Use of Turnip (*Brassica rapa* var. *rapifera*) and Rutabaga (*B. napus* var. *napobrassica*) for the Improvement of Clubroot Resistance in Spring *B. napus* Canola

by

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Abstract

Clubroot disease, caused by *Plasmodiophora brassicae*, is one of the most serious threats to spring *Brassica napus* canola production in Canada. Growing of clubroot-resistant cultivars is the key to control this disease. The genetic base of the Canadian spring *B. napus* canola for clubroot resistance genes is narrow, and a strong resistance to the newly evolved pathotypes cannot be achieved using the available resistance genes. In this study, two advanced-generation breeding populations of spring *B. napus* canola, a BC_1F_8 population derived from spring *B. napus* canola × *B. rapa* subsp. *rapifera* European fodder turnip accession ECD 01 interspecific cross and a doubled haloid (DH) population developed from spring *B. napus* canola × Canola line carrying clubroot resistance of *B. napus* var. *napobrassica* rutabaga cv. Polycross, were accessed for resistance to different pathotypes of *P. brassicae*. Several canola lines carrying resistance to multiple *P. brassicae* pathotypes including 2B, 3A and 5x (L-G1) were identified in both populations, and resistance to these pathotypes showed significant positive correlation suggesting that the genetic control of resistance to these two pathotypes might be under a similar genetic control. QTL mapping by using the DH population identified the chromosomes A03 and A08 carry clubroot resistance. In case of the A03, a genomic region located at about 32-54 cM region conferred resistance to *P. brassic*ae pathotypes 2B, 3A, 5x (L-G1), 3H and 3D, and a region at about 210-220 cM conferred resistance to pathotypes 3H and 3D; these two genomic regions could be positioned at about 16 and 25 Mb of the physical map of A03. In case of A08, a region located at 0.0-2.35 cM contributed resistance to pathotypes 2B, 5x (L-G1), 3H and 3D, and a region located at 2.35-5.55 cM contributed resistance to pathotype 3A; these two genetic regions could be positioned at about 11-13 Mb of A08. Thus, results from this thesis research demonstrated that the turnip accession ECD 01 and rutabaga cv. Polycross carry resistance to different *P. brassicae* and can be used to broaden the genetic base of spring *B. napus* canola for resistance to clubroot disease.

The knowledge of the genomic regions of A03 and A08 carrying clubroot resistance can be used in the breeding, as well as for fine-mapping of the QTL regions and map-based cloning of the clubroot resistance genes.

Preface

This thesis is an original work by Yingyi Liu, who performed all of the experiments, analyzed the research data, wrote the first draft of all chapters and incorporated the valuable comments and suggestions from her supervisors Drs. Habibur Rahman and Guanqun (Gavin) Chen. Dr. Habibur Rahman reviewed the first draft of all thesis chapters and provided suggestions for improvements. Dr. Guanqun (Gavin) Chen also reviewed and contributed to the revisions of this dissertation.

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List of Symbols and Abbreviations

±	Plus/minus
=	Equal
>	Greater than
<	Less than
≤	Less than or equal
~	About
%	Percent
°C	Degrees Celsius
2*n*	Diploid number of chromosomes
µl	Microlitre
µmol	Micromole
µmol/g	Micromole per gram
AAFC	Agriculture and Agri-Food Canada
ABI	Applied Biosystems
AFLP	Amplified fragment length polymorphism
BC1	First backcross generation
BC1F8	xth generation of BC1-derived population
BLAST	Basic local alignment
bp	Base pair
BRAD	*Brassica* Database
CAPS	cleaved amplified polymorphic sequence
CCD	Canadian Clubroot Differentials
C.I.	Confidence Interval
cm	Centimeter

cM	Centi-Morgan
CR	Clubroot resistance
cv.	Cultivar
cvs.	Cultivars
dai	Days after inoculation
DH	Doubled haploid
DNA	Deoxyribose nucleic acid
dNTP	Deoxynucleotide triphosphate
DSI	Disease severity index
ECD	European Clubroot Differentials
ET	Ethylene transferase
F_1	First generation
F_x	xth generation of F2-derived population
g	Gram
GT	genotype by trait
Hi-Di	Highly Deionized Formamide
JA	Jasmonic acid
ICIM	Inclusive Composite Interval Mapping
LOD	Logarithm of odds
LRR	Leucine-rich repeats
MAS	marker-assisted selection
Mb	Million base pair
mM	Millimole
ml	Millilitre
min	Minute

NBS	Nucleotide-binding site
ng	Nanogram
ng/µl	Nanogram per microliter
nt	Nucleotide
p	Probability value
PC1	First principal component
PC2	Second principal component
PCA	Principle component analysis
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
r	Pearson's correlation coefficient
R	R project for statistical computing
RAPD	Random amplified polymorphism DNA
REC	recombination frequency
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SA	Salicylic Acid
SCAR	design sequence-characterized amplified region
SDS	Sodium dodecyl sulfate
S.E.	Standard Error
SNP	Single nucleotide polymorphism
SRAP	Sequence related amplified polymorphism
SSR	Simple sequence repeat
SMA	Single Marker Analysis
STS	sequence-tagged site

TAIR	The Arabidopsis Information Resource
TIR	Toll-interleukin-1
U (μmol/min)	Enzyme unit (micromole per minutes)
UCSC	University of California Santa Cruz
USDA-FAS	United States Department of Agriculture, Foreign Agricultural Service
var.	Variety

1 Chapter 1: Literature review

1.1 Introduction

Brassica napus L. is a profitable oilseed crop in the genus *Brassica* of the Brassicaceae (Cruciferae) family. The Brassicaceae family includes more than 4,000 species belonging to 360 genera (Jabeen 2020). The genus *Brassica* includes several economically important species, such as *B. rapa* L. (bok choy, Chinese cabbage, and turnip), *B. oleracea* L. (broccoli, Brussels sprouts, cabbage, cauliflower, collard, kale, and kohlrabi), *B. napus* L. (canola and rutabaga), and *B. juncea* (L.) Czern. (brown mustard) (Rakow 2004; Warwick et al. 2013; Jabeen 2020). These well-known plant species are normally grown for vegetables, fodders, edible oils, and condiments. Furthermore, the *Brassica* rapeseed oil can also be used for non-edible purposes, such as cosmetics, lacquers, lamp oil, manufacture of soaps, biodiesel, and industrial lubricants (McVetty and Duncan 2015, 2016; McKeon et al. 2016).

The term "canola" refers to specific varieties of *Brassica* oilseed crops that contain less than 2% of erucic acid in seed oil and less than 30 μmol/g of glucosinolates in oil-free seed meal (Bonnardeaux 2007). Canola cultivars were first developed from the species *B. napus* and *B. rapa* in 1974 and 1977 (Barthet 2016), respectively, and later from *B. juncea* in 2007 (Burton et al. 2007), to produce nutritional vegetable oil for human consumption and protein-rich seed meal for animal feed (for review, see Huhtanen et al. 2011; Jahreis and Schäfer 2011; Lin et al. 2013; for review, see Mejicanos et al. 2016). Due to the excellent fatty acid profiles of canola oil, its market demand is growing globally (McVetty and Duncan 2016). Currently, canola is the second-leading source of vegetable oil after soybean in the world (Daun and Unger 2016).

Canola is one of the most valuable crops in Canada, especially in the Prairie Provinces of Alberta, Saskatchewan and Manitoba. Currently, Canada is the leading producer of canola and it

exports more than 50% of its canola products to different countries including China, the United States, South Korean, Mexico, and Japan (USDA-FAS 2021; Statistics Canada 2021a). The canola industry creates tremendous benefits to Canada's economy. However, production of this crop is always under the threat of multiple environmental stresses, and this includes the biotic stresses such as diseases, weeds and insects, and the abiotic stresses which includes the negative impacts of climate change such as drought and heat stresses (Gavloski et al. 2011; Kutcher et al. 2013; Qian et al. 2018; for review, see Asaduzzaman et al. 2020). Among the biotic stresses, clubroot disease, caused by *Plasmodiophora brassicae*, is one of the most important as it causes significant losses in Canadian canola production (for review, see Dixon 2009; Tewari et al. 2005; Pageau et al. 2006; for review, see Howard et al. 2010).

In Canada, clubroot disease was first identified in canola field in Alberta in 2003 (Tewari et al. 2005), and in a few years this disease spread to southern Alberta (for review, see Howard et al. 2010), Saskatchewan (Dokken-Bouchard et al. 2012), Manitoba (McLaren et al. 2014) and Ontario (Al-Daoud et al. 2018). In the past decade, multiple strategies have been investigated to lower the incidence and severity of clubroot on canola, and this includes cultural, chemical and biological control methods, and the development of clubroot-resistant cultivars (for review, see Rahman et al. 2014; Peng et al. 2014b). However, the hardy resting spores of *P. brassicae* are capable of surviving in the soil and retain their virulence for at least 17 years (Wallenhammer 1996). The dissemination of *P. brassicae* from field to field occurs rapidly. Therefore, traditional clubroot management approaches become less efficient; hence, integrated management strategies by growing clubroot-resistant cultivars together with traditional control strategies are required for sustainable management of clubroot in infested canola fields (for review, see Hasan et al. 2021a).

In the past decade, extensive studies have been conducted to investigate the genetic basis of clubroot resistance in *Brassica* species. Several major clubroot resistance genes and numerous minor effect quantitative trait loci (QTL) have been identified and mapped on the chromosomes of *B. napus*, *B. rapa*, *B. oleracea*, and *B. nigra* (for review, see Hasan et al. 2021a). Clubroot resistance in the European winter canola cv. Mendel has been used in the breeding of clubroot-resistant *B. napus* canola cultivars in Canada (Rahman et al. 2011). Unfortunately, clubroot resistance in most of the commercial cultivars is based on a single dominant resistance gene; genetic resistance often become ineffective due to the emergence of new *P. brassicae* pathotypes (Strelkov et al. 2016, 2021; Askarian et al. 2021; Hollman et al. 2021). Hence, pyramiding of multiple clubroot resistance genes in a canola cultivar has been considered as an effective strategy to improve the durability of resistance to multiple pathotypes of *P. brassicae* (for review, see Rahman et al. 2014).

Molecular markers linked to desired genomic regions have been extensively used in genetic diversity analysis, genomic mapping studies and plant breeding research (for review, see Nadeem et al. 2018). Simple sequence repeat (SSR) markers are powerful tools for genetic mapping of genomic regions associated with clubroot resistance in *Brassica* populations and pyramiding multiple clubroot resistance genes into one *B. napus* cultivar through marker-assisted selection (MAS) (Kuginuki et al. 1997; Sakamoto et al. 2008; Chu et al. 2014; Hasan et al. 2021b). This MSc thesis research will evaluate two advanced-generation spring *B. napus* canola breeding populations for resistance to multiple *P. brassicae* pathotypes, map the clubroot resistance loci of the rutabaga cv. Polycross, and identify SSR markers linked to clubroot resistance for use in spring *B. napus* canola breeding.

1.2 Canola (*Brassica napus*)

1.2.1 Origin and evolution of *Brassica napus*

B. napus (AACC, 2*n* = 38) is an allotetraploid species that derived from interspecific crosses between two diploid species, *B. rapa* (AA, 2*n* = 20) and *B. oleracea* (CC, 2*n* = 18) (Allender and King 2010; Chalhoub et al. 2014). The phylogenetic relationship between the six main *Brassica* species was described as a triangle which is commonly called the "Triangle of U" or "U's Triangle" (U 1935, cited by McVetty and Duncan 2016) (Figure 1.1). Recent genetic studies revealed that the evolution of this allopolyploid species occurred about 7500 years ago (Chalhoub et al. 2014), 6700 years ago (Sun et al. 2017), or 1910 to 7180 years ago (Lu et al. 2019).

The geographical location where the initial hybridization event occurred between *B. rapa* and *B. oleracea* is unclear as no genuinely wild *B. napus* populations can be found in nature (Gomez-Campo 1999). It is commonly accepted that *B. napus* originated in the coastal Mediterranean and European Atlantic regions, where the two progenitors, *B. rapa* (European turnip) and *B. oleracea* (kohlrabi, broccoli, cauliflower, and Chinese kale), can be found in nature (Rakow 2004; Lu et al. 2019). However, this allopolyploid species is believed to have multiple origins as naturalized forms were discovered in New Zealand where its two ancestor species grow wild (Rakow 2004). Recent molecular marker-based studies have also provided evidence that the present-day *B. napus* germplasms developed from different interspecific hybridization events occurred in multiple geographical regions (Allender and King 2010).

1.2.2 *Brassica* oilseed crop in Canada

In Canada, *B. rapa* was first introduced from Poland in 1936 and *B. napus* was first introduced from Argentina in 1942 (Downey 2021); therefore, these two species are commonly

called ‘Polish canola’ and ‘Argentine canola’, respectively. On the contrary, *Brassica* oilseeds have been grown for oil in China, India, Japan, and Europe for centuries. During the Second World War, Canada started commercial-scale cultivation of *Brassica* oilseed crops (Barthet 2016). At that time, *Brassica* seed oil with a high level of erucic fatty acid was primarily used as lubricants in steam engines and ships (Downey 2021). In 1974, the first low erucic acid (< 2% erucic acid in oil) and low glucosinolates (< 30 μmol/g) *B. napus* canola cv. Tower was developed through conventional breeding by Canadian researchers, and thereafter the canola-quality *B. rapa* and *B. juncea* were developed (Stefansson and Kondra 1975; Barthet 2016). Among the different canola-quality *Brassica* oilseed crop species, the spring-type Argentine canola which has high yield potential, high oil content, and excellent tolerance to environmental stresses, is the most widely grown species of canola in Western Canada (Mendham and Robertson 2016).

In Canada, *B. napus* canola occupies most of the *Brassica* oilseed production. According to growth habit types, canola-quality *B. napus* can be categorized as winter-type, semi-winter-type, and spring-type. The winter-type *B. napus* requires a relatively long vernalization period (eight weeks) for flower initiation and is mostly cultivated in Europe (Pullens et al. 2019). Semi-winter-type *B. napus* requires mild vernalization (four weeks) and is mainly grown in China and Japan (Mendham and Robertson 2016). Due to the extreme cold winter conditions, the winter-type or semi-winter-type *B. napus* growth cannot be grown in Western Canada. By contrast, spring-type *B. napus* flower without vernalization and is primarily grown in Canada and Australia (Mendham and Robertson 2016).

1.2.3 Uses and benefits of canola

Canola oil accounts for about 35-45% of the weight of dry seeds (Daun and Unger 2016). This oil is free of cholesterol and trans fats, very low in saturated fatty acids (~ 4%), rich in

polyunsaturated fatty acids (~ 10% of alpha-linolenic fatty acid and ~ 25% of linoleic fatty acid), and high in monounsaturated fatty acid [~ 55%, low in erucic acid (< 2%)] (Dupont et al. 1989; Zambiazi et al. 2007). Because of its excellent fatty acid composition, canola oil is one of the world's healthiest dietary oils for use as a salad oil, cooking oil, shortening, or margarine (Daun and Unger 2016). Several studies have revealed the beneficial effects of diets enriched in canola oil. For example, Liu et al. (2016) reported that high-oleic acid canola oil consumption reduces abdominal fat mass and prevents metabolic syndrome in severe overweight people. Ghobadi et al. (2018) reported that consumption of canola oil lowers the content of serum lipids, including the total cholesterol and low-density lipoprotein cholesterol which decreases the risk of cardiovascular diseases. In contract, a high content of erucic acid in diets can increase the risk of heart disease in mammals (Knutsen et al. 2016). Traditionally, rapeseed oil containing a high level of erucic fatty acid is mainly used for non-edible purposes, such as lamp oil, soap, industrial lubricants, hydraulic fluids, or manufacture of soaps and plastics (Jahreis and Schäfer 2011; McVetty and Duncan 2015, 2016; McKeon et al. 2016). Additionally, canola oil is a clean, natural, and renewable source of biodiesel (for review, see Ge et al. 2017). Compared to petroleum diesel, biodiesel can reduce greenhouse gas emissions (Ge et al. 2018).

Canola meal is the by-product of canola seed after oil extraction. The crude protein content of canola meal is around 36-40% (Daun and Unger 2016); amino acid composition of this protein is excellent and it is high in sulphur-containing amino acids (methionine and cysteine) (for review, see Tan et al. 2011). Traditional rapeseed meal, with high concentrations of glucosinolates, can cause adverse effects on animals, and this includes reduced feed intake, reduced growth, decreased reproductive performance, abnormality of thyroid, liver, and kidney (for review, see Tripathi and Mishra 2007; Prieto et al. 2019). On the contrary, canola meal contains low glucosinolate content,

therefore, it is widely used as a protein component in animal feeds (Bonnardeaux 2007; for review, see Tan et al. 2011). Furthermore, canola meal is a more economical choice for protein supplement as compared to soybean meal. Several studies have confirmed that canola meal can be used as an excellent protein alternative for dairy cows (for review, see Huhtanen et al. 2011), swine (Yun et al. 2018), and poultry (Leeson et al. 1987).

1.2.4 Economic value of canola

Brassica oilseed is the world's second most important oilseed after soybeans, which are followed by peanuts, sunflower seed, cottonseed, palm-kernel, and copra (USDA-FAS 2021). In 2020/2021, global vegetable oil production reached 603.32 million metric tons where *Brassica* oilseed occupied more than 10% of this production (USDA-FAS 2021) (Figure 1.2). *Brassica* oilseed crops are primarily cultivated in Canada, Europe, China, and India. In 2020/2021, the total global production of this oilseed was 72,660 thousand metric tons (Figure 1.2), where Canada was the leading producer with a production volume of 19,485 thousand metric tons (USDA-FAS 2021) (Figure 1.3).

