. Influence of soil moisture, seeding date, and canola cultivars (Tobin and Westar) on the germination and rotting of sclerotia of *Sclerotinia sclerotiorum*. Can. J. Pl. Pathol. 11:393-399.

Toroser, D., Thormann, C.E., Osborn, T.C. and Mithen, R. 1995. RFLP mapping of quantitative trait loci controlling seed aliphatic glucosinolate content in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet. 91:802-808.

Trick, M., Long, Y., Meng, J. and Bancroft, I. 2009. Single nucleotide polymorphism (SNP) discovery in the polyploid *Brassica napus* using Solexa transcriptome sequencing. Pl. Biotech. J. 7:334-46.

Tsunoda, S. 1980. Eco-physiology of wild and cultivated forms in *Brassica* and allied genera. p. 109-120. In: *Brassica* crops and wild allies. Tsunoda, S., Hinata, K. and Gomez-Campo, C. eds. Japan Sci. Sot. Press, Tokyo.

Turkington, T.K. and Morrall, R.A.A. 1993. Use of petal infestation to forecast Sclerotinia stem rot of canola: The influence of inoculum variation over the flowering period and canopy density. Phytopath. 83:682-689.

Twengstrijm, E., Sigvald, R., Svensson, C. and Yuen, J. 1998. Forecasting Sclerotinia stem rot in spring sown oilseed rape. Crop Prot. 17(5):405-411.

Uloth, M., You, M. and Barbetti, M. 2015. Comparison of host resistance to Sclerotinia stem rot in historic and current *Brassica napus* and *B. juncea* and critical management implications. Crop Pasture Sci. 66(8):841-848.

USDA. 2015. Oilseeds: world markets and trade. URL: https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf (3 November 2015).

Uzunova, M., Ecke, W., Weissleder, K. and Röbbelen, G. 1995. Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theor. Appl. Genet. 90(2):194-204.

Van Ooijen, J.W. and Voorrips, R.E. 2001. Joinmap® 3.0. Software for the calculation of genetic linkage maps. Pl. Res. Int. Wageningen, the Netherlands.

Velasco, P., Soengas, P., Vilar, M. and Cartea, M.E. 2008. Comparison of glucosinolate profiles in leaf and seed tissues of different *Brassica napus* crops. J. Amer. Soc. Hort. Sci. 133(4):551-558.

Viana, J.M.S. 2005. Dominance, epistasis, heritabilities and expected genetic gains. Genet. Mol. Biol. 28(1):67-74.

Vinjamoori, V., Byrum, J.R., Hayes, T. and Das, P.K. 2004. Challenges and opportunities in the analysis of raffinose oligosaccharides, pentosans, phytate, and glucosinolates. J. Anim. Sci.82:319-328.

Wakefield, E.N. 1924. On the names *Sclerotinia sclerotiorum* (Lib.) Massee, and *S. libertiana* Fuckel. Phytopathol. 14:126-127.

Walker, K.C. and Booth, E.J. 2001. Agricultural aspects of rape and other *Brassica* products. Eur. J. Lipid Sci. Technol. 103:441-446.

Walz, A., Zingen-Sell, I., Theisen, S. and Kortekamp, A. 2008. Reactive oxygen intermediates and oxalic acid in the pathogenesis of the necrotrophic fungus *Sclerotinia sclerotiorum*. Eur. J. Pl. Pathol. 120:317-330.

Wang, S., Basten, C.J. and Zeng, Z.B. 2011a. Windows QTL Cartographer Version 2.5_009. North Carolina State University, Raleigh, NC, USA.

Wang, N., Li, F., Chen, B., Xu, K., Yan, G., Qiao, J., Li, J., Gao, G., Bancroft, I., Meng, J., King, G.J. and Wu, X. 2014. Genome‐wide investigation of genetic changes during modern breeding of *Brassica napus*. Theor. Appl. Genet. 127:1817–1829.
DOI 10.1007/s00122-014-2343-6

Wang, H., Liu, G., Zheng, Y., Wang, X. and Yang, Q. 2004. Breeding of a *Brassica napus* cultivar Zhongshuang No. 9 with high-resistance to *Sclerotinia sclerotiorum* and dynamics of its important defense enzyme activity. Agr. Sci. China, 37(1):23-28.

Wang, H., Wu, J., Sun, S., Liu, B., Cheng, F., Sun, R. and Wang, X. 2011b. Glucosinolate biosynthetic genes in *Brassica rapa*. Gene, 487(2):135-142.

Warwick, S.I., Francis, A. and Al-Shehbaz, I.A. 2006. Brassicaceae: species checklist and database on CD-Rom. Pl. Syst. Evol. 259:249-258.

Warwick, S.I., Francis, A. and Gugel, R.K. 2009. Guide to wild germplasm of *Brassica* and allied crops (tribe *Brassiceae, Brassicaceae*). URL: https://brassica.info/info/publications/guidewild/Guide_ed.3_Introd_16July2009.pdf (7 November 2015).

Weber, S., ÜNker, F. and Friedt, W. 2005. Improved doubled haploid production protocol for *Brassica napus* using microspore colchicine treatment *in vitro* and ploidy determination by flow cytometry. Pl. Breed. 124(5):511-513.

Weese, A., Pallmann, P., Papenbrock, J. and Riemenschneider A. 2015. *Brassica napus* L. cultivars show a broad variability in their morphology, physiology and metabolite levels in response to sulfur limitations and to pathogen attack. Pl. Physiol. 6(9):1-18.

Wei, D., Mei, J., Fu, Y., Disi, J.O., Li, J. and Qian, W. 2014. Quantitative trait loci analyses for resistance to *Sclerotinia sclerotiorum* and flowering time in *Brassica napus*. Mol. Breed. 1-8.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18(24):7213-7218.

Willetts, H.J. and Wong, J.A.L. 1980. The biology of *Sclerotinia sclerotiorum, S. trifoliorum,* and *S. minor* with emphasis on specific nomenclature. Bot. Rev. 46:101-165.

Williams, J.R. and Stelfox, D. 1980. Influence of farming practices in Alberta on germination and apothecium production of sclerotia of *Sclerotinia sclerotiorum* causal organism of Sclerotinia stem rot and head blight of rapeseed. Can. J. Pl. Pathol. 2:169-172.

Wittstock, U. and Halkier, B.A. 2000. Cytochrome P450 CYP79A2 from *Arabidopsis thaliana* L. catalyzes the conversion of L-phenylalanine to Phenylacetaldoxime in the biosynthesis of benzylglucosinolate. J. Biol. Chem. 275:14659-14666.

Wu, J., Cai, G., Tu, J., Li, L., Liu, S., Luo, X., Zhou L., Fan, C. and Zhou, Y. 2013. Identification of QTLs for resistance to Sclerotinia stem rot and *BnaC.IGMT5.a* as a candidate gene of the major resistant QTL SRC6 in *Brassica napus*. PLoS ONE, 8(7):1-12. e67740. doi:10.1371/journal.pone.0067740.

Wu, Q., Chen, Y., Zhou, S., Fu, L., Chen, J., Xiao, Y., et al. 2015. High-Density Genetic Linkage Map Construction and QTL Mapping of Grain Shape and Size in the Wheat opulation Yanda1817 × Beinong6. PLoS ONE 10(2): e0118144. doi:10.1371/journal.pone.0118144

Wu, J., Wu, L., Liu, Z., Qian, L., Wang, M., Zhou, L., Yang, Y. and Li, X. 2009. A plant defensing gene from *Orychophragmus violaceus* can improve *Brassica napus*' resistance to *Sclerotinia sclerotiorum*. Afr. J. Biotech. 8(22):6101-6109.

Yang, R., Han, Y., Li, G., Jiang, D. and Huang, H. 2007. Suppression of *Sclerotinia sclerotiorum* by antifungal substances produced by the mycoparasite *Coniothyrium minitans*. Eur. J. Pl. Pathol. 119:411-420.