In Canada, canola is the second most profitable agricultural crop after wheat; some other important crops are corn, barley, and soybeans (Statistics Canada 2021b) (Figure 1.4). Currently, Canada is the top exporter of canola products in the world. Canadian canola products, which includes seed, refined oil, crude oil, and seed meal, are exported to more than 50 countries (USDA-FAS 2021; Statistics Canada 2021a). China, the United States, Japan, Mexico, South Korea, and Philippines are the main markets for the Canadian canola products (Statistics Canada 2021a). The United States is the primary importer of Canadian canola meal; more than 2.5 million tons of canola meal are exported to this country every year (Barthet 2016). In 2019, Canada's share to the global canola export market was about 61.3% (USDA-FAS 2021). Based on a report developed

by the agri-business research firm LMC International (2020), the canola industry contributes $29.9 billion annually to the economic activities in Canada. Additionally, the canola sector creates more than 207,000 jobs across the country, and the total annual wage impact was estimated to be $12 billion (LMC International 2020).

Nevertheless, canola production in Canada is affected by multiple threats. In the period of 2017 to 2020, the Canadian canola production was decreased from 21.5 million metric tons to 18.7 million metric tons (Statistics Canada 2021b) (Figure 1.4). According to a survey report developed by Statistics Canada (2019), this reduction was primarily attributable to a decline in harvested area. In addition to this, environmental stresses including biotic stresses, such as weeds, insects, and diseases, and the abiotic stresses, such as water and heat, may have also contributed to this lower the productivity of canola (Kutcher et al. 2010; Gavloski et al. 2011; for review, see Asaduzzaman et al. 2020; for review, see Dolatabadian et al. 2021). The incidence and severity of various diseases, such as clubroot, blackleg, sclerotinia stem rot, and verticillium stripe, significantly affects canola production in Canada (Tewari et al. 2005; Kutcher et al. 2013; for review, see Dolatabadian et al. 2021). Among these, clubroot is one of the most devastating diseases that can destroy canola production resulting in significant economic losses in Canada (for review, see Dixon 2009; Pageau et al. 2006; for review, see Howard et al. 2010).

1.3 Clubroot (*Plasmodiophora brassicae*)

1.3.1 Background

Clubroot disease, caused by *Plasmodiophora brassicae* Woronin, is an increasingly important disease that affects cruciferous plants throughout the world (for review, see Dixon 2009). This pathogen belongs to the class Plasmodiophorids of the family Plasmodiophoraceae (for review, see Braselton 1995). Clubroot infection is described by the formation of club-shaped

galls or swellings on the roots of infected canola plants (for review, see Hwang et al. 2011a). These galls can restrict water and nutrition uptake in host plants, leading to above-ground symptoms such as wilting, stunting, yellowing, premature ripening, and uneven ripening of the plants (for review, see Hwang et al. 2011a; Robin et al. 2019). Consequently, clubroot disease can negatively affects seed yield and oil content and its quality (Pageau et al. 2006). According to Dixon (2009), about 10-15% yield loss can occur on cruciferous vegetables worldwide. On severely infected canola fields, this disease can decrease yield by about 30-100% (Tewari et al. 2005; Pageau et al. 2006), and decline the seed oil content by around 6% (Pageau et al. 2006).

P. brassicae is an obligate biotrophic parasite that lives entirely on a suitable host for its survival (Braselton 1995; for review, see Hwang et al. 2011a). Most of the clubroot infection studies primarily focused on the genera *Brassica*, *Raphanus*, and *Arabidopsis*; however, all plants belonging to the family Brassicaceae could be potential host of this pathogen (for review, see Dixon 2009). Clubroot disease was first reported in Spain in the thirteenth century (Harani and Li 2015). After that, this disease spread into the rest of the Europe, and a severe outbreak of this disease devastated the cabbage industry in St. Petersburg, Russia in late 19th century (for review, see Howard et al. 2010). During this time, the causal organism of clubroot was identified as *P. brassicae* by Woronin (Woronin 1878, cited by Howard et al. 2010). To date, the incidences of clubroot disease have been detected on cruciferous vegetables in more than 60 countries (CABI/EPPO 2011).

A review on the introduction of the pathogen causing clubroot disease in Canada and its spread and importance has been published by Howard et al. (2010). According to this review, *P. brassicae* was probably introduced into Canada with clubroot infected turnips by the early 20th century. Since then, clubroot became a threat to the production of cruciferous crops in the Atlantic

Provinces, Ontario, Quebec, and British Columbia. During this time, this disease was only confirmed in a few home gardens and cruciferous vegetable fields in the Prairie Provinces. In 2003, this disease was first observed in 12 commercial canola fields in Central Alberta (Tewari et al. 2005). By 2018, clubroot infection had been identified in a total of 3,044 canola fields in Alberta (Strelkov et al. 2019). Furthermore, an increasing occurrence of clubroot disease has also been reported in other canola producing provinces, Saskatchewan (Dokken-Bouchard et al. 2012; Ziesman et al. 2019) and Manitoba (McLaren et al. 2014; Faroese et al. 2019). Most recently, clubroot was also observed on canola in Ontario (Al-Daoud et al. 2018).

1.3.2 Clubroot life cycle

The life cycle of *P. brassicae* consists of three distinct phases, survival of resting spores in soil, root hair infection (primary infection), and cortical infection (secondary infection) (for review, see Kageyama and Asano 2009) (Figure 1.5). *P. brassicae* is a fungus-like, soil-borne pathogen that remains dormant in the soil as long-lasting resting spores, and reacts rapidly when living hosts are available (Friberg et al. 2005). The resting spores of *P. brassicae* is highly tolerant to environmental stresses due to the presence of chitin in its cell wall (Schwelm et al. 2015). These spores can survive in the soil for up to 17 years without growing host plants, with a half-life of around 4 years (Wallenhammer 1996; Hwang et al. 2013). Previous researchers demonstrated that the primary transmission route of *P. brassicae* is through the movement of resting spores in soil or plant tissues via agricultural machinery, water, or windblown dust (Cao et al. 2009; Gossen et al. 2015; Rennie et al. 2015).

The life cycle of *P. brassicae* starts with the germination of resting spores in the soil. Temperature, soil moisture content, pH value, calcium level, and root exudates significantly effect on resting spore germination (Friberg et al. 2005; Sharma et al. 2011; Rashid et al. 2013). The

percentage of root hair infection and the development of clubroot symptoms were suppressed at 10 and 15°C (Sharma et al. 2011). Soil pH values ranging from 5.0 to 7.0 were found to be the most favourable for *P. brassicae* resting spore germination (Rashid et al. 2013). Furthermore, both host and non-host exudates stimulates the germination of resting spores (Friberg et al. 2005; Rashid et al. 2013). Non-host root exudates of perennial ryegrass (*Lolium perenne*) results in a greater germination of *P. brassicae* resting spores than the root exudates from host plants, such as *B. napus* canola and Chinese cabbage (*B. rapa* var. *pekinensis*) (Friberg et al. 2005; Rashid et al. 2013).

Under optimal conditions, *P. brassicae* resting spores are stimulated to germinate and produce biflagellate primary zoospores (Ayers 1944; for review, see Kageyama and Asano 2009). The biflagellate structure increases the mobility of the zoospores in high humidity soil. The primary infection stage begins with the penetration of the primary zoospores penetrate in root hairs and epidermal cells (for review, see Kageyama and Asano 2009; Liu et al. 2020a). Root-hair infection occur in almost all root hairs of both resistant and susceptible *B. napus* canola cultivars at about 7 days after inoculation (dai) (Fei et al. 2016). Within the infected root hairs, primary zoospores are differentiated into primary plasmodia, and then developed into zoosporangia (Tommerup and Ingram 1971; Liu et al. 2020a). Subsequently, secondary zoospores are formed from zoosporangia and released back to the soil or the lumen (for review, see Kageyama and Asano 2009; Liu et al. 2020a).

The secondary infection stage is responsible for clubroot symptoms in susceptible hosts (Liu et al. 2020b). The secondary zoospores are differentiated into secondary plasmodia in the root cortex (Tommerup and Ingram 1971; Liu et al. 2020a). Secondary plasmodia are rare in the initial stage of inoculation but are commonly observed in infected root cortical tissues at about 14 dai (Fei et al. 2016). The resistance mechanism in the resistant plants suppresses the development of

the secondary plasmodia (Fei et al. 2016). In contrast, cortical infection in the susceptible cultivars alters auxin and cytokinin metabolisms, and this leads hypertrophy (cell division) and hyperplasia (cell elongation) of the infected cells, resulting in the formation of visible galls or club-like swellings on the infected roots (for review, see Kageyama and Asano 2009; Robin et al. 2019). Consequently, the clubbed root symptoms block the absorption of water and nutrients by the susceptible plants (for review, see Hwang et al. 2011a). The mature secondary plasmodia are cleaved into a significant number of resting spores within the infected host roots (Hwang et al. 2013). After the decomposition of the galls or swellings, the newly formed resting spores are released into the soil (fore review, see Kageyama and Asano 2009). Under field conditions, a gram of gall from a clubroot-susceptible canola plant can release up to 1×10^{10} resting spores per gram of soil (Hwang et al. 2013).

1.3.3 Pathotypes of *P. brassicae*

The pathotypes of *P. brassicae* are differentiated depending on their virulence on different host plants (Strelkov et al. 2018). To date, several clubroot differential sets have been developed to classify the pathotypes of *P. brassicae*. Among them, the differential systems of Williams (1966), European Clubroot Differential (ECD) set (Buczacki et al. 1975), and Somé et al. (1996) are most commonly used to characterize *P. brassicae* populations worldwide (for review, see Strelkov and Hwang 2014). Williams' (1966) differential set comprises two hosts of *B. napus* and two hosts of *B. oleracea*, and this set can differentiate at least 16 pathotypes. The ECD set consists of five hosts of each of the three subsets, *B. napus*, *B. rapa*, and *B. oleracea*, and it is capable of differentiating 34 isolates of European *P. brassicae* (Buczacki et al. 1975). The system developed by Somé et al. (1996) with three cultivars of *B. napus* has a relatively low capacity for differentiating pathogens. In the initial stage of the identification of *P. brassicae* pathotypes in

Canada, Williams's (1966) classification system was extensively used to identify the strains of *P. brassicae*. Based on Williams's (1966) differential system, pathotypes 2, 3, 5, 6, and 8 were confirmed on canola in Canada (Strelkov et al. 2006, 2007; Xue et al. 2008; Cao et al. 2009). Among these five pathotypes, pathotypes 3 was identified as the most prevalent and virulent pathotype on canola (Strelkov et al. 2006; Xue et al. 2008).

However, the differential sets of Williams (1966), the ECD set (Buczacki et al. 1975), and Somé et al. (1996) are not sufficient for distinguishing the new *P. brassicae* populations evolved in Canada in the recent years (for review, see Strelkov and Hwang 2014). In 2013, new *P. brassicae* pathotypes (or populations), such as L-G1, L-G2, L-G3, and D-G3, with the capability of infecting resistant canola cultivars were detected in Alberta (Strelkov et al. 2016). These new populations of *P. brassicae* were differentiated as pathotype 5 based on Williams's (1966) differential set; however, their virulence on the first-generation clubroot-resistant cultivars was different from the original pathotype 5 (Strelkov et al. 2016). Hence, the Canadian Clubroot Differential (CCD) set was introduced to distinguish the 'old' and 'new' strains of *P. brassicae* from Canada (Strelkov et al. 2018).

The CCD set includes 13 hosts of *B. napus*, *B. oleracea*, and *B. rapa*. By using this set, 17 *P. brassicae* pathotypes were identified from field populations collected in 2014-2016 in Western Canada (Strelkov et al. 2018). The pathotypes 2F, 3H, 5I, 6M, and 8N identified on the CCD set, correspond to the previously identified Williams's pathotypes 2, 3, 5, 6, and 8, respectively (Strelkov et al. 2018). While comparing with other differential sets, the CCD set exhibit a more precise and reliable differentiating ability. For instance, the Williams's pathotype 3 is assigned as pathotypes 3A, 3D, 3H, and 3O on the CCD set, where the pathotype 3A is virulent on the first-generation clubroot-resistant *B. napus* cvs. Mendel and 45H29, while the pathotype 3H is not able

to overcome the resistance of these cultivars (Strelkov et al. 2018). In 2017 and 2018, five novel virulent pathotypes including 5A, 6C, 8A, 8B, and two isolates of 8C were detected in the Peace Country of Alberta (Strelkov et al. 2021); additionally, nine new pathotypes (2C, 6D, 8D, 9A, 9B, 9C, 11A, and 13A) were identified from 166 canola fields in Western Canada (Hollman et al. 2021). Most recently, six new virulent pathotypes (2A, 2F, 4A, 6A, 6B, and 7A) were discovered from nine commercial canola fields in Alberta (Askarian et al. 2021). The CCD set, thus, significantly contributes to the understanding of the population diversity of *P. brassica* — a knowledge important for breeding of clubroot-resistant canola cultivars for Canada's canola industry.

1.3.4 Clubroot management strategies

Different cultural control strategies, such as crop rotation, application of soil amendments, planting bait crops, and manipulating seeding date have been used to control clubroot on canola (for review, see Hwang et al. 2014). Crop rotations refer to the cultivation of non-hosts, such as barley and pea, while growing susceptible canola cultivars (Hwang et al. 2019). Peng et al. (2014b) suggested at least 2-year rotation for sustainable management of this disease, while Hwang et al. (2019) reported that a 2- or 3-year non-host break can greatly decline clubroot severity. Soil amendments, such as limestone (calcium carbonate), calcium cyanamide and boron, could be employed to manipulate soil properties and subsequently create unfavorable conditions for clubroot disease development (Hwang et al. 2011b; McGrann et al. 2016). Other strategies, such as planting bait crops has also been reported to slightly reduce the inoculum potential of resting spores and disease severity (Ahmed et al. 2011). Nonetheless, the results of cultural control were not consistently effective when soil inoculum load was high (Ahmed et al. 2011; Hwang et al. 2011b; for review, see Hwang et al. 2014).

Chemical control refers to the application of fungicides and soil fumigants that can effectively get rid of the pathogens or pests in the soil. The application of fungicides, such as fluazinam and cyazofamid, can efficiently suppress clubroot on cruciferous crops with an efficacy of more than 50% (Peng et al. 2011; Liao at al. 2021). Soil fumigants, such as Vapam and dazomet, are effective agents in controlling *P. brassicae* on canola (Hwang et al. 2014b; Hwang et al. 2017). Nevertheless, the application of fungicides and soil fumigations are impracticable for the management of the soil-borne pathogen *P. brassicae* in large-scale field conditions (Hwang et al. 2014b; for review, see Peng et al. 2104b).

Biological control is an environmentally friendly approach to suppress *P. brassicae* population through the use of microorganisms. In recent decades, many microorganisms including *Bacillus subtilis*, *Gliocladium catenulatum*, *Lysobacter antibioticus*, *Bacillus velezensis*, and *Bacillus amyloliquefaciens* have been evaluated for controlling *P. brassicae* on crucifer vegetable crops (Peng et al. 2011; Zhou et al. 2014; Zhu et al. 2020). However, the efficiency of biocontrol agents is highly dependent on environmental conditions (for review, see Peng et al. 2014b). Thereby, additional studies are required to overcome the challenges associated with the efficiency of the biological control agents.

Genetic resistance appears to be the most convenient and effective method to control clubroot on canola. In Canada, the first clubroot-resistant canola cv. 45H29 was developed in 2009; as of 2021, 55 additional clubroot-resistant cultivars have become available on the Canadian market (Canola Encyclopedia 2021). However, most of the resistant cultivars carry a narrow genetic base of resistance, and the resistance exhibits a pathotype- or race-specific effect, as their genetic resistance was primarily derived from the winter canola cv. ‘Mendel’ or *B. rapa* (Rahman et al. 2011; for review, see Rahman et al. 2014). The resistant cultivars based on single-gene

resistance are not effective when multiple pathotypes of *P. brassicae* are present in soil (Strelkov et al. 2016). In 2013, new virulent pathotypes with the capability of overcoming genetic resistance of the first-generation clubroot-resistant cultivars was identified in several canola fields in Alberta (Strelkov et al. 2016). Based on the CCD set, a total of 37 *P. brassicae* pathotypes including 5 original and 32 newly evolved pathotypes were detected through Western Canada (Strelkov et al 2018, 2021; Askarian et al. 2021; Hollman et al. 2021). Thereby, integrated management strategies, combing traditional control strategies in addition to durable genetic resistance, should be used for long-term management of clubroot on canola (for review, Hasan et al. 2021a).

1.3.5 Clubroot resistance in *Brassica*

Clubroot disease resistance in *Brassica* germplasm is needed for developing clubroot-resistant canola quality *B. napus* cultivars for Western Canada. In the past decade, several large-scale screening projects have been carried out to assess a large number of accessions of the amphidiploid *Brassica* species (*B. juncea*, *B. carinata*, and *B. napus*) and the diploid *Brassica* species (*B. oleracea*, *B. rapa*, and *B. nigra*) for resistance to specific *P. brassicae* pathotypes (Hasan et al. 2012; Peng et al. 2014a; Zhang et al. 2015; Liu et al. 2018; Farid et al. 2020). The amphidiploid *Brassica* species are members of the primary gene pool; introgression of clubroot resistance from the primary gene pool into *B. napus* canola through crossing can be accomplished easily as the progeny plants exhibit normal chromosome pairing and gene segregation, and good fertility (for review, see Rahman et al. 2014). On the other hand, gene transfer from the secondary gene pool (*B. oleracea*, *B. rapa*, and *B. nigra*) to the amphidiploid species through interspecific hybridization is more complex (for review, see Rahman 2013); however, this can be achieved for many traits including clubroot resistance (Rahman et al. 2011, 2015; Attri and Rahman 2018; Iftikhar et al. 2018; Hasan and Rahman 2016; Nikzad et al. 2020).

Resistance to Canadian *P. brassicae* pathotypes is rare in the amphidiploid *Brassica* species. A large collection of *B. juncea* and *B. carinata* accessions were evaluated for clubroot resistance; however, only one *B. juncea* accession which exhibited strong resistance to pathotype 4 was found (Hasan et al. 2012; Peng et al. 2014a; Liu et al. 2018). Ten of the 93 *B. juncea* accessions were reported to possess low or moderate resistance to pathotype 3 (Peng et al. 2014a). Most of the canola-quality *B. napus* cultivars are highly susceptible to various strains of *P. brassicae* (Hasan et al. 2012; Peng et al. 2014a). However, strong resistance to clubroot disease has been reported in rutabaga (*B. napus* subsp. *napobrassica*). Six rutabaga genotypes, including four Newfoundland-developed cvs. IRPT, Kingston, Polycross, and Brookfield, and two European cvs. Marian and Invitation, showed strong resistance to Canadian isolates of *P. brassicae* (Spaner 2002). Ayers and Lelacheur (1972) reported that the rutabaga cvs. York and Wilhelmsburger were responsible for resistance to two races of *P. brassicae*. Hasan et al. (2012) reported that three rutabaga cvs. Wilhelmsburger, Brookfield and Polycross carried resistance to multiple Canadian *P. brassicae* pathotypes. On the other hand, Peng et al. (2014a) found clubroot resistance in two rutabaga cvs. Wilhelmsburger and Askegarde. It is apparent that the rutabaga cultivars are valuable sources for use in clubroot-resistant *B. napus* canola breeding programs. Nevertheless, when incorporating clubroot resistance loci into spring *B. napus* canola, the other undesirable traits such as late flowering, late maturity, rich in erucic acid content, and high level of glucosinolates could also be introgressed from the rutabaga into *B. napus* canola cultivars (for review, see Rahman et al. 2014).

Previous studies demonstrated that many of the *B. rapa* cultivars possess resistance against multiple Canadian isolates of *P. brassicae* (for review, see Hasan et al. 2021a). Of the five European turnip accessions (*B. rapa* subsp. *rapifera*) evaluated by Hasan et al. (2012), all of them

exhibited resistance to *P. brassicae* pathotypes 2, 3, 5, 6 and 8. Peng et al. (2014a) found strong resistance against pathotype 3 in the turnip cvs, Siloga, Taronda, Vedette, and Vollenda. Ten of 14 Chinese cabbage cultivars showed resistance to pathotypes 3, 5, and 6 were reported by Zhang et al. (2015). To date, many major clubroot resistance loci conferring resistance to different strains of *P. brassicae* have been mapped to the A-genome chromosomes of the Chinese cabbage (*B. rapa* ssp. *chinensis* and *pekinensis*) and European fodder turnips (Hirai et al. 2004; Kato et al. 2013; Chu et al. 2014; Yu et al. 2017; Hirani et al. 2018; Pang et al. 2018; Fredua-Agyeman et al. 2020a). Furthermore, several clubroot resistance loci have been successfully used in the breeding of clubroot-resistant Chinese cabbage, rutabaga, and *Brassica* oilseed crops (Yoshikawa 1981; Spaner 2002; Rahman et al. 2011; Matsumoto et al. 2012; Zhang et al. 2012; Hasan and Rahman 2016; Liu et al. 2018).

A large number of *B. oleracea* accessions were tested for clubroot resistance in the past decade; however, resistance in this species has been found less frequently than in *B. rapa* (Hasan et al. 2012; Pang et al. 2014a; Liu et al. 2018; Farid et al. 2020; as reviewed by Hasan et al. 2021a). Of the 49 *B. oleracea* accessions studied by Hasan et al. (2012), only two cabbage cvs., Badger Shipper and Bindsachsener, showed resistance against different pathotypes of *P. brassicae*. Five of the 30 *B. oleracea* accessions tested by Peng et al. (2014a) were resistant to pathotype 3. Similarly, among the 135 *B. oleracea* accessions accessed by Farid et al. (2020), only 24 accessions carried strong resistance to pathotype 3A and 5x (field isolate L-G2). So far, resistance to *P. brassicae* was primarily found in two *B. oleracea* accessions, cabbage and kale (Liu et al. 2018; Farid et al. 2020).

In addition to *B. rapa* and *B. oleracea*, resistance to *P. brassicae* has also been found in *B. nigra* (Hasan et al. 2012; Peng et al. 2014a; Chang et al. 2019). Of a total of 77 *B. nigra* accessions

tested by Hasan et al. (2012), 60 individuals showed strong resistance to the five original Canadian *P. brassicae* pathotypes (2, 3, 5, 6, and 8). Peng et al. (2014a) reported three *B. nigra* cultivars carried a high level of resistance to pathotype 3; among these, 'BRA 192/78', possessed resistance to the five old *P. brassicae* pathotypes.

1.3.6 The genetic basis of resistance

Clubroot resistance has been extensively studied in the genus *Brassica*. So far, more than 20 major dominant clubroot resistance loci and a good number of QTL have been identified and mapped on the chromosomes of *B. napus*, *B. rapa*, *B. oleracea*, and *B. nigra* (for review, see Hasan et al. 2021a). Genes relating to plant-pathogen interaction and plant hormone signaling transduction pathways are important for response of the resistant plants to clubroot infection (Fu et al. 2019). Many of the major clubroot resistance genes reported in the genus *Brassica* encode toll-interleukin-1 receptor, nucleotide binding site, leucine-rich repeat (TIR-NBS-LRR class) proteins (Ueno et al. 2012; Hatakeyama et al. 2013; Yuan et al. 2015; Huang et al. 2017; Chang et al. 2019; Fu et al. 2019; Zhu et al. 2019). Fu et al. (2019) found that genes associated with the jasmonic acid (JA), ethylene(ET) and brassinosteroid (BR) were only up-regulated in the clubroot-resistance cultivars.

Genetic analysis and QTL mapping studies have reported more than 20 major clubroot resistance loci on the chromosomes A01, A02, A03, A05, A06, A07 and A08 of *B. rapa* (for review, see Hasan et al. 2021a). *Crr2*, *CR6a*, and *PbBa1.1* mapped on chromosome A01 (Lee et al. 2002; Suwabe et al. 2003, 2006); *CRc* and *Rcr8* were identified on A02 (Sakamoto et al. 2008; Yu et al. 2017); *Bcr1*, *Crr3*, *CRa/CRb*, *CRd*, *CRk*, *CRq*, *CR6b*, *PbBa3.1*, *PbBa3.2, PbBa3.3*, *Rcr1*, *Rcr2*, *Rcr4*, *Rcr5*, and *Rcr10* ^{ECD01} were positioned on A03 (Lee et a., 2002; Hirai et al. 2004; Piao et al. 2004; Saito et al. 2006; Cho et al. 2008; Sakamoto et al. 2008; Ueno et al. 2012; Chen et al.

2013; Kato et al. 2013; Chu et al. 2014; Yuan et al. 2015; Yu et al. 2016, 2017, 2022; Hatakeyama et al. 2017; Huang et al. 2017, 2019; Pang et al. 2018; Zhang et al. 2022); *CrrA5* was mapped on A05 (Nguyen et al. 2018); *Crr4* was located on A06 (Suwabe et al. 2006); *qBrCR38-1* was mapped on A07 (Zhu et al. 2019); and *Bcr2*, *Crr1* (*Crr1a, Crr1b*), *CRs*, *Rcr3*, *Rcr9*, *Rcr9* ^{ECD01}, *PbBrA08*^{Banglim}, *PbBa8.1*, and *qBrCR38-2* were detected on A08 (Suwabe et al. 2003, 2006; Chen et al. 2013; Hatakeyama et al. 2013; Yu et al. 2017, 2022; Laila et al. 2019; Zhu et al. 2019; Choi et al. 2020; Karim et al. 2020; Zhang et al. 2022). Since these clubroot resistance loci were reported by different researchers using different pathotypes of *P. brassicae*, some of them might be the same locus.

B. oleracea accessions, such as cabbage, broccoli, kale, and cauliflower, have been evaluated for resistance against *P. brassicae* (Hasan et al. 2012; Pang et al. 2014a; Liu et al. 2018; Farid et al. 2020). To date, only one dominant major CR loci, *Rcr7*, has been mapped on chromosome C07 of cabbage cvs., Tekila and Kilaherb (Dakouri et al. 2018). Many minor clubroot resistance loci which carry resistance to multiple pathotypes have been identified on all C-genome chromosomes of *B. oleracea* (for review, see Hasan et al. 2021a). As compared to *B. rapa*, genetic resistance in *B. oleracea* is more complicated. Clubroot resistance in *B. oleracea* is quantitative and controlled by many genes (Nagaoka et al. 2010; Lee et al. 2016; Peng et al. 2018; Farid et al. 2020; Ce et al. 2021). Therefore, it is important to strengthen clubroot resistance in spring *B. napus* canola cultivars using the clubroot resistance loci from A-genome of *B. rapa* and C-genome of *B. oleracea* (for review, see Rahman et al. 2014).

In the case of other *Brassica* species, several major clubroot resistance loci and minor QTL have been identified in *B. napus* and *B. nigra* (for review, see Hasan et al. 2021a). Ayers and Lelachur (1972) found a single dominant gene in the rutabaga cv. York and three major genes in

the rutabaga cv. Wilhemsburger. Manzanares-Dauleux et al. (2000) located a major loci *Pb-Bn1* on linkage group DY4 of *B. napus*, which was responsible for resistance to two single spore isolates of *P. brassicae*. A major gene on chromosome A08 and a minor locus on A03 were identified in the rutabaga cv. Brookfield (Hasan and Rahman 2016; Hasan et al. 2021b). Furthermore, a major resistance gene, *Rcr6*, conferring resistance to pathotype 3, was mapped to the B-genome chromosome B03 of *B. nigra* (Chang et al. 2019).

In Canada, most of the clubroot-resistant *B. napus* canola cultivars were controlled by a single major resistance gene derived from the winter-type *B. napus* cv. Mendel (for review, see Rahaman et al. 2014; Canola Encyclopedia 2021). Due to the evolution of new virulent pathotypes of *P. brassicae*, ineffectiveness of this major gene resistance has been reported in canola field by Strelkov et al. (2016). Therefore, pyramiding multiple clubroot resistance genes into a canola cultivar is an important approach to improve the durability of disease resistance (for review, see Hasan et al. 2021a). Matsumoto et al. (2012) has combined three major genes *CRa*, *CRk*, and *CRc* through MAS and developed clubroot-resistant *B. rapa* lines for resistance to six isolates of *P. brassicae*. Tomita et al. (2013) has pyramided five QTLs through MAS and increase resistance to six isolates of *P. brassicae* in *B. oleracea*. Recently, accumulation of two genes *CRb* and *PbBa8.1* has also been reported to reinforce resistance to multiple *P. brassicae* pathotypes in *B. napus* (Shah et al. 2019). In this regard, identification of additional clubroot resistance genes and development of polymorphic molecular markers linked to these resistance genes are the primary tasks for the development of clubroot-resistant *B. napus* canola cultivars through MAS.

1.4 Molecular markers

Molecular markers have been broadly used as valuable tools to access genetic variations or polymorphisms among individuals in a population at the DNA level (for review, see Nadeem et

al. 2018). Many molecular markers co-segregating with clubroot resistance genes have been identified and used in genetic diversity analysis, genetic linkage mapping and MAS in the genus *Brassica*; these include amplified fragment length polymorphism (AFLP), cleaved amplified polymorphic sequence (CAPS), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), design sequence-characterized amplified region (SCAR), single nucleotide polymorphism (SNP), sequence-tagged site (STS) and SSR markers (Kuginuki et al. 1997; Voorrips et al. 1997; Piao et al. 2004; Saito et al. 2006; Werner et al. 2008; Yu et al. 2016; Huang et al. 2017, 2019; Hasan and Rahman 2018).

SSR markers, also known as microsatellites, are tandem repeated DNA motifs composed of one to six nucleotides (for review, see Kalia et al. 2011). SSR markers are regarded as efficient and cost-effective molecular markers for use in genetic analysis and plant breeding, since they are genetic co-dominant, multi-allelic, with high reproducibility and broad coverage of the genome (Powell et al. 1996; for review, see Kalia et al. 2011). SSR markers have been used to evaluate genetic variability among six *Brassica* species and provided insight into the genetic relationship between the diploid and amphidiploid species (Thakur et al. 2018; Raza et al. 2019). Similarly, SSR markers can be used to examine the genetic diversity of different families within a specific population of *B. napus* (Hasan et al. 2006), *B. oleracea* (El-Esawi et al. 2016) or *B. juncea* (V. et al. 2013). Previous studies have developed various SSR makers for use in genetic mapping of clubroot resistance loci in *Brassica* species (Suwabe et al. 2002; Tamura et al. 2005; Chang et al. 2009; Kato et al. 2013). For example, Suwabe et al. (2003) mapped two major clubroot resistance loci between two SSR markers, BRMS-088 and BRMS-096. Fredua-Agyeman and Rahman (2016) mapped the *CRa*/*CRb*^{Kato} locus on the chromosome A03 of the European winter-type *B. napus* canola cv. Mendel by using 12 SSR markers. Based on 48 polymorphic SSR markers, Hasan

and Rahman (2016) constructed a linkage map of chromosome A08 of *B. napus* and identified a genetic region conferring resistance to pathotypes 2, 3, 5, 6 and 8 in the rutabaga cv. Brookfield. Therefore, the SSR markers co-segregated with clubroot resistance are valuable tools for use in the accumulation of multiple clubroot resistance genes into one *B. napus* canola cultivar through MAS (Shah et al. 2019).

1.5 Research objectives

This MSc thesis research aims to extend our knowledge on resistance to different pathotypes of *P. brassicae* in canola, evaluate clubroot resistance in two *B. napus* advance-generation populations, and identify clubroot resistance loci and their corresponding SSR markers for use in canola breeding.

1) Evaluation of a *B. napus* BC_1F_8 population derived from canola × *B. rapa* interspecific cross for resistance to *P. brassicae* pathotypes 2B, 2A, 5x (L-G1) and 3H.

2) Assessment of a *B. napus* DH population derived from susceptible canola × resistant canola carrying resistance of rutabaga for resistance to *P. brassica* pathotypes 2B, 3A, 5x (L-G1), 3H and 3D.

3) Mapping of clubroot resistance using the above-mentioned *B. napus* DH population segregating for clubroot resistance of rutabaga.

4) Identification of SSR markers linked to clubroot resistance of rutabaga.

1.6 Figures

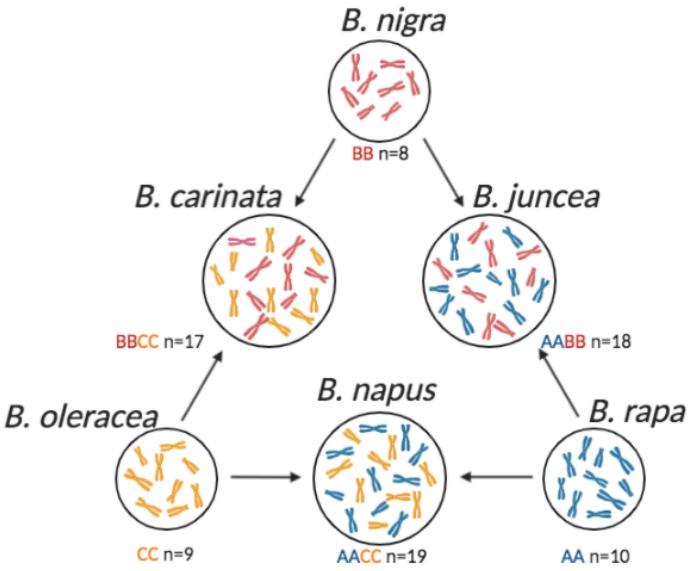

Figure 1.1 "Triangle of U" describes the relationship among six major *Brassica* species (adapted from U 1935).

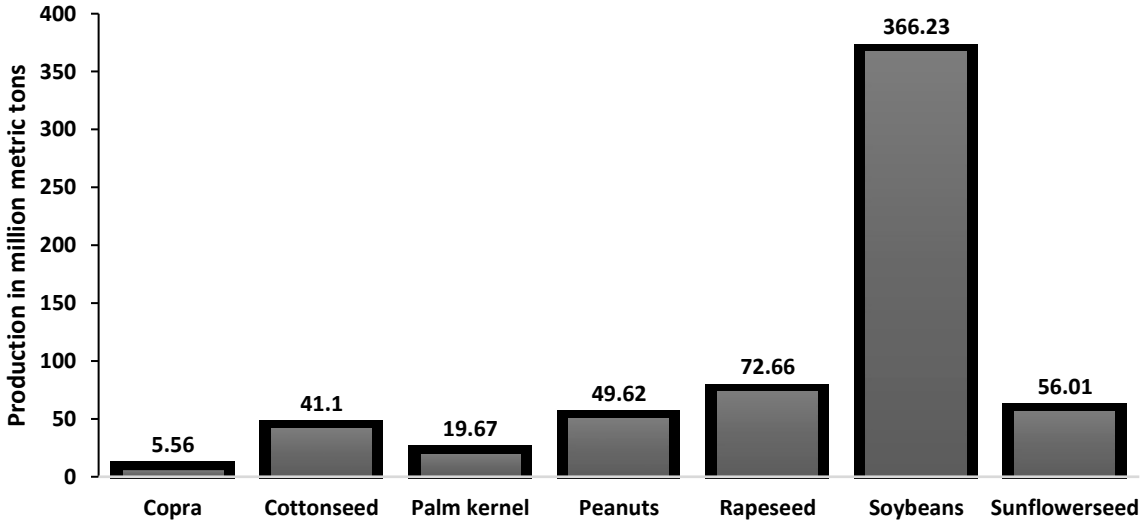

Figure 1.2 The global production of major oilseed crops in million metric tons in 2021/2022 (adapted from USDA-FAS 2021).