Yang, Y.W., Lai, K.N., Tai, P.Y. and Li, W.H. 1999. Rates of nucleotide substitution in angiosperm mitochondrial DNA sequences and dates of divergence between *Brassica* and other angiosperm lineages. J. Mol. Evol. 48:597-604.

Yin, X., Yi, B., Chen, W., Zhang, W., Tu, J., Fernando, W.G.D. and Fu, T. 2010. Mapping of QTLs detected in a *Brassica napus* DH population for resistance to *Sclerotinia sclerotiorum* in multiple environments. Euphytica, 173:25-35.

Young, C.S. and Werner, C.P. 2012. Infection routes for *Sclerotinia sclerotiorum* in apetalous and fully petalled winter oilseed rape. Pl. Pathol. 61(4):730-738.

Young, C.S., Clarkson, J.P., Smith, J.A., Watling, M., Phelps, K. and Whipps, J.M. 2004. Environmental conditions influencing *Sclerotinia sclerotiorum* infection and disease development in lettuce. Pl. Pathol. 53:387-397.

Yue, B., Radi, S.A., Vick, B.A., Cai, X., Tang, S., Knapp, S.J., Gulya, T.J., Miller, J.F. and Hu, J. 2008. Identifying quantitative trait loci for resistance to Sclerotinia head rot in two USDA sunflower germplasms. Phytopathol. 98:926-931.

Zhang, Y. 2004. Biocontrol of Sclerotinia stem rot of canola by bacterial antagonists and study of biocontrol mechanisms involved. MSc. thesis. Plant Science, University of Manitoba.

Zhao, J. and Meng, J. 2003a. Detection of loci controlling seed glucosinolate content and their association with *Sclerotinia* resistance in *Brassica napus*. Pl. Breed. 122:19-23.

Zhao, J. and Meng, J. 2003b. Genetic analysis of loci associated with partial resistance to *Sclerotinia sclerotiorum* in rapeseed (*Brassica napus* L.). Theo. Appl. Genet. 106:759-764.

Zhao, J., Buchwaldt, L., Rimmer, S.R., Sharpe, A., Mcgregor, L., Bekkaoui, D. and Hegedus, D. 2009. Patterns of differential gene expression in *Brassica napus* cultivars infected with *Sclerotinia sclerotiorum*. Mol. Pl. Pathol.10(5):635-649.

Zhao, J., Peltier, A.J., Meng, J., Osborn, T.C. and Grau, C.R. 2004. Evaluation of Sclerotinia stem rot resistance in oilseed *Brassica napus* using a petiole inoculation technique under greenhouse conditions. Pl. Dis. 88:1033-1039.

Zhao, J., Udall, J.A., Quijada, P.A., Grau, C.R., Meng, J. and Osborn, T.C. 2006. Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homeologous non-reciprocal transposition in *Brassica napus* L. Theor. Appl. Genet. 112:509-516.

Zhao, J., Wang, J., An, L., Doerge, R.W., Chen, Z.J., Grau, C.R., Meng, J. and Osborn, T.C. 2007. Analysis of gene expression profiles in response to *Sclerotinia sclerotiorum* in *Brassica napus*. Planta, 227:13-24.

Zukalova, H. and Vasak, J. 2002. The role and effects of glucosinolates of *Brassica* species - a review. Rostlinna Vyroba, (48):175-180.

APPENDICES

Table S1. SRAP Primer Pairs in PCR for a *B. napus* DH Population M730

Forward Primer	Dye	Reverse Primer	Number of Markers
		BG1	1
		BG11	1
		BG23	1
		BG32	1
		BG33	1
Odd		BG54	3
		BG23	1
		BG29	2
		BG55	1
		BG66	1
FC	FAM	BG86	2
		BG23	1
		BG77	1
EM		BG80	1
GA		BG23	1
		BG33	1
		BG34	1
		BG35	1
SA7	VIC	BG37	1
		BG2	1
		BG4	1
		BG68	2
		BG69	1
BG23		BG89	1
		BG25	1
PRO		BG46	2
		BG18	1
		BG23	1
SAB	NED	BG56	1

Table S2. Percentage of SNPs per Unique SNP Position (UP) in the Genome of a *B. napus* DH Population M692

Chromosome	1 SNP /UP	2 SNPs /UP	3 SNPs /UP	4 SNPs /UP	5 SNPs /UP	6 SNPs /UP	7 SNPs /UP	8 SNPs /UP	9 SNPs /UP	10 SNPs /UP	>10 SNPs /UP
A1	56.83	15.83	7.91	5.04	5.76	0.00	3.60	1.44	2.16	0.00	1.44
A2	64.55	10.91	10.00	5.45	0.00	2.73	1.82	0.00	1.82	0.91	1.82
A3	61.86	16.53	7.20	5.51	2.12	2.97	1.27	0.42	0.85	0.42	0.85
A4	47.11	27.27	6.61	4.96	4.96	1.65	1.65	0.83	3.31	0.00	1.65
A5	56.56	17.21	6.56	4.10	3.28	3.28	0.82	0.00	1.64	0.82	5.74
A6	60.53	19.30	7.02	6.14	1.75	1.75	0.88	0.00	0.88	0.00	1.75
A7	67.25	12.28	8.19	4.09	4.68	0.58	0.58	0.00	1.17	0.00	1.17
A8	36.36	13.64	13.64	9.09	2.27	2.27	4.55	4.55	0.00	0.00	13.64
A9	56.99	12.90	9.68	3.23	3.23	1.08	2.15	4.30	0.00	2.15	4.30
A10	61.36	13.64	6.82	5.68	0.00	3.41	2.27	1.14	1.14	0.00	4.55
A Subgenome	56.94	15.95	8.36	5.33	2.80	1.97	1.96	1.27	1.30	0.43	3.69
C1	57.78	12.59	5.19	5.19	4.44	1.48	1.48	1.48	1.48	0.74	8.15
C2	51.00	13.00	5.00	3.00	6.00	3.00	1.00	1.00	1.00	0.00	16.00
C3	46.25	18.75	5.00	5.00	3.13	2.50	1.88	1.88	1.88	0.63	13.13
C4	41.91	20.59	9.56	3.68	5.88	2.94	1.47	2.94	2.94	0.74	7.35
C5	55.56	16.67	5.56	1.39	4.17	5.56	2.78	1.39	0.00	2.78	4.17
C6	64.91	10.53	5.26	0.00	3.51	0.00	3.51	1.75	1.75	3.51	5.26
C7	47.45	16.06	9.49	8.03	2.19	3.65	2.19	0.73	0.73	0.73	8.76
C8	64.35	13.04	5.22	2.61	1.74	5.22	1.74	0.87	0.00	0.87	4.35
C9	58.49	13.21	5.66	5.66	1.89	1.89	3.77	0.00	1.89	1.89	5.66
C Subgenome	54.19	14.94	6.21	3.84	3.66	2.91	2.20	1.34	1.30	1.32	8.09
AC Genome	55.56	15.44	7.29	4.58	3.23	2.44	2.08	1.30	1.30	0.87	5.89

Table S3. Glucosinolate Content (μmol/g Seed) of a *B. napus* DH Population M692 and Their Parents*