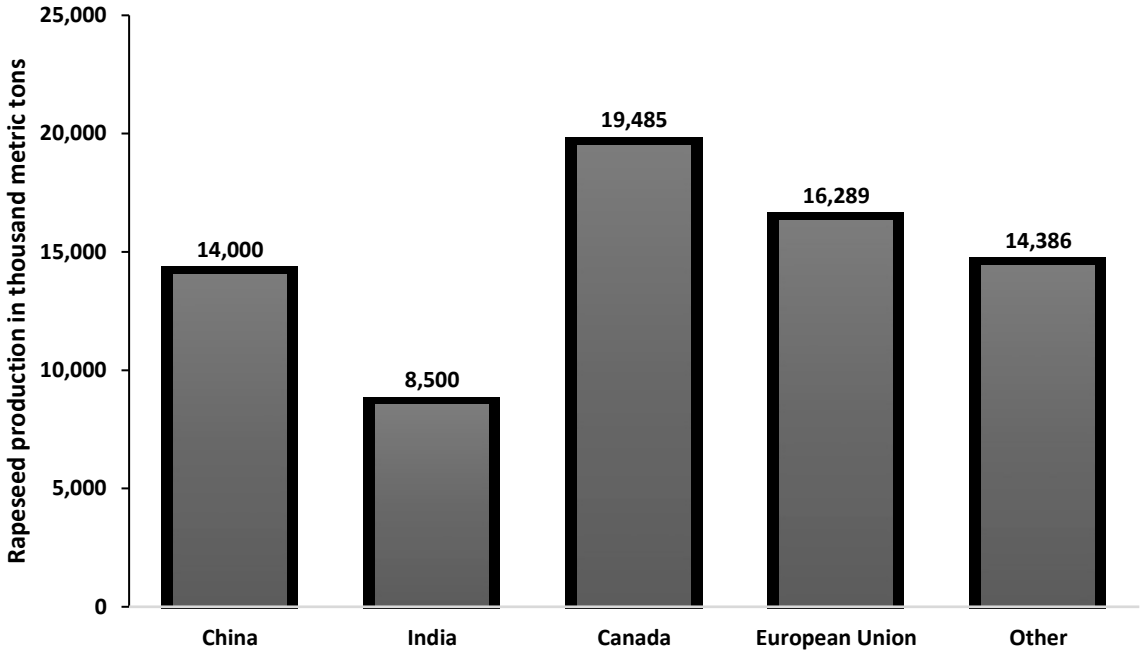

Figure 1.3 Production of *Brassica* oilseeds in thousand metric tons in 2020/2021 in major producing countries (adapted from USDA-FAS 2021).

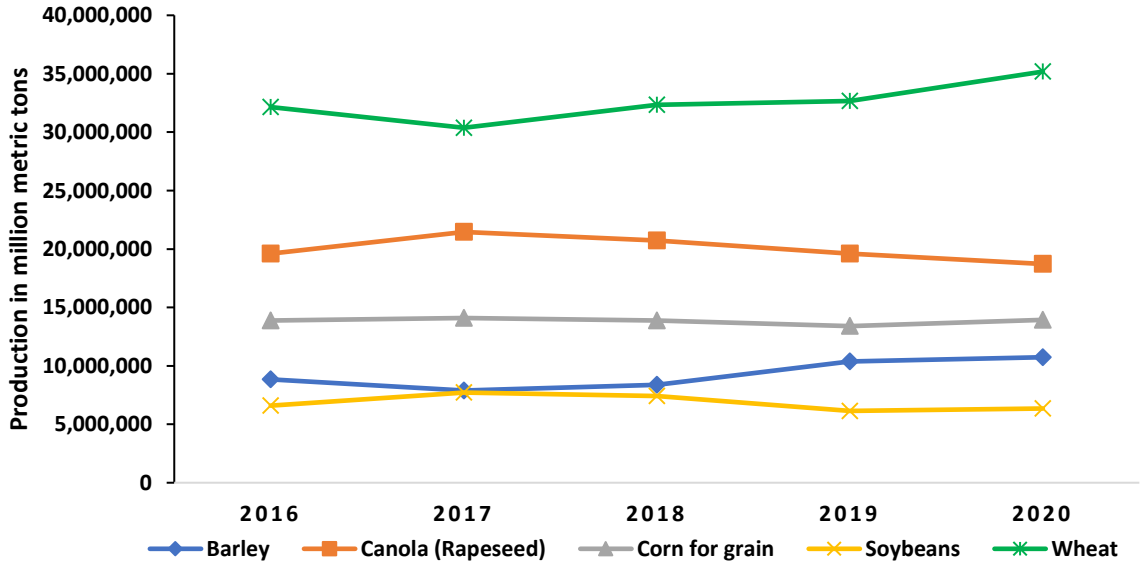

Figure 1.4 Production of major field crops in million metric tons in Canada during the period of 2016 to 2020 (adapted from Statistics Canada 2021b).

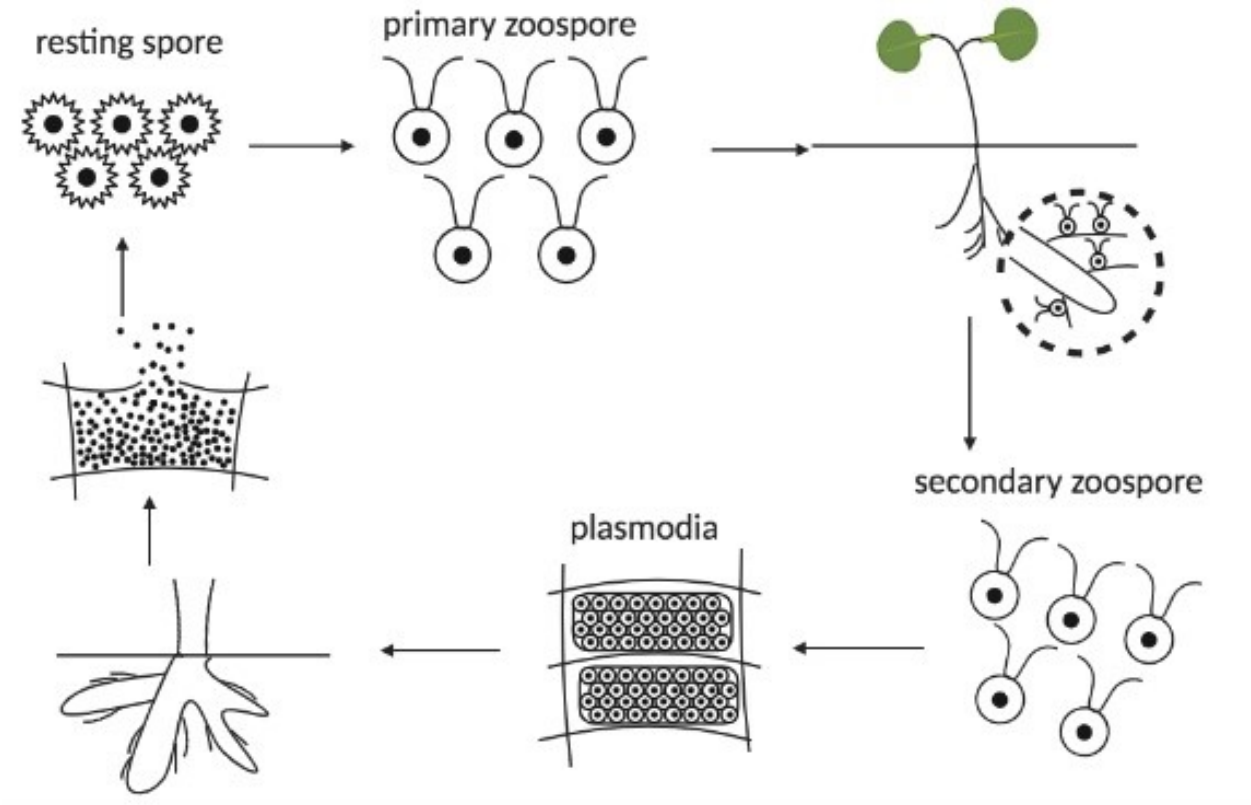

Figure 1.5 Life cycle of *Plasmodiophora brassicae* (adapted from Kageyama and Asano 2009).

2 Chapter 2: Evaluation of two advanced-generation spring *Brassica napus* populations for resistance to multiple *Plasmodiophora brassicae* pathotypes

2.1 Introduction

Clubroot disease, caused by *Plasmodiophora brassicae* Woronin, is one of the most devastating diseases that adversely affects the production of cruciferous crops including *Brassica napus* canola (Braselton 1995; for review, see Dixon 2009). The incidence of this disease was documented in Spain in the 13th century, and this disease has been found in more than 60 countries (CABI/EPPO 2011; for review, see Hirani and Li 2015). In Canada, clubroot disease in canola field was first reported in Alberta in 2003 (Tewari et al. 2005); since then, additional clubroot-infested canola field have been confirmed in this province (Strelkov et al. 2007) as well as in Saskatchewan (Dokken-Bouchard et al. 2012), Manitoba (McLaren et al. 2014), and Ontario (Al-Daoud et al. 2018).

P. brassicae is an obligate biotrophic protist, which causes the formation of club-like swellings or galls on the roots of the susceptible plants (for review, see Hwang et al. 2012). The galls inhibit the uptake of water and nutrients, which leads to wilting, stunting, yellowing, and premature ripening of the plants, and ultimately results in yield loss and low seed quality (Pageau et al. 2006; for review, see Hwang et al. 2012). On a global scale, the pathogen *P. brassicae* accounts for an annual yield loss of 10-15% in cruciferous crops (for review, see Dixon 2009). In Canada, yield losses in the range of 30-100% have been reported in canola under moderate to heavy infestation conditions (Tewari et al. 2005; Pageau et al. 2006); aside from yield loss, this disease can also reduce seed oil content by about 3.6-6.1% (Pageau et al. 2006).

As a fungus-like, soil-borne pathogen, *P. brassicae* survives in soil as long-lasting resting spores (for review, see Kageyama and Asano 2009). The hardy resting spores of *P. brassicae* can

persist in soil and remain virulent for more than 17 years (Wallenhammer 1996). Early studies have shown that traditional strategies such as cultural practices, and the use of chemical and biological agents were not sustainable for the management of clubroot disease in large-scale field conditions (Ahmed et al. 2011; for review, see Peng et al. 2104b). Genetic resistance was regarded as the most convenient and environmentally friendly approach of controlling this disease; therefore, studies focused on finding clubroot resistance genes and understanding the genetic and molecular basis of this resistance to improve canola for resistance to *P. brassicae*.

So far, several independent dominant clubroot resistance genes and many quantitative trait loci (QTL) have been identified and mapped to the A-genome of *Brassica rapa* and *B. napus*, C-genome of *Brassica oleracea* and *B. napus*, and B-genome of *Brassica nigra* (for review, see Hasan et al. 2021a). Since 2009, at least 55 clubroot-resistant canola cultivars have been developed and released in Canadian market (Canola Encyclopedia 2022). However, most of the clubroot-resistant cultivars carry a narrow genetic base of resistance with a race-specific effect. Since wide diversity exists in *P. brassicae* population and emergence of new virulent pathotypes can occur in a short period of time, the narrow genetic resistance can become ineffective shortly (for review, see Diederichsen et al. 2009; Strelkov et al. 2016). For example, the clubroot-resistant *B. napus* cvs. Mendel and 45H29 were resistant to the original pathotype 3H; however, these cultivars showed susceptibility to the newly emerged virulent pathotypes such as 2B, 3A and 5x as classified on the Canadian Clubroot Differential (CCD) set (Strelkov et al. 2018). Therefore, further research effort is needed to identify additional clubroot resistance loci in *Brassica* crops for introgression into *B. napus* canola. The European fodder turnips (*B. rapa* subsp. *rapifera*) and rutabaga (*B. napus* var. *napobrassica*) have been reported to carry resistance to different pathotypes of *P. brassicae* and these germplasms can be used to broaden the genetic base of spring *B. napus* canola for

clubroot resistance (Ayers and Lelacheur 1972; Suwabe et al. 2003; Hasan et al. 2012; Peng et al. 2014a; for review, see Rahman 2014; for review, see Hasan et al. 2021a).

The objective of this study was to investigate two advanced-generation breeding populations [BC_1F_8 and doubled haploid (DH)] of spring *B. napus* canola for resistance to multiple pathotypes of *P. brassicae*. Among these, the BC_1F_8 population was derived from an interspecific cross involving a spring *B. napus* canola breeding line and a European fodder turnip accession. The DH population was developed from crosses involving three spring *B. napus* canola breeding lines and four clubroot-resistant spring *B. napus* lines which had a rutabaga cv. Polycross in their parentage. It was hypothesized that resistance to different *P. brassicae* pathotypes could be identified in both populations.

2.2 Materials and Methods

2.2.1 Plant material

Two spring *B. napus* (2n = 38; AACC) populations used in this study, BC_1F_8 and DH, were obtained from the Canola Breeding Program of the University of Alberta. The BC_1F_8 population was derived from a cross between a clubroot-susceptible spring *B. napus* canola line A04-73NA (zero erucic acid in oil and < 15 μmol/g glucosinolates in seed meal) and a clubroot-resistant *B. rapa* var. *rapifera* European turnip accession ECD 01 (cv. Debra). The interspecific cross was made by using A04-73NA as female, and the F_1 plants were backcrossed to the *B. napus* parent A04-73NA to produce the BC_1 population: (A04-73NA × *B. rapa* var. *rapifera* turnip accession ECD 01 or cv. Debra) × A04-73NA. The BC_1 population was subjected to pedigree breeding with selection for resistance to pathotype 3H from which the BC_1F_8 population was developed through self-pollination.

In the case of the DH population, F_1 plants were produced by crossing three clubroot-susceptible spring *B. napus* canola breeding lines A01-104NA, A04-73NA, and A06-19NA, which were used as male parents, and four *B. napus* lines, viz. 1IA1190.030, 1IA1190.044, 1IA1190.052 and 1IA1190.064, carrying clubroot-resistance of the rutabaga cv. Polycross as female. These female parent lines were derived from the following crosses: (A05-17NI × Polycross) × A04-73NA; (A05-17NI × Polycross) × A01-104NA; (A05-17NI × Polycross) × A06-19NA. A04-73NA, A01-104NA, A06-19NA, and A05-17NI are spring *B. napus* breeding lines developed by the Canola Breeding Program of the University of Alberta. DH lines were developed through microspore culture of the F_1 plants; the details of this technique can be found elsewhere (Hasan and Rahman 2016).

Evaluation of the BC_1F_8 and DH populations for resistance to *P. brassicae* pathotypes was carried out in a greenhouse maintained at 20-22/15 °C temperature (day/night) with a 16-hour photoperiod. This greenhouse is located at the top of the Agriculture/Forestry building of the University of Alberta and is used for clubroot resistance tests. For this, seedlings were grown in 72-cell trays (tray size: 52 cm × 26 cm × 5 cm, L × W × D; cell size: 4 cm × 4 cm × 5 cm, L × W × D) filled with Sunshine Professional growing mix (Sun Gro Horticulture Sunshine Horticulture, 15831 N.E. Bellevue, USA). In the case of the population developed from *B. napus* × *B. rapa* interspecific cross through selection for resistance to *P. brassicae* pathotype 3H in early generations, 68 BC_1F_8 lines were assessed for resistance to pathotypes 2B, 3A, and 5x (L-G1) in two replications and for resistance to pathotype 3H in one replication. As for the DH population, 106 lines were evaluated for resistance to pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D in three replications. In each replication, eight plants per line were grown together with a highly susceptible *B. napus* canola cv. Hi-Q, which served as the negative control.

2.2.2 Inoculum preparation and inoculation

Single-spore isolates of *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D were obtained from Dr. Stephen Strelkov, Department of Agricultural, Food and Nutritional Science, University of Alberta. The isolates were multiplied by inoculating the highly susceptible cv. Hi-Q, and the galls were preserved at −20 °C until use. The pathogen inoculum was prepared following the protocol described by Hasan et al. (2012) with some modification. Briefly, the frozen clubroot galls were thawed at room temperature for about 30 min and 37 g galls were homogenized with 1000 ml distilled water using an electric blender (Ninja® Professional Blender 1100W). The homogenate was filtered through eight layers of cheesecloth into a conical flask, and the concentration of resting spores was adjusted with distilled water to approximately 1.0×10^7 resting spores per ml. The resting spore suspension of each pathotype of *P. brassicae* was prepared separately on the day of inoculation.

Inoculation of canola seedlings was performed following the pipette inoculation method described by Voorrips and Visser (1993). In brief, seedlings at the age of seven to ten days after germination were inoculated by pipetting 1 ml resting spore suspension at the base of each seedling using an Eppendorf Repeater Plus pipette. To ensure a successful inoculation, the seedlings were inoculated again on the following day. After inoculation, the trays were placed on a larger tray filled with water to create a favorable condition for the development of *P. brassicae*. After two weeks, the seedlings were watered daily and fertilized (20 N: 20P: 20K) as required.

2.2.3 Phenotyping for clubroot resistance

At 45-50 days after inoculation, the plants were removed from the cells and the roots were washed with water and evaluated for galling. Scoring for clubroot resistance was carried out using a 0 to 3 scale according to Kuginuki et al. (1999), where 0 = no galling; 1 = a few tiny galls on less

than 1/3 of the lateral roots; 2 = moderate galling on 1/3 to 2/3 of the lateral roots; and 3 = severe galling on more than 2/3 of the main and lateral roots. Disease severity index (DSI) was calculated using the following formula of Horiuchi and Hori (1980) modified by Strelkov et al. (2006):

$$DSI\ (\%) = \frac{\Sigma(n_0 \times 0 + n_1 \times 1 + n_2 \times 2 + n_3 \times 3)}{N \times 3} \times 100$$

Where ***0***, ***1***, ***2***, and ***3*** are the disease severity classes, ***n*** is the total number of plants in each class, and ***N*** is the total number of plants in each replication. The line was designated as resistant (R) (DSI ≤ 20%), moderately resistant (MR) (DSI > 20 to ≤ 40%), moderately susceptible (MS) (DSI > 40 to ≤ 70%), or susceptible (S) (DSI > 70%).

2.2.4 Statistical analysis

Basic descriptive statistical analysis, such as mean and standard error (S.E.) were carried out using Microsoft Excel. Pearson's correlation coefficient (*r*) between DSI values for resistance to two different pathotypes were calculated using the software program R and cor.test function (https://www.r-project.org/) (Benesty et al. 2009). Principal component analysis (PCA) and genotype by trait (GT) biplot analysis for grouping of the lines for resistance to different pathotypes was carried out using prcomp function and ggplot2 package in R (Bro and Smilde 2014; Wickham 2016). Venn diagrams were generated using the VennDiagram package in R (Chen and Boutros 2011).