Line	PR	GN	4C	GL	GB	5C	Al	GBC	TG	Line	PR	GN	4C	GL	GB	5C	Al	GBC	TG
1	12	3	16	1	1	1	17	3	19	**50**	1	0	1	0	0	0	1	2	3
2	16	7	23	3	3	6	28	1	30	**51**	10	3	14	2	2	4	18	2	20
3	3	1	4	1	0	1	5	2	7	**52**	6	4	10	1	0	1	11	3	13
5	9	2	10	0	0	1	11	1	12	**53**	55	8	62	4	4	9	71	2	73
6	36	10	46	5	6	11	57	2	58	**54**	3	1	4	1	0	1	5	2	7
8	24	9	33	4	3	7	40	2	42	**56**	3	2	5	1	1	1	6	2	9
10	11	5	16	4	3	7	22	2	24	**57**	5	1	6	0	0	1	6	3	9
11	17	6	23	2	3	5	28	1	29	**58**	15	6	21	0	1	1	22	3	25
12	37	14	51	6	9	14	65	1	66	**59**	31	8	38	2	1	3	41	3	44
13	1	1	2	0	0	0	2	2	4	**60**	26	10	36	3	3	6	42	2	44
14	1	0	1	0	0	0	1	1	2	**61**	24	8	32	1	1	2	34	3	37
15	15	4	19	1	1	2	21	2	23	**62**	14	3	18	3	4	6	24	3	27
16	20	2	22	1	1	1	23	2	26	**63**	6	2	8	1	1	2	9	2	12
17	13	4	17	2	2	4	21	1	22	**64**	11	3	14	3	2	5	19	3	22
18	22	7	29	2	2	4	33	1	34	**65**	10	2	12	3	2	5	17	3	20
19	2	1	3	0	0	0	3	3	7	**66**	3	1	4	0	0	1	5	2	7
20	52	10	62	3	3	6	68	2	70	**67**	33	11	44	1	2	3	47	3	49
21	5	2	7	0	0	0	7	2	9	**68**	36	11	47	2	3	5	52	2	54
23	3	2	5	1	1	2	7	3	10	**69**	4	4	8	0	0	0	8	2	10
24	6	3	8	1	0	2	10	4	14	**70**	0	0	0	0	0	0	0	2	2
26	20	7	28	9	4	13	41	3	43	**71**	2	1	4	0	0	0	4	2	6
27	0	0	0	0	0	0	0	2	2	**73**	24	11	35	4	6	10	45	2	47
28	21	3	24	3	4	7	31	2	33	**75**	16	3	19	4	4	7	26	2	28
29	35	4	38	6	5	11	50	1	51	**77**	5	2	7	0	0	0	7	2	9
30	15	3	18	0	0	1	18	2	20	**78**	37	6	43	8	10	19	61	2	63
31	17	5	21	3	3	5	27	2	29	**79**	2	1	3	0	0	0	3	2	5
32	8	2	10	0	0	0	10	2	12	**80**	7	2	9	1	0	1	10	3	13
33	16	4	20	1	1	2	22	2	24	**81**	13	5	18	4	1	6	23	2	25
34	6	1	7	0	0	1	8	2	10	**82**	6	2	8	1	0	1	10	2	11
35	3	1	5	0	0	0	5	2	7	**83**	8	2	10	2	1	3	13	1	14
36	8	3	11	1	1	1	12	2	14	**84**	43	11	54	10	9	19	73	2	75
37	1	1	2	0	0	0	2	4	6	**86**	2	1	3	0	0	0	3	2	5
38	3	1	4	0	0	0	4	4	8	**87**	2	1	3	0	0	0	3	2	5
39	12	4	16	1	0	1	17	3	20	**88**	21	2	23	1	0	1	24	2	27
40	23	7	29	7	5	13	42	5	46	**89**	29	7	36	2	1	3	39	3	41
41	0	0	0	0	0	0	0	3	3	**90**	6	2	8	1	1	2	10	1	10
42	39	12	51	4	3	7	58	3	61	**93**	2	1	3	0	0	1	3	1	5
44	32	8	40	4	3	6	47	2	49	**95**	0	0	0	0	0	0	0	2	2
45	11	5	16	2	0	2	19	3	22	**96**	18	5	23	4	3	7	30	1	32
46	3	1	4	1	0	1	5	4	9	**97**	1	0	1	0	0	0	1	3	3
47	7	2	9	1	1	2	11	3	14	**M29**	11	5	16	1	1	2	18	3	21
48	11	5	16	2	2	4	20	3	23	**M69**	2	1	2	0	0	1	3	4	7
49	5	2	7	1	0	1	8	1	9										

*: PR, progoitrin; GN, gluconapin; 4C, four carbon aliphatic glucosinolates; GL, glucoalyssin; GB, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; Al, total aliphatic glucosinolates; GBC, glucobrassicin and TG, total glucosinolate content.

Table S4. Simple Sequence Repeat Primer Pairs in PCR for a *B. napus* DH Population ZT

Oligo Name	Forward Primer Sequence 5'-3'	Reverse Primer Sequence 5'-3'	Repeat	Ch*
BRAS041	CCAGACTCCGATAAAACC	ATGCAAGCTTAGACAGAGATT	CT	1
BRAS083	GATGTTGTTGGGGAGAATG	AAAAAGTAGGCAAGTTCAAGC	AAG	2
BRAS002	CACTCACAGCCCTCTTCTTCT	CCTCCAGCTTCCTTTACCA	TC	3
Na10-b11	TTTAACAACAACCGTCACGC	CTCCTCCTCCATCAATCTGC	$(CT)_{28}$	3
CB10347	ATCTGAACACTTTCGGCA	GGAAGCACCATGTCAGC	CT	4
Na10-C06	TGGATGAAAGCATCAACGAG	ATCAATCAACACAAGCTGCG	$(GA)_{58}$	7
Ra2-E12	TGTCAGTGTGTCCACTTCGC	AAGAGAAACCCAATAAAGTAGAACC	$(GA)_{32}$	8
CB10199	CTCATCATATTCGGCGAC	GCTTGAGTTTCCATGGTG	GT	9
Na14-F11	CTATGGTTCATCTTTCGCCG	CATGCTCCAACCACAGTTTG	$(GT)_7$	11
CB10277	ACAAATGCTTGAGTGATA	TCTTCGTAAACTTGTTCTTGA	GA	11
CB10493	TGACGTGTGAGCAACAGA	CTGAGTCACAAGCCGAGT	GA	14
BRAS026	ATTACAAAAATGCCCTGAC	TAAGTGATCTTCTCTCCAACA		17
CB10028	CTGCACATTTGAAATTGGTC	AAATCAACGCTTACCCACT	CTT	18
Na12-G04	CGAATTGAAGGATGAGTTTGG	CACATGTTTTATCATTCACAAGTCC	$(GA)_{25}$	19

*: Proposed chromosome

Table S5. Glucosinolate Content (μmol/g Seed) of a *B. napus* DH Population ZT and Their Parents*