2.3 Results

2.3.1 BC_1F_8 population of *B. napus* × *B. rapa* interspecific cross

Sixty-eight BC_1F_8 lines of *B. napus* × *B. rapa* interspecific crosses, which have been selected for resistance to *P. brassicae* pathotype 3H in early generations, were evaluated for resistance to pathotypes 2B, 3A, 5x and 3H. As shown in Figure 2.1A, 19 (27.9%) were classified

as R, 11 (17.6%) as MR, eight (11.8%) as MS, and 29 (42.7%) as S to pathotype 2B. In the case of resistance to pathotype 3A, 24 (35.3%) were R, six (8.8%) were MR, and 38 (55.9%) were S (Figure 2.1B). As for resistance to pathotype 5x, 15 (22.1%) were R, 12 (17.6%) were MR, three (4.4%) were MS, and 38 (55.9%) were S (Figure 2.1C). All 68 (100%) lines were resistant to pathotype 3H. A joint analysis of data of the 68 lines for resistance to multiple pathotypes showed that 10 (14.7%) lines were resistant to all four pathotypes, 29 (42.6%) were resistant to pathotype 3H but susceptible to pathotypes 2B, 3A and 5x, and the remaining 29 (42.6%) lines were resistant to pathotype 3H but moderately resistant or susceptible to pathotypes 2B, 3A, or 5x (Figure 2.1D). Thus, the results indicated that resistance to multiple *P. brassicae* pathotypes could be introgressed from European turnip into *B. napus* canola through *B. napus* × *B. rapa* interspecific cross.

Subsequently, PCA was carried out to understand the nature of genetic variability among the BC_1F_8 lines for resistance to different *P. brassicae* pathotypes. The GT biplot analysis explained about 98.2% of the total variance, of which the first principal component (PC1) explained 96.4% of the total variance and the second principal component (PC2) explained 1.8% of the total variance, where PC1 represents variation for genotype and PC2 represents variation for resistance to the pathotypes (Figure 2.2). The size of the vector for resistance to pathotype 3A was longer as compared to resistance to other pathotypes, suggesting that this pathotype was the major discriminator of the BC_1F_8 population. The vectors for resistance to 3A and 5x had an angle of nearly zero, indicating that these two pathotypes accounted for a similar type of variation. The trait pairs of resistance to pathotype 2B vs. 3A and 2B vs. 5x had acute (< 90°) angles, suggesting that their variation was positively correlated. The trait pairs of resistance to pathotype 2B vs. 3H, 3A vs. 3H and 5x vs. 3H had near-right angles, indicating that the resistance to pathotype 3H was weakly or not correlated with that to the other pathotypes. Consistently, the result was also evident

from the correlation analysis (Table 2.1). Correlation between the traits of resistance to 2B vs. 3A (r = 0.94**), 2B vs. 5x (r = 0.95**), and 3A and 5x (r = 0.97**) was positive and significant, whereas, no significant correlation was found between 2B vs. 3H (r = 0.08), 3A vs. 3H (r = 0.01), and 5x vs. 3H (r = 0.08).

2.3.2 DH population of *B. napus* × rutabaga interspecific cross

A total of 106 DH lines, which were expected to segregate for clubroot resistance of the rutabaga cv. Polycross, were tested for resistance to pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D (Figure 2.3). Among these lines, 19 (17.9%) were R, 14 (13.2%) were MR, 11 (10.4%) were MS, and 62 (58.5%) were S to pathotype 2B (Figure 2.3A). As for resistance to pathotype 3A, 40 (37.7%) lines were R, seven (6.6%) were MR, seven (6.6%) were MS, and 52 (49.1%) were S (Figure 2.3B). In the case of resistance to pathotype 5x, 36 (34.0%) were R, 17 (16.0%) were MR, seven (6.6%) were MS, and 46 (43.4%) were S (Figure 2.3C). Amongst these 106 DH lines, 26 (24.5%) lines were identified as R, six (5.7%) as MR, 12 (11.3%) as MS, and 62 (58.5%) as S for resistance to pathotype 3H (Figure 2.3D), while 26 (24.5%) were R, six (5.7%) were MR, 11 (10.4%) were MS, and 63 (59.4%) were S to pathotype 3D (Figure 2.3E). Of the total number of DH lines, 14 (13.2%) lines were resistant to all five pathotypes (Figure 2.4A), six (5.6%) lines were resistant to pathotype 3A but moderately resistant or susceptible to the other four pathotypes (Figure 2.4A), ten (9.4%) lines were resistant to pathotype 5x but moderately resistant or susceptible to the other four pathotypes (Figure 2.4A), 45 (42.5%) lines were susceptible to all five pathotypes (Figure 2.4B), and the rest 31 (29.2%) lines carried a different level of resistance or susceptibility to pathotypes 2B, 3A, 5x, 3H, or 3D. The identification of DH lines carrying resistance to multiple pathotypes demonstrates that clubroot resistance of the rutabaga cv.

Polycross can be used to broaden the genetic base of resistance to *P. brassicae* pathotypes in spring *B. napus* canola.

The existence of variation in the DH population for resistance to different *P. brassicae* pathotypes could also be visualized by using PCA and GT biplot analysis. The GT biplot analysis accounted for approximately 95.9% of the total variation, where the first two principal components, PC1 (genotype) and PC2 (resistance to different pathotypes), explained 89.6% and 6.3% of the total variation, respectively (Figure 2.5). Resistance to pathotype 5x had a relatively longer vector than the others, indicating that this made relatively a greater contribution to the total variation in this population for resistance to the five pathotypes. The trait pairs of resistance to 2B vs. 3H, 2B vs. 3D and 3H vs. 3D had angles of nearly zero suggesting that the resistance to these three pathotypes were strongly correlated. The trait pairs of resistance to 2B vs. 3A, 2B vs. 5x, 3A vs. 5x, 3A vs. 3H, 3A vs. 3D, 5x vs. 3H, and 5x vs. 3D had acute (< 90°) angles, indicating that the resistance to all five pathotypes were positively correlated. Similarly, positive correlation was found for resistance to 2B vs. 3H ($r = 0.95$**), 2B vs. 3D ($r = 0.93$**), 3H vs. 3D ($r = 0.97$**), 2B vs. 3A ($r = 0.88$**), 2B vs. 5x ($r = 0.79$**), 3A vs. 5x ($r = 0.86$**), 3A vs. 3H ($r = 0.89$**), 3A vs. 3D ($r = 0.87$**), 5x vs. 3H ($r = 0.80$**), and 5x vs. 3D ($r = 0.77$**) (Table 2.2).

2.4 Discussion

In this study, two *B. napus* populations derived from crosses involving clubroot resistant European turnip accession ECD 01 (cv. Debra) and rutabaga cv. Polycross were evaluated for their resistance to five Canadian *P. brassicae* pathotypes, with the aim to investigate the prospect of developing a canola line carrying resistance to multiple pathotypes including the recently evolved ones, such as pathotypes 2B, 3A, and 5x. Resistance to *P. brassicae* have been found in multiple rutabaga cultivars and different forms of *B. rapa*, including the European fodder turnips and

Chinese cabbage (*B. rapa* ssp. *chinensis* and *pekinensis*) (Ayers and Lelacheur 1972; Shattuck and Proudfoot 1990; Spaner 2002; Hasan et al. 2012; Peng et al. 2014a; Chu et al. 2014; Yu et al. 2017; Hirani et al. 2018; Pang et al. 2018; Zhang et al. 2022).

Genetic analysis and molecular mapping of resistance revealed that several major clubroot resistance loci conferring resistance to different *P. brassicae* isolates can be found in European fodder turnips (for review, see Piao et al. 2009). For example, three major loci *Crr1* (*Crrla* and *Crr1b*), *Crr2*, and *Crr4* were identified in the turnip cv. Siloga, and they were mapped on chromosomes A08, A01 and A06, respectively (Suwabe et al. 2003, 2006; Hatakeyama et al. 2013). The locus *Crr3* exhibiting resistance to pathotype 2 was mapped on chromosome A03 of the turnip cv. Milan White (Hirai et al. 2004; Saito et al. 2006). The *CRa*/*CRb* locus of A03 was identified in the turnip cvs. Gelria R, Debra, and ECD 02. (Piao et al. 2004; Ueno et al. 2012; Kato et al. 2013; Fredua-Agyeman et al. 2020). Two dominant loci *CRk* and *CRc* showing resistance to isolates M85 and K04 were identified in the turnip cv. Debra, and they were mapped on chromosomes A03 and A02, respectively (Sakamoto et al. 2008; Matsumoto et al. 2012). Two QTL, *Rcr8* on A02 and *Rcr9* on A08, conferring resistance to pathotype 5x and a QTL *Rcr4* on A03 conferring resistance to pathotypes 2, 4, 5, 6, and 8 were identified in the turnip cv. Pluto (Yu et al. 2017). A resistance gene *Rcr5* was fine-mapped on A03 of the turnip cv. Purple Top White Globe, which was responsible for resistance to pathotype 3 (Huang et al. 2019). Two major genes *Rcr3* and $Rcr9^{wa}$, for resistance to pathotype 3 and 5x, respectively, were mapped on A08 of the turnip cv. Waaslander (Karim et al. 2020). Hirani et al. (2018) mapped two independent dominant resistance loci on A03 and one locus on A08 in the ECD set, where ECD 01 carrying two of the three loci showed resistance to multiple Canadian *P. brassicae* field isolates. Two dominant genes, *Bcr1* of A03 and *Bcr2* of A08, conferring resistance to pathotype 4 were identified in the turnip

accession ECD 04 (Zhang et al. 2022). Recently, two major loci were identified in the turnip accession ECD 01, where *Rcr9* ^{ECD01} of A08 confer resistance to pathotype 3A, 5x, 3H and 3D, and *Rcr10* ^{ECD01} of A03 confer resistance to pathotype 3A, 3H, and 3D (Yu et al. 2022). Thus, it is apparent that the turnips such as ECD 01 (cv. Debra) carry multiple clubroot resistance genes which can confer resistance to multiple *P. brassicae* pathotypes. Previously, several researchers (Leflon et al. 2006; Matsumoto et al. 2012; Chu et al. 2013; Attri and Rahman 2018; Hasan et al. 2021b) have demonstrated that alleles from *B. rapa* can be introgressed into *B. napus* through *B. napus* × *B. rapa* interspecific cross. The results from evaluation of the BC_1F_8 lines derived from *B. napus* × *B. rapa* turnip ECD 01 (cv. Debra) interspecific cross demonstrated that resistance to *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), and 3H can be introgressed from turnip into spring *B. napus* canola.

The BC_1F_8 population that was used in this study was selected for resistance to pathotype 3H in early generations. Interestingly, some of the advanced-generation BC_1F_8 lines exhibited a high level of resistance to the pathotypes 2B, 3A and 5x as well (Figure 2.1). Resistance to these pathotypes in this population could be due to a pleiotropic effect of a major gene or a cluster of co-localized genes or QTL. A genomic region conferring resistance to multiple pathotypes has also been reported by Hasan and Rahman (2016) in the case of the clubroot resistance introgressed into canola from rutabaga cv. Brookfield. Results from PCA and GT biplot analysis indicated that resistance to pathotypes 2B, 3A and 5x could be under a similar genetic control or multiple genes from the same genomic regions; on the other hand, a different genetic mechanism might be involved in the control of resistance to pathotype 3H (Figure 2.2). This was also evident from correlation between resistance to different pathotypes. Resistance to pathotypes 2B, 3A and 5x were strongly correlated (r = 0.94-0.97), while the resistance to pathotype 3H showed almost no

correlation with resistance to 2B, 3A and 5x (r = 0.01-0.08) (Table 2.1). Taken together, the results demonstrated that, although the early generations populations of this *B. napus* × *B. rapa* interspecific cross were subjected to selection for resistance to pathotype 3H, additional clubroot resistance loci for resistance to pathotypes 2B, 3A and 5x have also been introgressed into the BC_1F_8 *B. napus* inbred lines.

Rutabaga, which carries the same genome of canola-quality *B. napus*, is genetically diverse from spring *B. napus* canola (Bus et al. 2011). Crossing of canola with rutabaga produces fertile hybrids without any anomalies in chromosome pairing and gene segregation (Shiranifar et al. 2020). Therefore, an interspecific cross between clubroot resistant rutabaga and canola is not only expected to introduce clubroot resistance genes into canola, but also expected to broaden the genetic base of this crop (Shiranifar et al. 2020; Hasan et al. 2021c). Several rutabaga cultivars, such as Askegarde, Chignecto, Fortune, Kingston, York, Wilhelmsburger, Brookfield, and Polycross, were reported to carry resistance to different *P. brassicae* pathotypes cultivars (Ayers and Lelacheur 1972; Shattuck and Proudfoot 1990; Spaner 2002; Hasan et al. 2012; Peng et al. 2014a); therefore, they are excellent germplasm for use in the breeding of clubroot-resistant canola cultivars. Ayers and Lelachur (1972) reported the rutabaga cv. York carry a single dominant gene confer resistance to races 2 and 3, while the rutabaga cv. Wilhemsburger carry a major gene for resistance to race 3 and two major genes for resistance to race 2. Fredua-Agyeman et al. (2020b) evaluated 124 rutabaga accessions for resistance to 15 Canadian *P. brassicae* pathotypes and reported forty-five SNPs and four PCR-based markers to be associated with resistance to 13 pathotypes (2F, 3H, 5I, 6M, 8N, 2B, 3A, 3O, 5C, 5G, 5K, 5L, and 8P) on chromosomes A03 and A08. Hasan et al. (2012) reported that the rutabaga cv. Wilhelmsburger, as well as the cvs. Polycross and Brookfield exhibit resistance to multiple pathotypes. Genetic mapping of resistance

of the rutabaga cv. Brookfield identified a genomic region of chromosome A08 exhibiting resistance to pathotypes 2, 3, 5, 6, and 8, which were identified in the initial stage of clubroot-resistance research in Canada (Hasan and Rahman 2016). Through the construction of a genetic linkage map for all 19 *B. napus* chromosomes, Hasan et al. (2021c) identified an additional minor effect QTL on chromosome A03 conferring resistance to pathotype 3. Transcriptome sequencing with this resistance showed that the cytokinin responsive factor (*CRF4*) might play a role in moderating clubroot resistance (Summanwar et al. 2021). Thus, it is apparent that the rutabaga cultivars carry one or more clubroot resistance genes and exhibit excellent resistance to multiple *P. brassicae* pathotypes. Consistently, the results from this study also showed that the rutabaga cv. Polycross might carry a single major resistance locus or multiple pathotype-specific clubroot resistance genes and can confer resistance to the *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D. Thus, the clubroot resistant lines identified in this thesis research can be used to broaden the genetic base of Canadian spring *B. napus* canola for resistance to multiple pathotypes.

Comparative analysis of resistance to multiple pathotypes in BC_1F_8 and DH populations showed that the genetic control of clubroot resistance in the turnip accession ECD 01 (cv. Debra) and rutabaga cv. Polycross to be different (Figure 2.2 & 2.5). Indeed, our PCA and correlation analysis for resistance to different pathotypes revealed a strong correlation between the resistance to pathotype 2B, 3A and 5x in both BC_1F_8 and DH populations, whereas correlation of resistance to pathotype 3H and resistance to the other pathotypes was different in these two populations. Correlation between resistance to pathotype 3H and to other pathotypes was highly positive and statistically significant (r = 0.80-0.97) in the DH population, while this correlation was almost zero in the BC_1F_8 population (r = 0.01-0.08) (Table 2.1 & 2.2). The relationship of resistance to different pathotypes could be resulted from the co-localization of a cluster of genes in a genomic

region or a pleiotropic effect of a gene conferring resistance to multiple pathotypes. For example, clubroot resistance loci *Rcr1* (Chu et al. 2014), *Rcr2* (Huang et al. 2017) and *Rcr4* (Yu et al. 2017) were co-localized with *CRa*/*CRb*^{Kato} (Ueno et al. 2012; Kato et al. 2013; Hatakayama et al. 2017) at ~25 Mb genomic region of the chromosome A03 of *B. rapa* Chiifu version 3.0 (Yu et al. 2022). The genomic region of chromosome A08 of *B. napus* rutabaga was responsible for resistance to pathotypes 2, 3, 5, 6, and 8 (Hasan and Rahman 2016), while three major genes *Crr1a*, *Rcr3* and *Rcr9* of A08 of *B. rapa* turnip were found to confer resistance to pathotypes 2, 3 and 5x, respectively (Suwabe et al. 2003, 2006; Hatakeyama et al. 2013; Yu et al. 2017; Karim et al. 2020). The genetic mechanisms of clubroot resistance of these two advanced-generation BC_1F_8 and DH populations need to be demonstrated with experiments in follow up studies.

2.5 Conclusion

In conclusion, results from this study demonstrate that the resistance of canola to multiple *P. brassicae* pathotypes can be introgressed from *B. rapa* var. *rapifera* European turnip accession ECD 01 (cv. Debra) as well as from rutabaga cv. Polycross into Canadian spring *B. napus* canola. The strong positive correlation between resistance to pathotypes 2B, 3A and 5x in both BC_1F_8 and DH populations indicates that the genetic control of resistance to these two pathotypes might be under a similar genetic control. In contrast, no correlation was found between the resistance to pathotype 3H and that to the other pathotypes in the BC_1F_8 population, while strong correlation was found between resistance to pathotype 3H and other pathotypes in the DH populations. Based on this, it could be inferred that the genetic control of clubroot resistance in the *B. rapa* European turnip accession ECD 01 (cv. Debra) and *B. napus* rutabaga cv. Polycross might be different. Therefore, these two resistance sources can be used to broaden the genetic base of clubroot resistance in spring *B. napus* canola. Practically, the BC_1F_8 and DH lines carrying resistance to

multiple pathotypes identified in this study can be used in breeding to develop clubroot resistant canola cultivars for sustainable production of this crop on the Canadian prairies. In addition, the results from this study can also be used for mapping of the resistances and to develop molecular markers associated with resistance for use in breeding.

2.6 Tables

Table 2.1 Pearson's correlation coefficients for resistance to different *Plasmodiophora brassicae* pathotypes [2B, 3A, 5x (L-G1) and 3H] in the pathotype 3H-resistant BC_1F_8 *Brassica napus* population derived from *B. napus* canola × *B. rapa* turnip interspecific cross.

P. brassicae pathotype	Pathotype 3A	Pathotype 5x	Pathotype 3H
Pathotype 2B	0.94**	0.95**	0.08
Pathotype 3A	-	0.97**	0.01
Pathotype 5x	-	-	0.08

**indicates significant level at $p < 0.01$.

Table 2.2 Pearson's correlation coefficients for resistance to *Plasmodiophora brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H and 3D in a *Brassica napus* doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross.

P. brassicae pathotypes	Pathotype 3A	Pathotype 5x	Pathotype 3H	Pathotype 3D
Pathotype 2B	0.88**	0.79**	0.95**	0.93**
Pathotype 3A	-	0.86**	0.89**	0.87**
Pathotype 5x	-	-	0.80**	0.77**
Pathotype 3H	-	-	-	0.97**

**indicates significance level at $p < 0.01$.

2.7 Figures

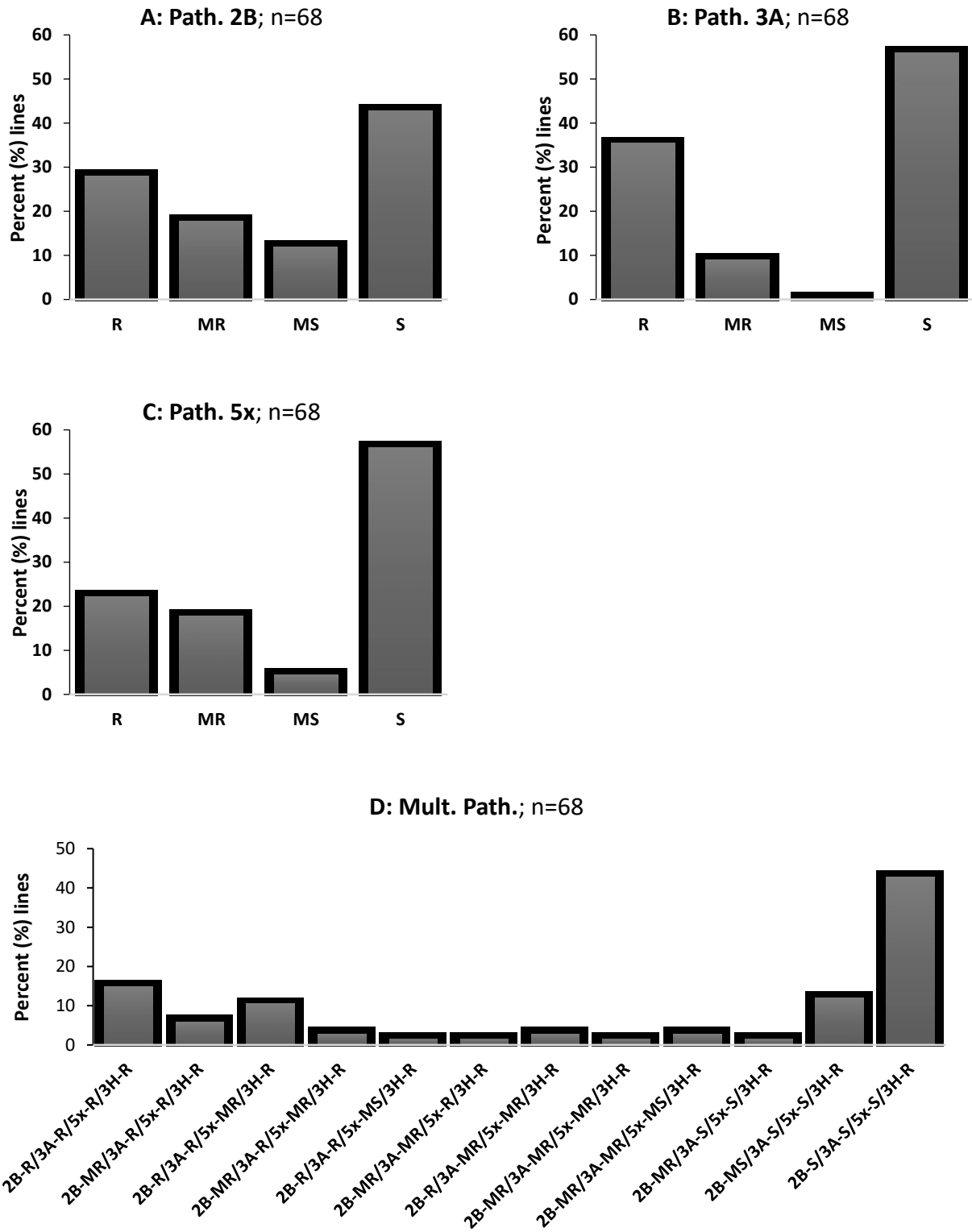
A: Path. 2B; n=68
B: Path. 3A; n=68
C: Path. 5x; n=68
D: Mult. Path.; n=68
Percent (%) lines
0
10
20
30
40
50
60
R
MR
MS
S
2B-R/3A-R/5x-R/3H-R
2B-MR/3A-R/5x-R/3H-R
2B-R/3A-R/5x-MR/3H-R
2B-MR/3A-R/5x-MR/3H-R
2B-R/3A-R/5x-MS/3H-R
2B-MR/3A-MR/5x-R/3H-R
2B-R/3A-MR/5x-MR/3H-R
2B-MR/3A-MR/5x-MR/3H-R
2B-MR/3A-MR/5x-MS/3H-R
2B-MR/3A-S/5x-S/3H-R
2B-MS/3A-S/5x-S/3H-R
2B-S/3A-S/5x-S/3H-R

Figure 2.1 Frequency distribution of 68 BC_1F_8 *Brassica napus* lines derived from *B. napus* × *B. rapa* interspecific cross for resistance to *Plasmodiophora brassicae* (A) pathotype 2B, (B) pathotype 3A, (C) pathotype 5x (L-G1), and (D) multiple pathotypes in greenhouse experiments. All lines were resistant to pathotype 3H. X-axis for figure D: 2B-R/3A-R/5x-R/3H-R = resistant to all four pathotypes (2B, 3A, 5x and 3H); 2B-MR/3A-R/5x-R/3H-R = resistant to pathotypes 3A, 5x and 3H while moderately resistant to pathotype 2B; 2B-R/3A-R/5x-MR/3H-R = resistant to pathotypes 2B, 3A and 3H while moderately resistant to pathotype 5x; 2B-MR/3A-R/5x-MR/3H-R = resistant to pathotypes 3A and 3H while moderately resistant to pathotypes 2B and 5x; 2B-R/3A-R/5x-MS/3H-R = resistant to pathotypes 2B, 3A and 3H while moderately susceptible to pathotypes 5x; 2B-MR/3A-MR/5x-R/3H-R = resistant to pathotypes 5x and 3H while moderately resistant to pathotypes 2B and 3A; 2B-R/3A-MR/5x-MR/3H-R = resistant to pathotypes 2B and 3H while moderately resistant to pathotypes 3A and 5x; 2B-MR/3A-MR/5x-MR/3H-R = resistant to pathotype 3H while moderately resistant to pathotypes 2B, 3A and 5x; 2B-MR/3A-MR/5x-MS/3H-R = resistant to pathotype 3H while moderately resistant to pathotypes 2B and 3A and moderately susceptible to pathotype 5x; 2B-MR/3A-S/5x-S/3H-R = resistant to pathotype 3H while moderately resistant to pathotype 2B and susceptible to pathotypes 3A and 5x; 2B-MS/3A-S/5x-S/2B-MS/3H-R = resistant to pathotype 3H while moderately susceptible to pathotype 2B and susceptible to pathotypes 3A and 5x; 2B-S/3A-S/5x-S/2B-S/3H-R = resistant to pathotype 3H and susceptible to other three pathotypes (2B, 3A and 5x). R = resistant (DSI ≤ 20%); MR = moderately resistant (DSI > 20 to ≤ 40%); MS = moderately susceptible (DSI > 40 to ≤ 70%); S= susceptible (DSI > 70%). *n* indicates the number of lines.

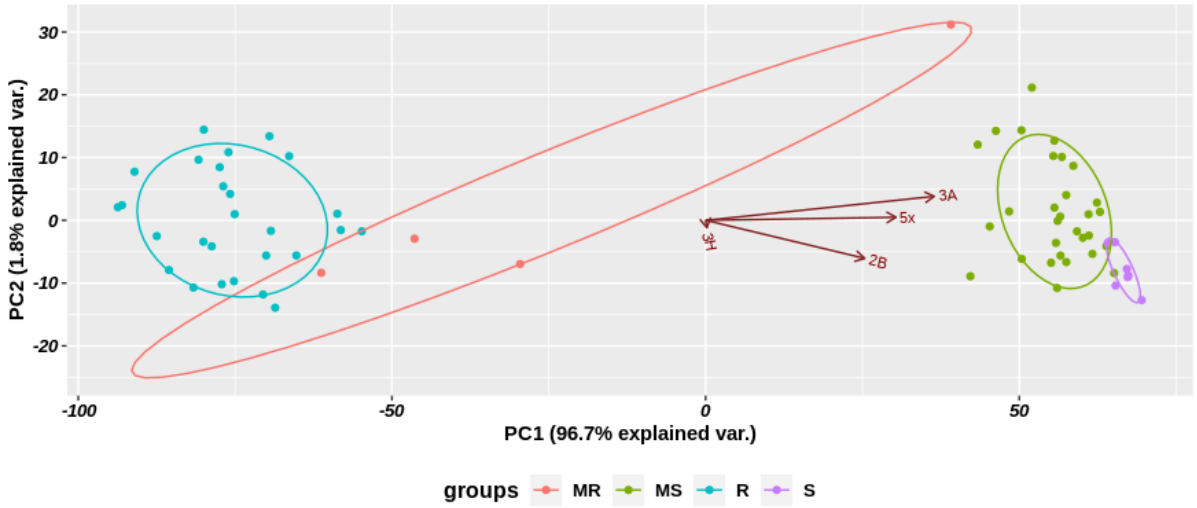

Figure 2.2 Principal component analysis (PCA) and genotypes by trait (GT) biplot analysis of the *Brassica napus* BC_1F_8 population (n = 68) derived from *B. napus* × *B. rapa* interspecific cross for resistance to *Plasmodiophora brassicae* pathotypes 2B, 3A, 5x (L-G1) and 3H. This analysis placed the population into four groups based on the average of DSI% for these pathotypes. R = resistant (DSI ≤ 20%); MR = moderately resistant (DSI > 20 to ≤ 40%); MS = moderately susceptible (DSI > 40 to ≤ 70%); S = susceptible (DSI > 70%).

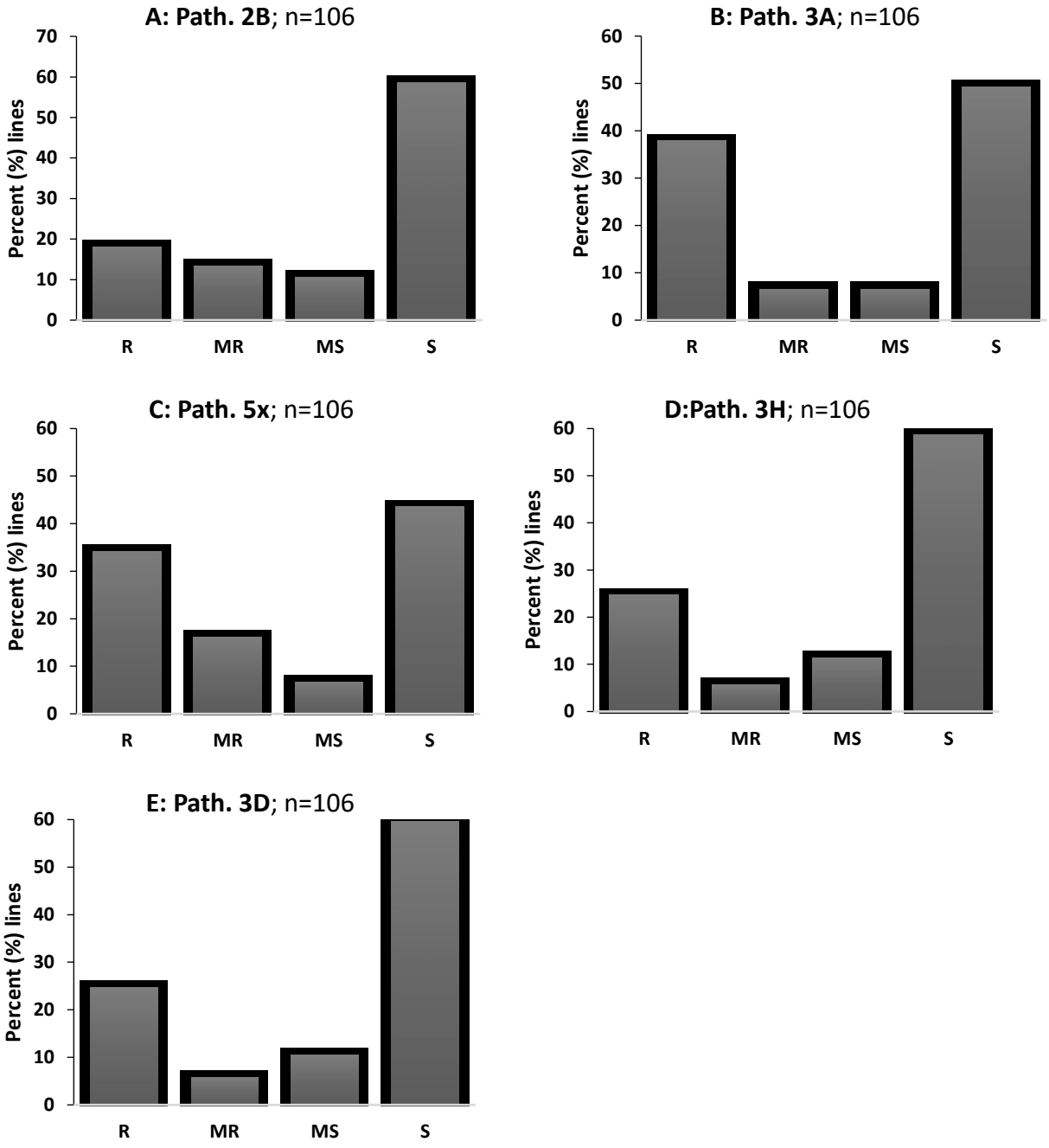

Figure 2.3 Frequency distribution of a *Brassica napus* doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross for resistance to *Plasmodiophora brassicae* (A) pathotype 2B, (B) pathotype 3A, (C) pathotype 5x (L-G1), (D) pathotype 3H and (E) pathotype 3D. R = resistant (DSI ≤ 20%); MR = moderately resistant (DSI > 20 to ≤ 40%); MS =moderately susceptible (DSI > 40 to ≤ 70%); S =susceptible (DSI > 70%). n indicates the number of lines.

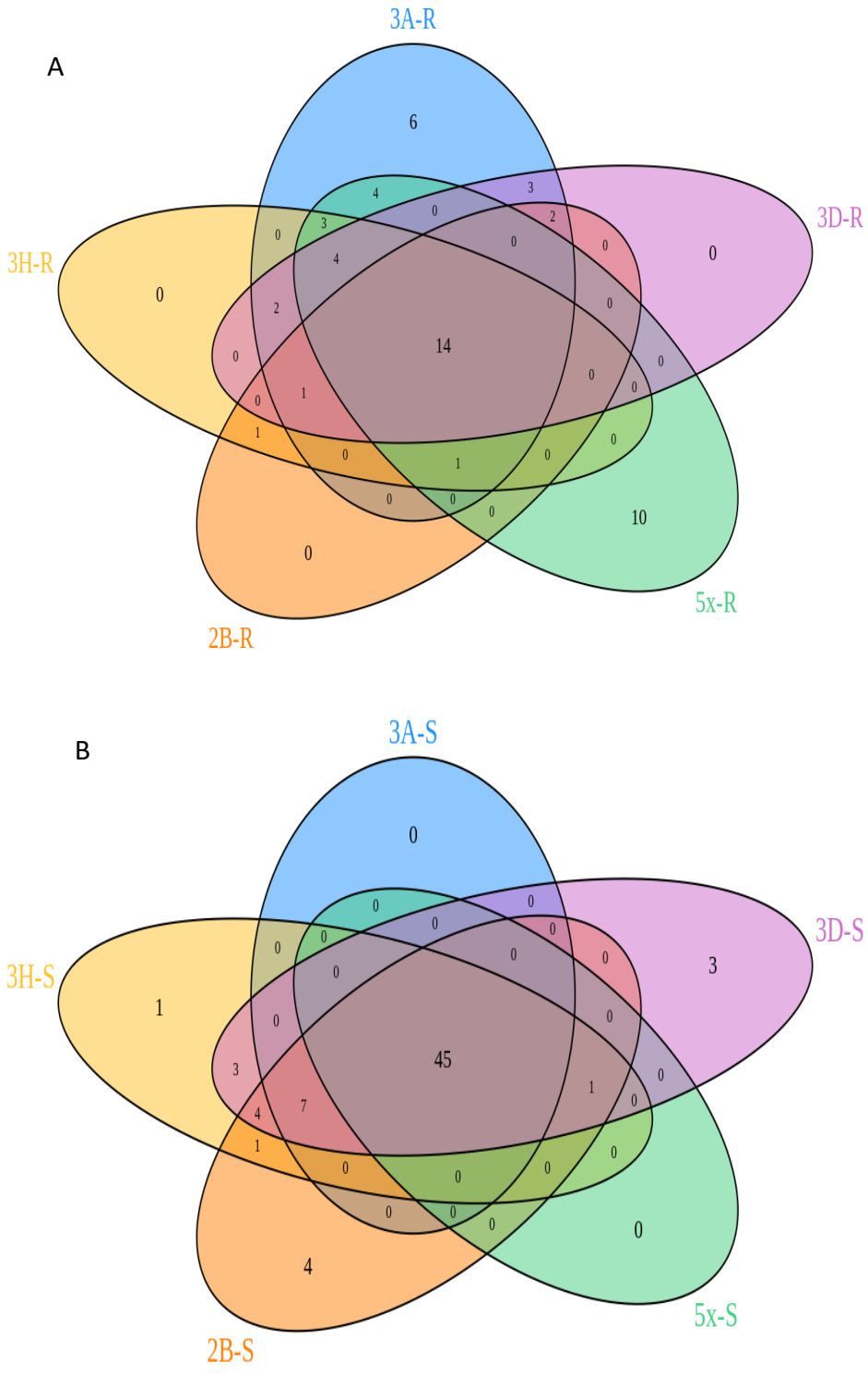

Figure 2.4 Venn diagrams showing the distribution of the *Brassica napus* doubled haploid (DH) lines carrying clubroot resistance of the rutabaga cv. Polycross for (A) resistance (DSI ≤ 20%) and (B) susceptibility (DSI > 70%) to *Plasmodiophora brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H and 3D (2B-R = resistance to pathotype 2B; 3A-R = resistant to pathotype 3A; 5x-R = resistant to

pathotype 5x; 3H-R = resistant to pathotype 3H; 3D-R = resistant to pathotype 3D; 2B-S = susceptible to pathotype 2B; 3A-S = susceptible to pathotype 3A; 5x-S = susceptible to pathotype 5x; 3H-S = susceptible to pathotype 3H; 3D-S = susceptible to pathotype 3D).