Line	PRO	GNP	4C	GLS	GBN	5C	T-Ali	GBC	TGC	Line	PRO	GNP	4C	GLS	GBN	5C	T-Ali	GBC	TGC
124	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.0	0.3	119	35.3	23.0	58.4	1.8	3.1	4.8	63.2	3.9	67.1
42	0.4	0.2	0.6	0.0	0.0	0.0	0.6	2.0	2.6	46	33.5	16.4	50.0	9.3	6.4	15.7	65.6	2.2	67.8
15	0.9	0.1	1.0	0.0	0.0	0.0	1.0	1.9	2.9	58	39.5	5.3	44.8	6.6	15.7	22.3	67.1	1.8	68.9
7	0.3	0.2	0.5	0.0	0.0	0.0	0.5	2.7	3.2	48	1.7	55.4	57.1	0.6	10.1	10.7	67.7	1.5	69.2
113	0.4	0.2	0.6	0.0	0.0	0.0	0.6	3.1	3.7	12	40.6	16.0	56.6	4.2	6.2	10.4	67.0	2.3	69.3
117	0.2	0.1	0.3	0.0	0.0	0.0	0.3	3.4	3.7	126	36.4	16.2	52.6	7.7	7.2	14.9	67.5	2.5	70.0
138	0.8	0.4	1.3	0.1	0.4	0.5	1.8	2.7	4.5	110	41.4	17.9	59.3	3.0	4.6	7.6	66.9	3.8	70.7
5	1.6	1.1	2.7	0.6	0.2	0.8	3.5	1.6	5.1	47	47.3	12.6	59.9	5.4	4.4	9.8	69.6	2.2	71.8
100	3.1	1.2	4.3	0.2	0.1	0.3	4.7	1.4	6.0	140	34.3	7.2	41.5	12.9	16.1	28.9	70.4	2.4	72.8
51	1.8	2.1	3.9	1.2	0.2	1.3	5.2	2.4	7.7	64	44.2	20.4	64.6	2.9	3.7	6.6	71.2	1.8	73.0
98	3.2	1.6	4.8	1.7	0.3	1.9	6.8	1.5	8.3	63	40.8	12.9	53.7	7.9	11.2	19.1	72.8	1.1	73.9
79	4.1	1.2	5.3	0.4	0.3	0.7	6.0	3.1	9.1	123	36.5	13.1	49.6	11.4	11.3	22.8	72.4	1.9	74.3
18	5.3	3.3	8.7	0.5	0.2	0.6	9.3	2.0	11.2	24	50.2	12.7	62.9	3.5	6.2	9.7	72.6	1.8	74.5
136	6.1	2.0	8.1	0.4	0.2	0.6	8.7	3.3	12.0	116	48.2	10.2	58.4	7.5	5.3	12.9	71.2	3.3	74.5
26	10.1	2.3	12.4	1.3	0.6	1.9	14.3	2.9	17.2	65	41.3	15.9	57.2	5.9	9.6	15.4	72.7	2.1	74.7
60	10.7	2.4	13.1	1.2	2.8	4.0	17.1	2.7	19.8	71	1.8	56.2	58.0	2.3	13.0	15.3	73.3	1.8	75.1
115	6.6	5.8	12.4	4.4	1.0	5.3	17.8	4.0	21.8	9	41.5	16.6	58.1	8.4	6.8	15.2	73.3	2.1	75.4
95	12.5	4.5	17.0	2.7	2.1	4.8	21.8	0.4	22.2	68	42.2	9.4	51.5	8.3	14.3	22.6	74.1	2.3	76.4
10	11.5	4.1	15.6	3.8	2.7	6.5	22.1	3.2	25.3	11	43.5	26.2	69.7	1.7	3.2	4.9	74.6	2.4	76.9
134	12.9	4.3	17.2	4.1	2.5	6.6	23.8	1.8	25.7	106	41.2	28.2	69.4	2.8	2.2	5.1	74.5	2.8	77.3
33	14.9	9.5	24.3	1.0	0.7	1.7	26.0	2.8	28.8	72	49.0	8.6	57.6	7.1	12.3	19.3	77.0	1.2	78.2
29	16.4	8.0	24.4	1.5	1.3	2.8	27.2	2.4	29.6	147	44.9	11.7	56.5	9.7	10.6	20.3	76.8	1.4	78.3
31	17.4	4.6	22.0	4.0	3.2	7.3	29.3	1.4	30.7	2	53.3	16.2	69.6	4.8	4.7	9.5	79.0	1.5	80.5
129	19.4	4.9	24.3	2.1	1.6	3.8	28.0	3.7	31.7	56	56.5	9.7	66.2	7.9	6.3	14.2	80.4	0.3	80.7
39	17.1	8.1	25.2	3.1	3.0	6.1	31.2	1.6	32.8	89	55.7	10.3	65.9	6.8	6.8	13.7	79.6	1.3	80.9
75	19.9	6.2	26.1	2.8	3.0	5.8	31.9	1.2	33.1	21	51.8	20.2	72.0	3.9	5.2	9.1	81.0	2.0	83.0
23	14.1	6.6	20.7	6.4	3.0	9.3	30.0	3.6	33.6	120	48.3	26.8	75.1	1.5	5.5	7.1	82.2	0.9	83.1
97	21.4	6.0	27.4	6.2	2.4	8.5	35.9	1.7	37.6	92	45.4	11.0	56.3	10.0	15.5	25.5	81.8	1.5	83.3
55	15.5	10.2	25.7	8.4	2.7	11.0	36.7	1.8	38.5	36	37.7	24.5	62.2	7.1	12.8	19.9	82.0	1.3	83.4
94	26.0	8.0	34.0	2.2	2.0	4.3	38.3	1.1	39.3	88	42.7	21.1	63.8	7.8	11.2	19.0	82.8	0.7	83.5
1	24.1	6.3	30.4	3.9	3.5	7.4	37.8	2.0	39.7	125	39.7	11.8	51.4	14.8	14.9	29.7	81.1	2.6	83.7
96	24.3	4.1	28.4	5.8	6.0	11.8	40.1	1.3	41.4	132	51.2	17.8	69.0	3.8	10.6	14.4	83.5	1.0	84.5
32	26.9	6.7	33.6	2.8	3.4	6.2	39.7	2.4	42.1	70	45.6	8.6	54.3	15.6	15.5	31.1	85.3	1.5	86.8
135	21.9	14.7	36.6	2.9	0.7	3.6	40.3	3.0	43.3	19	53.7	17.0	70.7	5.1	9.0	14.1	84.7	2.5	87.2
43	23.0	9.8	32.8	5.3	4.3	9.6	42.4	1.7	44.0	16	52.3	29.1	81.4	2.3	2.9	5.2	86.6	1.5	88.1
57	19.5	5.7	25.2	6.6	10.8	17.4	42.6	2.5	45.0	127	47.6	17.8	65.4	5.3	16.8	22.2	87.5	0.6	88.1
91	26.0	13.6	39.6	2.6	3.3	5.9	45.5	0.9	46.4	130	49.5	13.1	62.6	12.3	12.8	25.1	87.7	1.4	89.1
40	21.0	11.7	32.8	6.4	7.5	13.8	46.6	1.5	48.1	3	47.5	16.5	64.0	7.6	15.6	23.2	87.2	2.0	89.2
80	26.9	7.8	34.7	7.3	3.9	11.2	45.9	2.4	48.3	82	51.4	29.1	80.5	2.5	5.2	7.6	88.1	1.2	89.3
76	29.6	7.0	36.6	3.2	6.7	9.9	46.5	2.1	48.5	20	56.4	23.4	79.7	5.6	4.0	9.6	89.4	0.8	90.2
52	23.1	8.5	31.6	8.5	5.4	13.9	45.5	3.0	48.5	35	55.5	24.4	79.9	3.0	6.9	9.9	89.7	1.0	90.8
90	31.5	10.6	42.2	2.9	2.5	5.4	47.6	1.6	49.2	74	60.2	24.9	85.1	2.4	2.7	5.0	90.2	0.9	91.0
62	30.7	5.4	36.1	5.3	6.1	11.4	47.5	1.7	49.2	8	32.2	34.5	66.7	4.3	18.2	22.5	89.2	2.5	91.6
27	23.5	9.6	33.1	8.3	6.6	14.8	47.9	1.7	49.5	37	52.6	31.2	83.7	1.3	6.5	7.8	91.5	0.1	91.6
101	35.9	8.7	44.6	1.2	1.6	2.7	47.3	2.6	49.9	22	51.4	35.1	86.5	1.8	3.4	5.3	91.7	0.5	92.3
99	24.7	16.3	41.0	3.7	4.0	7.7	48.7	1.8	50.5	104	54.6	22.2	76.7	5.3	9.4	14.7	91.4	1.1	92.5
128	30.7	5.6	36.3	5.0	6.7	11.7	48.0	3.3	51.2	87	60.0	18.9	78.9	5.1	8.0	13.1	92.0	1.1	93.1
4	31.3	8.6	39.9	4.8	4.4	9.2	49.1	2.6	51.8	103	48.6	39.1	87.7	1.4	3.7	5.0	92.7	0.8	93.5
14	24.2	9.3	33.6	6.7	9.2	15.9	49.5	2.3	51.8	121	52.9	19.3	72.1	8.0	12.6	20.6	92.7	1.0	93.8
66	23.9	7.8	31.7	10.4	7.8	18.3	50.0	2.5	52.5	102	51.2	32.1	83.3	2.7	7.4	10.2	93.5	0.6	94.0
73	32.4	16.2	48.6	2.1	3.2	5.3	53.9	1.8	55.7	38	58.0	33.8	91.8	0.6	1.4	2.0	93.8	0.6	94.4
81	27.7	11.6	39.2	5.5	9.2	14.7	53.9	2.2	56.2	118	65.5	18.9	84.4	3.6	4.5	8.1	92.5	2.2	94.7
77	27.1	9.6	36.7	14.0	5.9	19.9	56.5	3.6	60.1	17	54.6	35.0	89.5	2.0	3.2	5.2	94.8	0.4	95.1
28	37.5	18.3	55.8	1.2	1.5	2.7	58.4	2.1	60.6	83	57.6	6.6	64.2	14.9	17.7	32.7	96.9	2.0	98.9
41	29.2	11.6	40.7	11.0	7.8	18.8	59.5	1.2	60.7	34	47.2	30.0	77.2	5.4	16.4	21.7	98.9	0.2	99.1
78	35.6	9.3	44.9	7.3	7.6	14.8	59.7	2.1	61.8	59	56.3	30.7	87.0	3.7	7.4	11.2	98.2	1.3	99.5
6	28.9	9.8	38.8	5.1	15.7	20.8	59.5	2.9	62.4	45	45.2	39.2	84.4	2.3	13.0	15.2	99.7	0.3	100.0
85	36.7	10.9	47.6	9.5	5.4	14.9	62.5	1.6	64.1	30	57.3	22.0	79.3	10.8	21.9	32.7	112.0	0.4	112.4
61	27.5	12.1	39.6	13.7	9.0	22.7	62.4	1.8	64.2	84	61.7	16.3	78.0	10.5	24.4	34.9	112.9	0.8	113.7
122	43.5	14.9	58.4	2.0	2.2	4.1	62.5	1.9	64.4	Topas	1.5	0.4	1.9	0.2	0.1	0.3	2.2	2.5	4.7
13	35.3	25.4	60.7	0.9	1.6	2.5	63.2	2.0	65.2	ZY821	49.3	32.4	81.8	4.4	10.8	15.2	97.0	0.3	97.2
111	40.1	9.5	49.6	8.8	4.9	13.8	63.3	3.6	67.0										