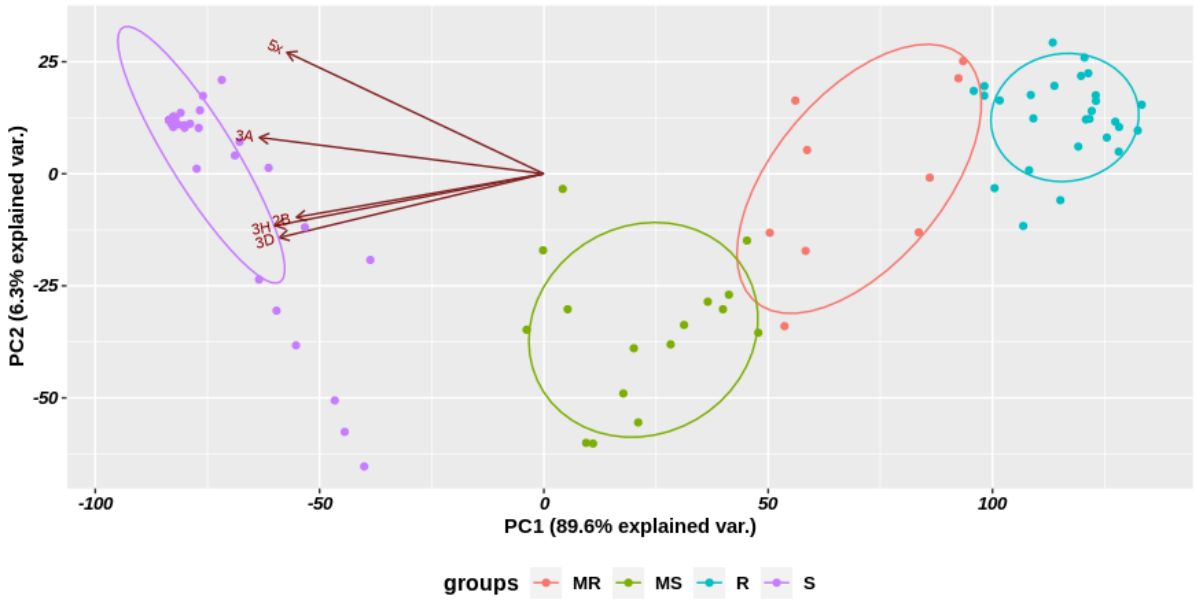

Figure 2.5 Principal component analysis (PCA) and genotypes by trait (GT) biplot analysis of the *Brassica napus* doubled haploid (DH) population (n = 106) carrying clubroot resistance of the rutabaga cv. Polycross for resistance to *Plasmodiophora brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H and 3D. This analysis placed the population into four groups based on the average of DSI% for resistance to these pathotypes. R = resistant (DSI ≤ 20%); MR = moderately resistant (DSI > 20 to ≤ 40%); MS = moderately susceptible (DSI > 40 to ≤ 70%); S = susceptible (DSI > 70%).

3 Chapter 3: Molecular mapping of clubroot resistance using a spring oilseed *Brassica napus* doubled haploid population carrying clubroot resistance of rutabaga

3.1 Introduction

Brassica napus (AACC, 2*n* = 38) is an allotetraploid species that originated from interspecific hybridization between two diploid species, *B. rapa* (AA, 2*n* = 20) and *B. oleracea* (CC, 2*n* = 18) (Allender and King 2010; Chalhoub et al. 2014). *B. napus* canola contains less than 2% of erucic acid in seed oil and less than 30 μmol/g of glucosinolates in oil-free seed meal, and it is primarily grown for edible oil and high-quality animal feeds (Bonnardeaux 2007; Daun and Unger 2016). Canada is the world's largest producer and exporter of canola (USDA-FAS 2022). The canola industry generates 29.9 billion annually to the economic activities in Canada (LMC International 2020). However, the production of this crop is always under the threat of multiple biotic and abiotic stresses, of which clubroot disease is one of the most devastating pathogens (Tewari et al. 2005; Pageau et al. 2006; for review, see Howard et al. 2010).

The obligate parasite *Plasmodiophora brassicae* Woronin is the causal agent of clubroot disease in cruciferous plants (for review, see Dixon 2009). Since the first discovery of this pathogen in canola fields in Alberta in 2003, clubroot disease became a severe threat to canola production in the Prairies Provinces (Tewari et al. 2005). In the initial stage, only five *P. brassicae* pathotypes, viz. 2F (2), 3H (3), 5I (5), 6M (6), and 8N (8) were identified from the field isolates where pathotype 3H has been the most prevalent and virulent (Strelkov et al. 2006, 2007; Xue et al. 2008; Cao et al. 2009). The first-generation clubroot-resistant canola cultivars carrying a single dominant resistance gene were developed and commercialized in Canada in 2009. However, the major resistance gene showed a race- or pathotype-specific resistance, and the resistance became

ineffective after the resistant cultivars were grown only for about four years (Diederichsen et al. 2009; Strelkov et al. 2016). Wide genetic diversity for virulence exists in *P. brassicae*, and multiple new virulent pathotypes such as 2B, 3A, 5x and 3D have been identified in canola fields where the first-generation clubroot-resistant cultivars were grown (Strelkov et al. 2016, 2021; Askarian et al. 2021; Hollman et al. 2021). Several studies have indicated that the traditional management strategies such as cultural, chemical and biological controls are not economic and effective for controlling this soil-borne disease in canola; growing of clubroot-resistant cultivars together with appropriate crop management practice has been regarded as the best approach for this (for review, see Hasan et al 2021a). Resistance to different *P. brassicae* pathotypes has been reported in *B. rapa*, *B. oleracea*, *B. nigra* and *B. napus* rutabaga (*B. napus* subsp. *napobrassica*), but rarely in the spring-type *B. napus* canola (for review, see Hasan et al. 2021a). Therefore, it is interesting to discover new clubroot resistance genes in different *Brassica* resources and elucidate the genetic and molecular basis of resistance, with the aim to develop clubroot-resistant canola cultivars.

Rutabaga (AACC, $2n = 38$) carries the same genome as *B. napus* canola (Bus et al. 2011). This is an excellent genetically diverse material for genetic improvement of clubroot resistance, agronomic and seed quality traits including seed yield in spring *B. napus* canola (Shiranifar et al. 2020, 2021; Hasan et al. 2021b). Several rutabaga cultivars such as Askegarde, Brookfield, Kingston, IRPT, Polycross and Wilhelmsburger carrying resistance to different isolates of *P. brassicae* have been found in Canada (Spaner 2002; Hasan et al. 2012; Peng et al. 2014; Fredua-Agyeman et al. 2020b). Ayers and Lelacheur (1972) identified one major gene for resistance to races 2 and 3 in the rutabaga cultivar York, and two dominant genes for resistance to race 2 and one gene for resistance to race 3 in the rutabaga cultivar Wilhemsburger. A genomic region of chromosome A08 of the rutabaga cv. Brookfield carries resistance to five *P. brassicae* pathotypes

(2F, 3H, 5I, 6M and 8N) was found in the rutabaga cultivar Brookfield (Hasan and Rahman 2016; Hasan et al. 2021b). Hasan et al. (2021b) also reported a minor locus on A03 conferring resistance to pathotype 3H in the rutabaga cv. Brookfield. Thus, it is apparent that the rutabaga cultivars can carry multiple clubroot resistance loci conferring resistance to multiple pathotypes. Therefore, clubroot resistance of other rutabaga accessions is highly valuable for further investigation to broaden the genetic base of resistance in spring *B. napus* canola.

Molecular markers are valuable tools for use in genetic diversity study and marker-assisted selection (MAS) for a trait (for review, see Nadeem et al. 2018). Among the different types of markers, such as restriction fragment length polymorphism (RFLP) and single nucleotide polymorphism (SNP), the simple sequence repeat (SSR) markers have been used extensively in plant breeding due to their co-dominance, high reproducibility and abundance in the genome (Powell et al. 1996; for review, see Kalia et al. 2011). For instance, several SSR markers co-segregating with clubroot resistance genes have been developed for use in MSA for clubroot resistance in *B. napus* (Cheng et al. 2009; Hasan and Rahman 2016) and its progenitor species *B. rapa* (Kato et al. 2013; Pang et al. 2018). Fredua-Agyeman and Rahman (2016) mapped the clubroot resistance locus of the European winter *B. napus* canola cv. Mendel (*CRa*/*CRb*^{Kato}) on A03 by using 12 SSR markers. Hasan and Rahman (2016) constructed a genetic linkage map of chromosome A08 using 48 SSR markers and mapped a clubroot resistance locus, likely corresponding to *Crr1a,* in the rutabaga cv. Brookfield. As such, introgression of clubroot resistance from other rutabaga accession and the development of SSR markers for increasing the pool of clubroot resistance genes and markers is needed for use in the breeding of spring *B. napus* canola through MAS.

The objectives of this study were to study the genetic basis of resistance to *P. brassicae* pathotypes 2B, 3A, 5x(L-G1), 3H, and 3D in a *B. napus* doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross, map the clubroot resistance loci, and identify molecular markers associated with resistance for use in marker-assisted breeding of clubroot-resistant spring *B. napus* canola.

3.2 Materials and methods

3.2.1 Plant material

The plant materials used in this study comprised 94 *B. napus* DH families derived from seven crosses involving four spring *B. napus* lines, viz. 1IA1190.030, 1IA1190.044, 1IA1190.052, and 1IA1190.064, carrying clubroot resistance of the rutabaga cv. Polycross, and three clubroot-susceptible spring *B. napus* canola lines, viz. A04-73NA, A06-19NA, and A01-104NA (Table 3.1). The four clubroot-resistant lines were derived from a cross involving a spring *B. napus* canola breeding line A05-17 NA and the rutabaga cv. Polycross. The three susceptible parents were spring-type *B. napus* breeding lines developed by the Canola Breeding Program of the University of Alberta. The seven parental lines were also assessed for resistance to all five pathotypes in two replications. The four female parents were resistant (DSI ≤ 20%) to *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D, while the three male parents were highly susceptible (DSI = 100%) to all five pathotypes. The DH population was evaluated for resistance to five *P. brassicae* pathotypes in Study 1 (Chapter 2) and the data is presented in Table 3.2. Of these 94 *B. napus* DH lines, 11 were resistant to all five pathotypes, 42 were highly susceptible and the remaining 41 lines carried a different level of resistance to different pathotypes.

3.2.2 DNA extraction

Leaf tissues were collected from two plants of each of the parents and the 94 DH lines (three to five weeks old plants) in 2.0 mL Eppendorf tubes, frozen in liquid nitrogen, and stored at −80°C until use. The leaf samples were ground with glass beads using a TissueLyser II (Qiagen, Hilden, Germany) and suspended in 500 µL of DNA lysis solution [200nM Tris-HCl pH 7.5, 25 mM EDTA pH 8.0, 0.5% sodium dodecyl sulfate (SDS), 250 mM NaCl]. The mixtures were incubated in a water bath at 65°C for 30 minutes with occasional inversion. After that, 30 µL of RNase was added to each sample, which was then incubated at 37°C for 30 minutes. After adding 150 µL of protein precipitation solution, the samples were placed on ice for 5 minutes, mixed with 500 µL of chloroform with repeated inversion, and centrifuged at 10,000 rpm for 10 minutes. After that, the aqueous layers were moved into new 1.5 microcentrifuge tubes, mixed well with 400 µL of chilled (-20°C) isopropanol by inversion, incubated on ice for more than 10 minutes and centrifuged at 10,000 rpm for 10 minutes at 4°C. Subsequently, the supernatant was discarded, and the pellets were washed with 500 µL of 70% cold ethanol and centrifuged at 10,000 rpm for 5 minutes. After that, the ethanol layer was discarded, and the pellets were air dried at room temperature for 15 minutes and resuspended in 50 µL of Nuclease-Free Water (Life Technologies, Austin, USA). The quality and concentration of the genomic DNA samples were analyzed using a ND-2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, USA). The DNA samples with a 260/280 nm absorbance ratio of about 1.8 were diluted to a final concentration of 25 ng/µL and stored at -20°C until use.

3.2.3 Simple sequence repeat (SSR) markers

In a research project using a few hundred canola lines carrying clubroot resistance of the rutabaga cv. Polycross and employing whole-genome resequencing of the bulk of resistant and

susceptible lines, seven genomic regions of the chromosomes A03, A04, A08, A09, C01, C04, and C05 contributing to clubroot resistance were identified (University of Alberta Canola Program, unpublished results). To identify markers from these genomic regions associated with resistance, SSR markers from chromosomes A03 and A08 were collected from three different sources: Markers obtained from the Agriculture and Agri-Food Canada (AAFC) through a material transfer agreement, the published markers reported to be associated with dominant clubroot resistance loci (Cheng et al. 2009; Li et al. 2010; Chu et al. 2014; Pang et al. 2018), and the markers designed by the Canola Breeding Program of the University of Alberta (unpublished results) based on the genome sequence information of *B. rapa* cultivar Chiifu-401-42 (Cheng et al. 2011) and *B. napus* cultivar Darmor-bzh (Chalhoub et al., 2014). A total of 253 SSR markers from A03 and A08 were selected from the genomic regions where clubroot resistance loci have been reported by different researchers (for review, see Hasan et al. 2021a) (Table 3.3). In case of the chromosomes A04, A09, C01, C04, and C05, a total of 211 SSR markers designed by the Canola Breeding Program of the University of Alberta based on the genome sequence information of *B. rapa* cultivar Chiifu-401-42 (Cheng et al. 2011) were used (Table 3.3). Thus, a total of 464 SSR markers were used to genotype the parents for polymorphism, from where 62 polymorphic markers were used for genotyping the 94 DH lines (Table 3.3).

3.2.4 PCR amplification

PCR amplification was carried out in a total volume of 12 µl reaction mixture, which included 2.4 µl of 5X Colorless GoTaq Flexi buffer, 0.125 µl of 5 U/µl GoTaq DNA Polymerase enzyme (Promega Corporation, Madison, USA), 1.2 µl of 25 mM $MgCl_2$, 0.3 µl of 25 nM fluorescently labelled M13 primer (FAM, NED, PET or VIC; Applied Biosystem, Foster City, USA), 2.4 µl of 2 mM dNTPs mix (Life Technologies, Carlsbad, USA), 1 µl of each of the 10 nM

forward and reverse primers, 2 µl of 25 ng/µl genomic DNA, and 1.575 µl of Nuclease-Free Water. PCR amplification reactions were performed in a SimpliAmp Thermal Cycler (Life Technologies Holdings Pte Ltd., Singapore) under the following conditions: 1 cycle of initial denaturation at 95°C for 3 minutes; 42 cycles where each cycle includes denaturation at 95°C for 30 seconds, annealing at 54-58°C for 30 seconds, and extension at 72°C for 45 seconds; followed by 1 cycle of final extension at 72°C for 10 minutes. The annealing temperature was chosen based on the length and composition of the primers. The PCR products were stored at −20°C until use.

3.2.5 ABI (Applied Biosystem Instruments) sequencing

The PCR products were labelled following the M13 primer genotyping protocol, as described by Schuelke (2000). For this, an M13 sequence 5'-CACGACGTTGTAAAACGAC-3' was attached to the 5' end of the forward primer of each SSR marker. The universal M13 primer sequences labelled with fluorescent dyes FAM, VIC, NED and PET were used to incorporate the fluorescent dyes into the amplification products. In a four-dye fluorescence-based ABI sequencing, 1 µl of each of the fluorescently labelled amplified products was added to 7.9 µl of highly deionized (Hi-Di) formamide (Thermo Fisher Scientific, Carlsbad, USA) and 0.1 µl of GeneScan-500 LIZ size standard (Applied Biosystems, Warrington, UK). Aliquots of the amplified DNA fragments were determined by size using a capillary electrophoresis system ABI 3730 analyzer (Applied Biosystems, Foster City, USA). The ABI genotyping results were analyzed using the software program GeneMapper version 6.0 (Applied Biosystems).

3.2.6 Linkage map construction and mapping the resistance loci

The SSR markers that showed polymorphism between clubroot-resistant and clubroot-susceptible parents were used to genotype the DH population. The genotyping data were analyzed using the software program QTL IciMapping version 4.0 (Meng et al. 2015) to construct linkage

map of the chromosomes. To achieve this, marker alleles from the resistant parents (female) were scored as "2", alleles from the susceptible parents scored as "0", and the missing alleles were scored as "-1". The linkage groups were established based on a minimum logarithm of odds (LOD) score of 3.0 and recombination frequency (RF) of 0.40. The Kosambi mapping function was applied to transform the recombination frequencies into centi-Morgan (cM) (Kosambi 1944). The physical positions of the SSR primers were determined by aligning the forward and reverse primers to the whole-genome assembly of *B. rapa* cultivar Chiifu-401-42 version 3.5 (Zhang et al. 2022) and *B. napus* cultivar Darmor-bzh version 10. (Rousseau-Gueutin et al. 2020) using BLAST search in the Brassicaceae Database (BRAD) (http://brassicadb.cn/), and the positions were determined when the length of the fragment between the forward and reverse primers matched with the known or predicated amplification product size. Based on this, the genetic linkage maps as well as their corresponding physical maps were developed using the software program MapChart version 2.32 (Voorrips 2002).