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content.

Table S6. The Percentage of Glucosinolate Content in Three *B. napus* DH Populations*

		PRO /4C	PRO/T-Ali	PRO/TGC	GNP /4C	GNP/T-Ali	GNP/TGC	4C/ T-Ali	4C/ TGC	GLS /5C	GLS/T-Ali	GLS/TGC	GBN/5C	GBN/T-Ali	GBN/TGC	5C/ T-Ali	5C/TGC	T-Ali /TGC	GBC/TGC
	Canola	74.7	65.3	40.3	25.3	21.7	15.3	87.0	55.5	63.9	8.2	5.7	35.8	4.8	3.4	13.0	9.1	64.6	35.3
	Rapeseed	67.6	60.3	58.3	32.4	28.9	27.9	89.2	86.1	46.3	5.1	4.9	53.7	5.7	5.5	10.8	10.4	96.6	3.4
M730	Population	68.4	60.8	56.3	31.6	28.1	26.5	89.0	82.8	48.1	5.4	5.0	51.9	5.6	5.3	11.0	10.3	93.1	7.0
	Canola	68.8	56.9	30.9	31.2	25.3	13.5	82.2	44.3	59.9	7.9	6.0	40.1	9.9	8.4	17.8	14.1	58.5	41.5
	Rapeseed	70.9	56.4	54.6	29.2	23.8	23.0	80.2	77.7	44.5	9.1	8.8	55.6	10.7	10.4	19.8	19.2	96.8	3.2
ZT	Population	70.6	56.5	51.2	29.4	24.0	21.6	80.5	72.8	46.2	8.9	8.3	53.9	10.6	10.1	19.5	18.4	91.2	8.8
	Canola	74.6	67.0	41.9	25.4	22.5	15.1	89.5	57.0	71.3	7.0	5.1	29.0	3.5	2.7	10.5	7.8	64.8	35.2
	Rapeseed	77.9	65.3	61.0	22.1	18.4	17.1	83.7	78.1	52.5	8.6	8.1	47.4	7.7	7.2	16.3	15.3	93.4	6.6
M692	Population	76.2	66.2	50.7	23.9	20.6	16.1	86.8	66.7	61.5	7.7	6.5	38.6	5.5	4.8	13.2	11.3	77.9	22.1

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content .

Table S7. Mean Stem Lesion Lengths (cm) Caused by *Sclerotinia sclerotiorum* in Two Replicates in a *B. napus* DH Population ZT

Line	2009	2010	Variance	Mean	Line	2009	2010	Variance	Mean
ZT008	1.7	1.6	0.0	1.7	ZT005	17.1	17.3	0.0	17.2
ZT147	1.5	4.0	3.1	2.8	ZT016	17.4	17.1	0.0	17.3
ZT062	1.5	5.3	7.1	3.4	ZT022	18.3	16.2	2.3	17.3
ZT020	3.9	3.3	0.2	3.6	ZT046	18.4	16.9	1.2	17.7
ZT111	2.3	5.2	4.0	3.7	ZT012	15.9	19.7	7.2	17.8
ZT059	3.6	3.9	0.1	3.8	ZT123	15.0	20.9	17.4	18.0
ZT070	3.6	4.9	0.8	4.3	ZT088	19.0	17.0	2.0	18.0
ZT072	3.6	5.3	1.5	4.4	ZT004	16.7	19.8	4.7	18.2
ZT119	3.6	5.4	1.7	4.5	ZT018	18.6	18.3	0.1	18.5
ZT096	3.3	6.4	4.9	4.8	ZT009	16.5	20.7	8.7	18.6
ZT030	6.3	4.4	1.9	5.4	ZT078	20.5	16.9	6.6	18.7
ZT100	6.5	4.6	1.9	5.5	ZT006	18.2	19.3	0.6	18.7
ZT113	7.0	4.6	2.9	5.8	ZT115	20.3	17.1	5.1	18.7
ZT098	6.0	6.0	0.0	6.0	ZT090	21.2	16.8	9.8	19.0
ZT136	10.3	4.4	17.5	7.3	ZT138	19.5	18.7	0.3	19.1
ZT080	5.5	10.7	13.6	8.1	ZT043	18.3	20.1	1.6	19.2
ZT082	10.6	9.8	0.4	10.2	ZT101	18.7	19.8	0.6	19.3
ZT001	12.3	8.7	6.3	10.5	ZT128	19.8	18.9	0.4	19.3
ZT031	12.7	8.6	8.5	10.7	ZT095	22.9	16.0	23.9	19.5
ZT021	13.8	8.5	14.2	11.2	ZT075	18.8	20.4	1.3	19.6
ZT011	9.0	14.0	12.5	11.5	ZT117	17.3	22.5	13.5	19.9
ZT036	10.7	12.4	1.5	11.5	ZT120	19.4	20.8	0.9	20.1
ZT037	10.5	13.4	4.2	11.9	ZT077	19.8	20.6	0.3	20.2
ZT047	11.3	13.8	3.1	12.2	ZT134	18.7	21.9	5.3	20.3
ZT039	10.8	13.7	4.4	12.2	ZT058	20.0	20.7	0.2	20.4
ZT127	11.5	13.0	1.1	12.3	ZT081	19.0	21.9	4.3	20.5
ZT052	13.0	11.8	0.7	12.4	ZT114	19.5	21.5	2.0	20.5
ZT048	14.3	10.0	9.4	12.6	ZT092	17.1	24.0	23.5	20.6
ZT002	14.4	11.3	4.9	12.9	ZT014	19.3	22.0	3.7	20.6
ZT099	12.0	14.1	2.2	13.1	ZT079	22.5	19.2	5.4	20.9
ZT007	13.8	12.4	1.0	13.1	ZT013	21.2	21.0	0.0	21.1
ZT104	12.0	15.8	7.3	13.9	ZT017	20.9	21.6	0.3	21.3
ZT125	11.3	17.0	16.5	14.1	ZT118	18.2	24.8	21.7	21.5
ZT015	14.8	13.7	0.6	14.2	ZT129	23.0	20.1	4.1	21.6
ZT066	16.1	12.8	5.4	14.5	ZT023	23.8	20.6	5.3	22.2
ZT042	14.7	14.5	0.0	14.6	ZT110	23.6	22.2	1.0	22.9
ZT089	16.0	13.7	2.7	14.8	ZT122	21.3	24.6	5.2	23.0
ZT124	14.8	15.0	0.0	14.9	ZT091	22.0	24.0	2.0	23.0
ZT010	16.6	13.4	5.2	15.0	ZT065	20.0	26.3	19.8	23.2
ZT055	14.4	16.4	2.1	15.4	ZT029	21.3	25.7	9.6	23.5
ZT033	17.2	13.8	6.0	15.5	ZT140	26.1	22.0	8.3	24.0
ZT038	16.7	14.4	2.6	15.5	ZT076	27.3	21.5	16.5	24.4
ZT027	18.0	13.2	11.5	15.6	ZT056	27.7	21.8	17.7	24.7
ZT061	14.2	17.1	4.1	15.6	ZT003	24.0	29.7	16.1	26.8
ZT097	16.5	15.2	0.8	15.9	ZT057	23.5	31.3	30.0	27.4
ZT083	13.1	18.6	14.9	15.9	ZT063	30.7	25.1	15.3	27.9
ZT094	17.9	14.0	7.7	16.0	ZT024	33.0	25.4	28.9	29.2
ZT068	15.1	17.1	1.9	16.1	ZT074	28.0	32.4	9.8	30.2
ZT041	19.3	13.7	15.6	16.5	Mean	15.8	16.0	6.1	15.9
ZT032	17.5	15.5	2.0	16.5	Topas	20.0	22.3	2.645	21.2
ZT060	16.4	16.8	0.1	16.6	ZY821	2.3	3.7	0.980	2.9

Table S8. SNP Clusters in the Genome of a *B. napus* DH Population M730

	Number of Markers per Cluster (Position) (%)										
Chromosome	1	2	3	4	5	6	7	8	9	10	>10
A1	51.1	17.8	11.9	6.7	3.0	2.2	2.2	0.7	0.0	0.0	4.4
A2	58.1	18.3	7.5	1.1	4.3	2.2	1.1	1.1	3.2	1.1	2.2
A3	55.0	19.6	7.1	5.4	2.5	2.1	1.1	1.4	1.1	0.4	4.3
A4	48.5	19.1	8.1	6.6	2.9	3.7	4.4	2.9	0.0	0.0	3.7
A5	50.7	22.5	6.3	2.1	2.1	3.5	4.2	2.8	0.0	0.0	5.6
A6	64.8	18.5	6.5	2.8	2.8	1.9	0.0	0.0	0.9	0.0	1.9
A7	57.9	21.0	5.1	5.6	0.9	2.3	0.9	2.8	0.0	0.0	3.3
A8	39.7	10.3	15.5	12.1	8.6	1.7	1.7	0.0	0.0	1.7	8.6
A9	54.6	14.9	12.1	6.4	1.4	0.0	2.1	2.1	1.4	1.4	3.6
A10	59.7	14.5	9.7	4.0	0.8	2.4	2.4	0.0	1.6	0.8	4.0
Subgenome A	54.0	17.7	9.0	5.3	2.9	2.2	2.0	1.4	0.8	0.5	4.2
C1	53.4	17.5	2.9	5.8	1.9	7.8	2.9	1.0	1.0	0.0	5.8
C2	45.6	14.8	6.0	7.4	3.4	2.0	2.0	0.0	1.3	1.3	16.1
C3	50.7	16.8	7.2	4.3	4.3	2.9	2.9	1.4	0.5	1.4	7.7
C4	41.4	13.3	7.8	7.8	6.3	2.3	4.7	0.8	1.6	2.3	11.7
C5	53.5	12.1	12.1	0.0	8.6	5.2	0.0	1.7	3.5	1.7	1.7
C6	62.2	2.7	2.7	0.0	2.7	5.4	0.0	2.7	5.4	0.0	16.2
C7	47.4	16.7	12.3	4.4	5.3	2.6	0.9	2.6	0.9	0.0	7.0
C8	52.9	20.2	10.1	2.5	2.5	1.7	2.5	1.7	1.7	0.0	4.2
C9	60.4	8.3	4.2	4.2	0.0	2.1	6.3	2.1	0.0	6.3	6.3
Subgenome C	51.9	13.6	7.3	4.0	3.9	3.6	2.5	1.6	1.8	1.4	8.5
Genome AC	53.0	15.7	8.2	4.7	3.4	2.8	2.2	1.5	1.3	1.0	6.2

Table S9. Replicates in Evaluation of Sclerotinia Stem Rot Resistance for a *B. napus* DH Population M730

Replicate	Stem lesion length measurements
D4AP12	4 DPI in the growthroom in April, 2012
D6MA12	6 DPI in the growthroom in May, 2012
D2JU12	2 DPI in the growthroom in July, 2012
F412	4 DPI in field nursery in 2012
F812	8 DPI in field nursery in 2012
D3SE12	3 DPI in the greenhouse in September, 2012
SE93	Lesion length difference between 9 DPI and 3 DPI in the greenhouse in September, 2012
D3OC13	3 DPI in the growthroom in October, 2013
JUN93	Lesion length difference between 9 DPI and 3 DPI in the growthroom in June, 2012
F813S3	8 DPI in field nursery in 2013

*: DPI: days post inoculation

Table S10. Replicates in Evaluation of Sclerotinia Stem Rot Resistance for a *B. napus* DH Population M692

Replicate	Stem lesion length measurements
J31C6	6 DPI in the greenhouse on Jan 31, 2012
J31G6	6 DPI in the growthroom on Jan 31, 2012
O3C6	6 DPI in the greenhouse on Oct 3, 2012
FC6	6 DPI in the greenhouse in Feb, 2013

*: DPI: days post inoculation

Table S11. Epistatic Effects of QTLs Regulating Seed Glucosinolates in Three *B. napus* DH Populations Identified Using SNP and SRAP Markers*

Pop	QTL	Type	Ch	Marker	Pos	LOD	Effect	Effect (%)	GSL
M730	3x6	AA				6	8.96	7.5	4C
M692	2	A	A3	40	158.3	8.1	-1.55	15.4	5C
	7	A	C7	39	101.5	21.3	3.72	54.4	5C
	9	A	C9	2	104.7	4.1	1.18	4	5C
	2x7	AA				3.6	-0.94	5.4	5C
	7x9	AA				3.9	1.2	-3.2	5C
	8	A	C9	1	101	8.1	-0.64	16.1	GBC
	6	A	C7	38	100.9	8.5	10.31	299.1	GBN
	6x8	AA				-3.9	0.69	-5.8	GBN
	8	A	C2	1	15.5	8.5	1.33	13.9	GLS
	10	A	C7	30	85.7	8.7	-1.03	-15.7	GLS
	11	A	C7	38	100.9	19.1	1.87	50.5	GLS
	4x11	AA				3.4	-0.34	3.6	GLS
	8x10	AA				4.9	-0.68	2.7	GLS
	8x11	AA				3.2	0.53	1.2	GLS
	5	A	A5	30	103.1	6.3	1.3	1.4	GNP
	5x10	AA				3.3	-0.78	4.5	GNP
	10	A	C7	36	95.9	13.2	11.86	63.3	PRO
	1x10	AA				3.3	-2.88	5.1	PRO
	5	A	C4	35	98.6	4.8	5	2.4	TGC
	7	A	C7	36	97.9	21.7	13.78	52.6	TGC
	5x7	AA				3.2	3.7	3.9	TGC
ZT	1	A	7	17	33.5	7.6	15.08	8.1	T-Ali
	3	A	9	8	32	4.5	18.23	26.7	T-Ali
	1x3	AA				3.6	-8.05	5.4	T-Ali
	1	A	7	17	33.5	8	15.22	8.5	TGC
	3	A	9	8	32	4.9	18.38	27.5	TGC
	1x3	AA				3.8	-8.19	5.5	TGC

*: SNP, single nucleotide polymorphism; SRAP, sequence related amplified polymorphism; Type: A, additive, AA, epistatic; Ch: chromosome; Pos: position in cM; LOD: logarithm of odds; GSL: glucosinolate; 4C, four carbon aliphatic glucosinolates; 5C, five carbon aliphatic glucosinolates; GBC, glucobrassicin; GBN: glucobrassicanapin; GLS; glucoalyssin; GNP, gluconapin; PRO, progoitrin; T-Ali, total aliphatic glucosinolates and TGC, total glucosinolate content.

Table S12. Correlation Coefficients of Seed Glucosinolate Content (μmol/g Seed) and Plant Stem Lesion Length (cm) Caused by *Sclerotinia sclerotiorum* in a *B. napus* DH Population M730*

Rep	PRO	GNP	4C	GLS	GBN	5C	T-Ali	GBC	TGC
D4AP12	0.02	0.03	0.03	-0.29	-0.03	-0.15	0.01	-0.08	0.00
D6MA12	-0.01	0.07	0.02	-0.14	-0.05	-0.10	0.01	0.08	0.01
D2JU12	-0.02	-0.09	-0.05	-0.10	-0.14	-0.15	-0.07	0.07	-0.07
F412	0.04	0.00	0.03	-0.05	0.02	0.00	0.02	0.06	0.03
F812	0.07	0.09	0.08	0.00	-0.02	-0.02	0.07	0.03	0.08
D3SE12	0.13	0.11	0.14	-0.08	0.07	-0.01	0.12	0.01	0.13
SE93	-0.01	0.03	0.00	-0.14	-0.09	-0.13	-0.01	-0.04	-0.02
D3OC13	0.23	0.25	0.26	-0.07	0.22	0.13	0.25	-0.06	0.25
Jun-93	-0.07	-0.11	-0.09	-0.31	-0.09	-0.20	-0.11	0.02	-0.11
F813S3	0.06	0.10	0.08	-0.18	0.11	0.00	0.08	0.04	0.08
Mean	0.05	0.05	0.05	-0.14	0.00	-0.06	0.04	0.01	0.04

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content.

Table S13. Correlation Coefficients of Seed Glucosinolate Content (μmol/g Seed) and Plant Stem Lesion Length (cm) Caused by *Sclerotinia sclerotiorum* in a *B. napus* DH Population M692*

Rep	PRO	GNP	4C	GLS	GBN	5C	T-Ali	GBC	TGC
J31C6	-0.10	-0.25	-0.13	-0.19	-0.11	-0.16	-0.15	-0.15	-0.15
J31G6	-0.03	-0.12	-0.05	-0.28	-0.18	-0.24	-0.09	-0.25	-0.10
O3C6	0.01	-0.03	0.01	-0.03	0.03	0	0	-0.23	0
FC6	0.32	0.41	0.34	0.32	0.37	0.36	0.36	-0.04	0.36

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content.

Table S14. Correlation Coefficients of Seed Glucosinolate Content (μmol/g Seed) and Plant Stem Lesion Length (cm) Caused by *Sclerotinia sclerotiorum* in a *B. napus* DH Population ZT*

Rep	PRO	GNP	4C	GLS	GBN	5C	T-Ali	GBC	TGC
2009	-0.02	-0.09	-0.05	-0.09	-0.12	-0.11	-0.08	0.05	-0.08
2010	0.08	-0.06	0.03	0.01	0.01	0.01	0.03	0.07	0.03
Mean	0.03	-0.08	-0.01	-0.04	-0.05	-0.05	-0.02	0.06	-0.02

*: PRO, progoitrin; GNP, gluconapin; 4C, four carbon aliphatic glucosinolates; GLS, glucoalyssin; GBN, glucobrassicanapin; 5C, five carbon aliphatic glucosinolates; T-Ali, total aliphatic glucosinolates; GBC, glucobrassicin and TGC, total glucosinolate content.

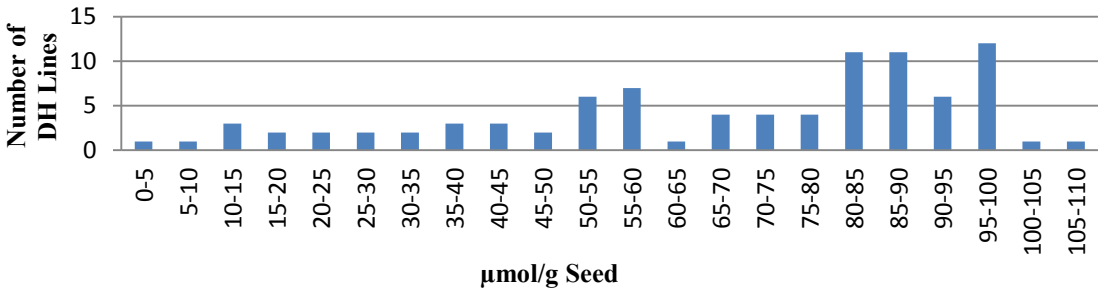


Figure S1. The Distribution of Total Seed Glucosinolate Content in a *B. napus* DH Population M730

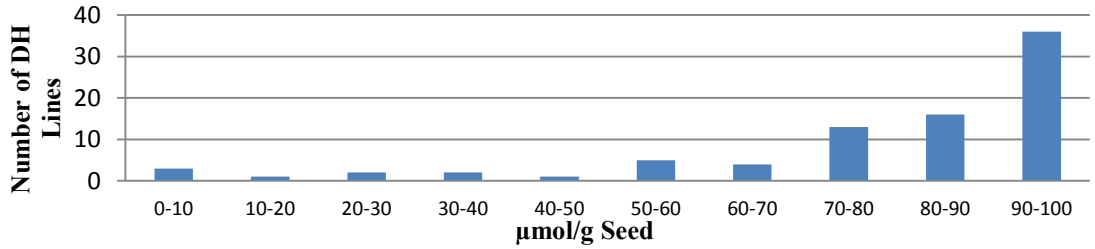


Figure S2. The Distribution of Total Seed Glucosinolate Content in a *B. napus* DH Population M692

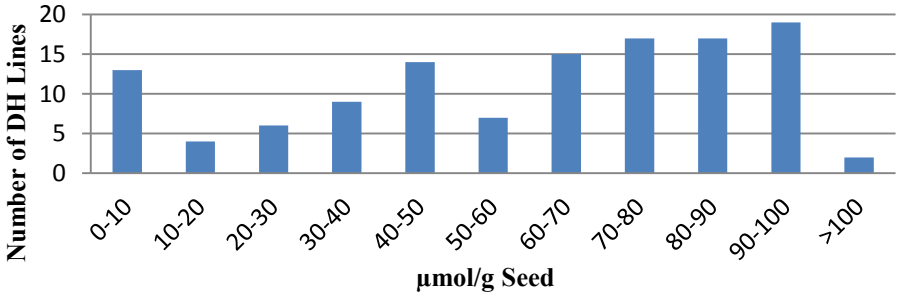


Figure S3. The Distribution of Total Seed Glucosinolate Content in a *B. napus* DH Population ZT

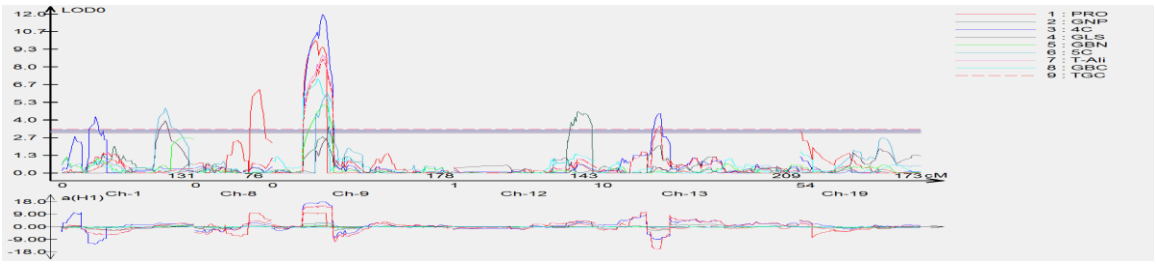


Figure S4. QTLs Identified for Seed Glucosinolates in a *B. napus* DH Population M730 Using SNP and SRAP Markers

Legend: PRO (Progoitrin), GNP (Gluconapin), 4C (Four Carbon Aliphatic Glucosinolates), GLS (Glucoalyssin), GBN (Glucobrassicanapin); 5C (Five Carbon Aliphatic Glucosinolates), T-Ali (Total Aliphatic Glucosinolates), GBC (Glucobrassicin) and TGC (Total Glucosinolate Content). **Upper Graph**: QTLs Identified by Peaks; LOD, Logarithm of Odds; Horizontal Axis, Chromosomes. **Lower Graph**: Additive QTL Effects.

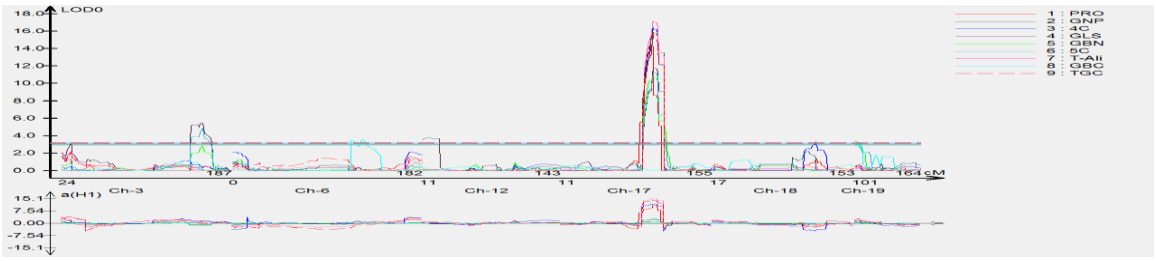


Figure S5. QTLs Identified for Seed Glucosinolates in a *B. napus* DH Population M692 Using SNP and SRAP Markers

Legend: PRO (Progoitrin), GNP (Gluconapin), 4C (Four Carbon Aliphatic Glucosinolates), GLS (Glucoalyssin), GBN (Glucobrassicanapin); 5C (Five Carbon Aliphatic Glucosinolates), T-Ali (Total Aliphatic Glucosinolates), GBC (Glucobrassicin) and TGC (Total Glucosinolate Content). **Upper Graph**: QTLs Identified by Peaks; LOD, Logarithm of Odds; Horizontal Axis, Chromosomes. **Lower Graph**: Additive QTL Effects.

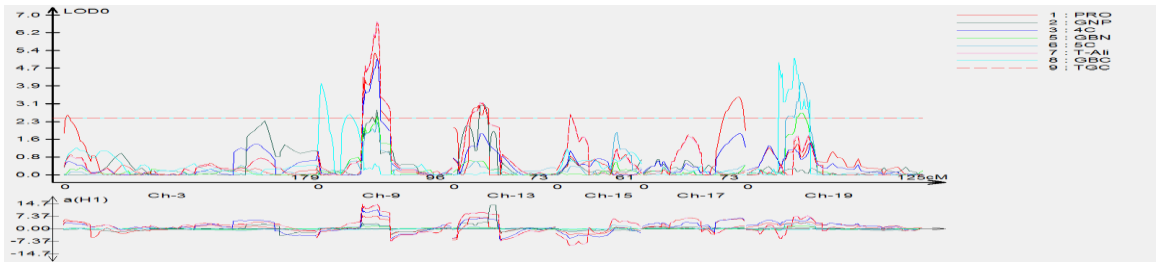
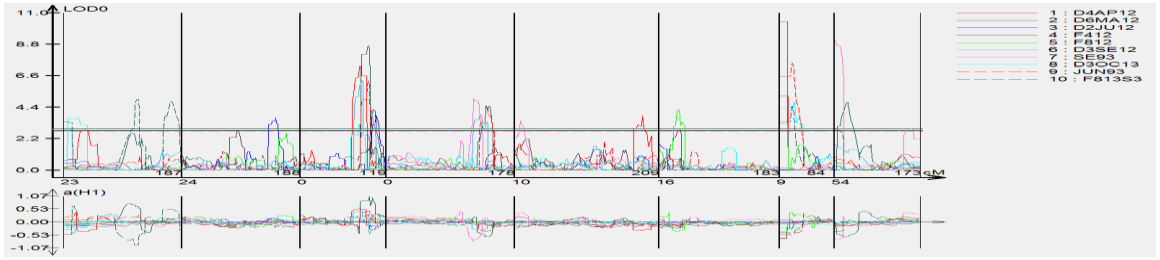


Figure S6. QTLs Identified for Seed Glucosinolates in a *B. napus* DH Population ZT Using SRAP Markers

Legend: PRO (Progoitrin), GNP (Gluconapin), 4C (Four Carbon Aliphatic Glucosinolates), GBN (Glucobrassicanapin); 5C (Five Carbon Aliphatic Glucosinolates), T-Ali (Total Aliphatic Glucosinolates), GBC (Glucobrassicin) and TGC (Total Glucosinolate Content). **Upper Graph**: QTLs Identified by Peaks; LOD, Logarithm of Odds; Horizontal Axis, Chromosomes. **Lower Graph**: Additive QTL Effects.



A2 *A3* *A7* *A9* *C3* *C4* *C6* *C9* --- *chromosome*

Figure S7. Identified QTLs Controlling Sclerotinia Stem Rot Resistance in a *B. napus* DH Population M730 in Ten Replicates Using SNP and SRAP Markers

Upper Graph: QTLs Identified by Peaks; LOD, Logarithm of Odds. **Lower Graph**: Additive QTL Effects.

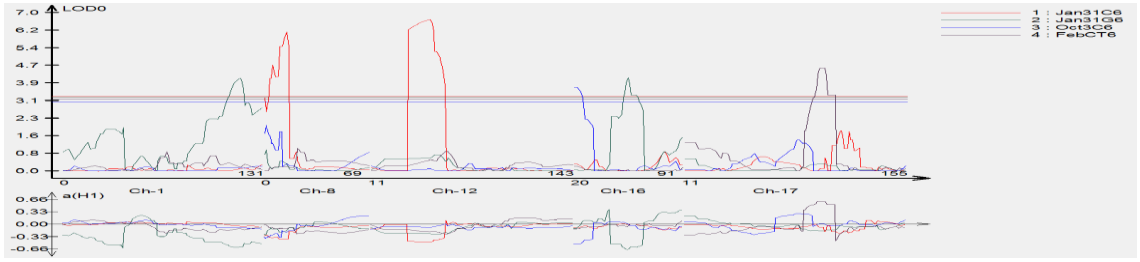


Figure S8. Identified QTLs Controlling Sclerotinia Stem Rot Resistance in a *B. napus* DH Population M692 in Four Replicates Using SNP and SRAP Markers

Upper Graph: QTLs Identified by Peaks; LOD, Logarithm of Odds; Horizontal Axis, Chromosomes. **Lower Graph**: Additive QTL Effects.