A composite interval mapping approach was carried out to estimate the likelihood of the genomic regions associated with clubroot resistance, as well as to identify the SSR markers linked to clubroot resistance for use in marker-assisted breeding. For this, genotypic and phenotypic data were subjected to Single Marker Analysis (SMA) and Inclusive Composite Interval Mapping-Additive (ICIM-ADD) using the software program QTL IciMapping version 4.0 (Meng et al. 2015). The walking speed for QTL mapping was set at a 0.1 cM interval with a probability of 0.001 in stepwise regression (Manichaikul et al. 2009). To declare a QTL, the empirical threshold of LOD with a significance level of 0.05 was obtained from 1000 permutations (Churchill and Doerge 1994). If two QTL overlapped within a 95% Confidence Interval (C.I.), they were regarded as the same QTL; otherwise, the QTL were considered independent. The QTL detected with LOD

scores of more than 3.0 and explained more than 10% of the total phenotypic variance were defined as major QTL (Lander and Kruglyak 1995). The QTL identified in this study were named following a modified nomenclature system used by Hasan et al. (2021b).

3.2.7 Candidate gene prediction

The genomic regions flanked by the SSR markers associated with clubroot resistance were scanned in the *B. rapa* cv. Chiifu-401-42 version 3.01 genome sequence (GCF_000309985.2) using the UCSC (University of California Santa Cruz) Genome Browser (https://genome.ucsc.edu/cgi-bin/hgGateway) (Lee et al. 2022) for annotated genes. The *ab initio* genes were predicted from the program Augustus version 3.1 (Stanke and Waack 2003) at the UCSC Genome Brower. The amino acid sequences predicted from Augustus genes were searched by BLASTp with the *Arabidopsis thaliana* genome as the reference (The Arabidopsis Information Resource TAIR10, https://www.arabidopsis.org/) to determine the potential protein functions.

3.3 Results

3.3.1 Molecular marker analysis

A total of 464 SSR markers from seven chromosomes of *B. napus* were assessed for polymorphism between the clubroot-resistant and clubroot-susceptible parental lines (Table 3.3). Amongst these 464 SSR primer pairs, 104 (22.4%) detected polymorphism between the parents, 220 (47.4%) produced fragments with the same size, while 140 (30.2%) failed PCR amplification of the genomic DNA. Of the 104 markers, 62 (59.6%) polymorphic markers detecting fragment differences of more than 5 bp between the parents were used to genotype the 94 *B. napus* DH lines (Table 3.3). Of the 62 SSR markers, 58 produced fragments with a clear difference and good reproducibility; genotypic data of these markers were used for genetic linkage analysis of the markers with resistance. For this, only markers from the same linkage group containing less than

10% of missing data were kept. Based on these results, 38 markers, which included 12 markers from chromosome A03, four from A04, 14 from A08, three from C01, two from C04, and three from C05, were used for further study.

3.3.2 Linkage map construction

Before constructing the linkage maps, SMA was carried out to identify the chromosomes carrying clubroot resistance. This analysis detected SSR markers from chromosomes A03 and A08 associated with clubroot resistance (Table 3.7). Therefore, genetic linkage maps were constructed using 12 markers from A03 and 14 markers from A08 (Figure 3.1a & 3.2b). The sequence information and origin of these SSR markers are presented in Table 3.4. The linkage map of A03 included two markers from AAFC, four publicly available markers (Cheng et al. 2009; Chu et al. 2014; Pang et al. 2018), and six markers designed based on the genome sequence information of *B. rapa* cultivar Chiffu-401-42 (Cheng et al. 2011). The linkage map of A08 included one marker from AAFC, one marker developed by Cheng et al. (2009), two markers developed based on the *B. napus* cv. Darmor-bzh genome sequence (Chalhoub et al., 2014), and 10 markers developed based on *B. rapa* cv. Chiffu-401-42 genome sequence (Cheng et al. 2011). The genetic map of A03 spanned 314.46 cM with a mean distance of 26.21 cM between markers (Figure 3.1b), while the A08 map covered 37.95 cM with an average marker interval of 2.71 cM (Figure 3.2b). The physical maps of A03 and A08 were constructed by using the physical locations of the SSR markers on the *B. rapa* cv. Chiifu-401-42 whole-genome sequence assembly version 3.5 (Zhang et al. 2022). The physical map of A03 spanned 16.0 to 25.6 Mb, while the map of A08 spanned 11.5 to 14.8 Mb regions (Table 3.6; Figure 3.1a & 3.2a).

3.3.3 QTL mapping for clubroot resistance

QTL mapping identified multiple genomic regions from A03 and A08 associated with resistance to different *P. brassicae* pathotypes (Table 3.5). These QTL were detected with LOD scores of 2.94 to 32.37 and explained 1.92 to 34.87% of the total phenotypic variance for resistance to pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D. Additive effect of these QTL varied from 7.76% to 37.41% DSI where the alleles of rutabaga cv. Polycross reduced the disease incidence.

In case of the chromosome A03, three QTL, viz. *qCRa_A03*, *qCRb_A03*, and *qCRc_A03*, were detected (Table 3.5). Among these, the locus *qCRa_A03*, conferring resistance to 2B, 3A, 5x (L-G1), 3H and 3D, is located at an interval of 31.45 to 49.85 cM, and detected with LOD scores of 27.63, 18.94, 14.87, 26.48, and 32.37, respectively, and explained about 31-35% of the total phenotypic variance for resistance to these pathotypes. The other major QTL, *qCRb_A03*, was positioned at 49.95 to 54.35 cM region, detected LOD score of 24.99, 25.42 and 30.07, and explained 28.77%, 26.91%, and 30.72% of the total phenotypic variance for resistance to pathotypes 2B, 3H and 3D, respectively. The locus *qCRa_A03* was flanked by the SSR markers yau376 and yau106, and the locus *qCRb_A03* was flanked by the SSR markers yau106 and sNRA85; therefore, it is possible that these two are a single QTL. In addition to this, a minor locus *qCRc_A03* affecting resistance to pathotypes 3H and 3D, was located at an interval of 209.95 to 220.55 cM and detected with LOD value of about 3.0, explained only about 2.0% of the total phenotypic variance. This minor QTL was located between the flanking markers A03_12778 and A03_12779.

As for chromosome A08, two QTL associated with clubroot resistance were identified (Table 3.5). The QTL *qCRa_A08* was positioned at 0.00 to 2.35 cM region, detected with LOD score of 8.73 to 14.55 and explained 7.31%, 31.16%, 9.88%, and 6.58% of the total phenotypic

variance for resistance to pathotypes 2B, 5x (L-G1), 3H, and 3D, respectively. The locus *qCRb_A08* conferring resistance to pathotype 3A was located at 2.35 to 5.55 cM region, detected with a LOD score of 17.96, and explained 27.92% of the phenotypic variance. The *qCRa_A08* was flanked by the SSR markers A08_3305 and A08_4450, while the *qCRb_A08* was positioned between the flanking markers A08_3314 and A08_4603. In addition to these SSR markers, the markers A08_4450, A08_4477 and A08_3314 were mapped on the same genetic region of the A08 linkage map (Figure 3.2b); therefore, it is also possible that these two genomic regions are a single QTL.

3.3.4 Evaluation of the flanking markers for co-segregation and candidate gene prediction

Nine flanking SSR markers from the A03 and A08 QTL, viz. yua376, yua106, sNRA85, A03_12778, and A03_12779 from A03, and A3305, A08_4450, A08_3314 and A08_4603 from A08 (Table 3.5) were evaluated for co-segregation with resistance to all five pathotypes (2B, 3A, 5x, 3H and 3D). Among the A03 markers, yau106 exhibited the strongest co-segregation (9.7% recombination) followed by the marker sNRA85 (10.6-12.8% recombination) with resistance to 2B, 3H and 3D. However, the marker yau106 showed greater recombination with resistance to 3A (20.4%) and 5x (25.8%). The remaining three A03 markers, yau376, A03_12778 and A03_12779 showed 32.3 to 41.5% recombination. As for the markers from chromosome A08, A08_3305 and A08_4450 showed 23.1% and 20.0% recombination, respectively, for resistance to pathotype 5x, while recombination between these two markers for resistance to 2B, 3H and 3D varied from 32.2 to 36.3%. The two markers A08_3314 and A08_4603 from the QTL region associated with resistance to pathotype 3A exhibited 20.2% and 21.3% recombination, respectively.

A total of 198 genes were predicted based on the *B. rapa* cv. Chiifu-401-42 version 3.01 genome sequence from the genomic regions on A03 and A08 associated with clubroot resistance. BLASTp alignment of the predicted amino acid sequences of the *ab initio* genes with the *A. thaliana* genome sequence identified two sequences encoding disease resistance proteins. These two sequences were identified with E-value of less than e-20, and thus, could be the potential candidates for clubroot resistance. One of the sequences was located at an interval of 16083342 to 16089681 bp region of A03, flanked by the SSR marker yau376 and yau106, exhibited similarity with 100 proteins of which 95 were toll-interleukin-1 receptor / nucleotide-binding site / leucine-rich-repeat (TIR-NBS-LRR) class of proteins (Table 3.8). The other sequence was positioned between 11576944 and 11577344 bp region of A08 and flanked by the SSR markers A08_3305 and A08_4450; this sequence was found to be encoding a TIR-NBS-LRR class protein (E-value = 1e-47).

Several additional genes were also identified in the present study including the ones encoding kinases, pectin lyases, germin-like proteins, chaperone proteins, ribosomal proteins, transcription factors, transferases, MYB domain proteins, ATP binding proteins and glycosyl hydrolases. These types of genes are involved in cellular and biological processes, and regulates plant vegetative and reproductive growth and development. Some of the genes encoded proteins such as temperature and salt responsive proteins, ubiquitin-like proteins, MD-2-related lipid recognition domain-containing proteins, drought-responsive family proteins, transducin/WD40 repeat-like superfamily proteins, glycosyl hydrolase family proteins, cysteine-rich RLK (RECEPTOR-like protein kinase), F-box proteins and cyclin family proteins, which have been reported to be involved in plant defence responses against abiotic and biotic stresses. In addition, about 9.1% of the matching genes encoded hypothetical proteins with unknown functions.

3.4 Discussion

The clubroot-resistant rutabaga cv. Polycross has been developed for growing in Newfoundland (Spaner 2002), and this cultivar was found to carry resistance to multiple *P. brassicae* pathotypes (2 or 2F, 3 or 3H, 5 or 5I, 6 or 6M, and 8 or 8N) (Hasan et al. 2012). Results from the present study revealed that the DH population derived from the crosses involving rutabaga cv. Polycross in their pedigree carries resistance to the recently evolved virulent pathotypes, such as 2B, 3A, 5x (L-G1), and 3D. Thus, this DH population is a valuable germplasm for genetic linkage analysis and QTL mapping of clubroot resistance of the rutabaga cv. Polycross.

Previous QTL mapping studies have identified and mapped at least nine major clubroot resistance loci in the genomic regions of 15.3-16.3 Mb and 23.8-26.0 Mb of A03 of *B. rapa* cv. Chiifu-401-42 whole-genome assembly version 3.5 (for review, see Hasan et al. 2021a). Among these, three major resistance loci, *CRk* (Sakamoto et al. 2008), *CRd* (Pang et al. 2018) and *Crr3* (Hirai et al. 2004; Saito et al. 2006) were identified in the 15.3-16.3 Mb genomic region, and six CR loci, viz. *Rcr4* (Yu et al. 2017), *Rcr5* (Huang et al. 2019), *Rcr1* (Chu et al. 2014; Yu et al. 2016), *Rcr2* (Huang et al. 2017), *CRa*/*CRb* (Piao et al. 2004; Ueno et al. 2012; Kato et al. 2013; Fredua-Agyeman and Rahman 2016; Hatakeyama et al. 2017), and *CRq* (Yuan et al. 2015), were found within the physical position of 23.8-26.0 Mb. In the present study, composite interval mapping analysis identified three clubroot resistance loci at about 39.7 to 52.0 and 214.4 to 216.4 cM positions. However, alignment of the three flanking SSR markers, yua376, yua106 and sNRA85, from 39.7 to 52.0 cM region of the *qCRa_A03* and *qCRb_A03* could be positioned at about 16 Mb position, while the other two flanking markers A03_12778 and A03_12779 from the 214.4 to 216.4 cM region of the *qCRc_A03* could be positioned at about 25 Mb region of A03 of *B. rapa* cv. Chiifu-401-42 (Table 3.6; Figure 3.1). Although the markers yau376 and yau106 were

positioned at about the same physical position, the genetic interval of these two markers was wide (49.91 cM) (Figure 3.1). Genetic distance was estimated based on 94 DH lines. A small mapping population could result in a wide genetic interval between two molecular markers that are physically close to each other. Fine-mapping by using a greater number of DH lines could narrow down the genetic distance. Based on the physical locations of the CR loci on A03 reported by other researchers, the QTL *qCRa_A03* and *qCRb_A03* are most likely the *CRd* or *Crr3*, while the minor loci *qCRc_A03* likely corresponds to *Rcr2* located at 25.3-25.6 Mb region.

In case of the chromosome A08, at least five clubroot resistance loci, viz. *CRs* (Laila et al. 2019), *Rcr3* (Karim et al. 2020), *Crr1a* (Suwabe et al. 2003, 2006; Hatakeyama et al. 2013), *Rcr9* (Yu et al. 2017; Karim et al. 2020) and *qBrCR38-2* (Zhu et al. 2019) were reported in *B. rapa*, and a major QTL was identified in the rutabaga cv. Brookfield (Hasan and Rahman 2016; Hasan et al. 2021b). Among these, the *qBrCR38-2* was positioned at the 20.2-21.8 Mb region of *B. rapa* cv. Chuiifu-401-42, while the remaining resistance loci were mapped in the 11.3-12.7 Mb region. In this study, QTL analysis detected *qCRa_A08* at 0.0 to 2.35 cM and *qCRb_A08* at 2.35 to 5.55 cM region of A08. BLAST search positioned the four flanking SSR markers from the *qCRa_A08* and *qCRb_A08* QTL at 11550186-11755827 bp and 11953416-12073493 bp, respectively. Thus, it is highly likely that clubroot resistance on A08 of the rutabaga cv. Polycross corresponds to *CRs* or *Rcr3* or *Crr1a* or *Rcr9*, or these two genomic regions could be a single locus.

In addition to clubroot resistance loci identified on chromosomes A03 and A08, at least eight major resistance loci and several QTL have been reported from the A-genome of *B. rapa* and C-genome of *B. oleracea* (for review, see Hasan et al. 2021a). In the present study, 52 SSR markers from A04 and 50 markers from A09 were screened for polymorphism between the clubroot-resistant and clubroot-susceptible parents (Table 3.3). Although 12 polymorphic markers were

identified from these two chromosomes, none of them co-segregated with clubroot resistance. The A-genome chromosomes A01, A02, A05, A06, and A07 have been reported to carry clubroot resistance loci; however, no resistance locus has so far been reported on A04 and A09 (for review, see Hasan et al. 2021a). Clubroot resistance in the C-genome of *B. oleracea* is under quantitative genetic control. To date, clubroot resistance loci have been identified in all C-genome chromosomes, but only a single major locus *Rcr7* (Dakouri et al. 2018) has been mapped on chromosome C07 (Nagaoka et al. 2010; Lee et al. 2016; Peng et al. 2018; Farid et al. 2020; Fredua-Agyeman et al. 2020b; Ce et al. 2021). In the present study, 109 SSR markers from C01, C04 and C05 were used to genotype the parental lines and 17 polymorphic markers were identified (Table 3.3). However, no association of these polymorphic markers could be detected with clubroot resistance through single marker analysis.

In this study, BLASTp search of the predicted amino acid sequences in the *A. thaliana* genome sequence database identified TIR-NBS-LRR encoding genes, receptor-like kinases, F-box genes, and other disease-responsive genes in the QTL regions conferring resistance to *P. brassicae* pathotypes 2B, 3A, 5x(L-G1), 3H, and 3D in the *B. napus* DH population carrying clubroot resistance of the rutabaga cv. Polycross. The NBS-LRR genes, including TIR-NBS-LRR class and coiled-coil (CC) motif (CC-NBS-LRR class), are the largest class of disease resistance genes (R genes) known in plants (for reviews, see Dangl and Jones 2001; Akira and Hemmi 2003; McHale et al. 2006). The NBS-LRR proteins encoded by R genes play an important role in plant defence responses against obligate biotrophic pathogens (for review, see Glazebrook 2005). At least 641, 249 and 443 NBS-LRR genes have been identified in *B. napus*, *B. rapa* and *B. oleracea*, respectively (Alamery et al. 2017). The genes encoding NBS-LRR proteins have been reported by several researchers to be the potential candidates for clubroot resistance (Ueno et al. 2012; Chu et

al. 2014; Huang et al. 2017; Chang et al. 2019; Choi et al. 2020; Karim et al. 2020). Additionally, the plant immune system has also been reported to be regulated by transmembrane receptor-like kinases (RLKs), transmembrane receptor-like proteins (RLPs) and receptor-like cytoplasmic kinases (RLCKs) (Tang et al. 2017; Sun and Zhang 2020). Previous studies found that RLKs, RLPs, glycosyl hydrolase family proteins and F-box proteins are putative candidate genes for clubroot resistance (Karim et al. 2020; Choi et al. 2020).

3.5 Conclusion

In conclusion, this study demonstrated the potential of the clubroot resistance alleles of the rutabaga cv. Polycross in the breeding of clubroot-resistant spring-type *B. napus* canola. The QTL regions contributed to resistance to the old *P. brassicae* pathotype 3H as well as the recently evolved pathotypes 2B, 3A, 5x(L-G1), and 3D, and the QTL were mapped on the chromosomes A03 and A08 where several clubroot resistance loci were identified previously. Although earlier studies reported these clubroot resistance loci mostly based on resistance to the original *P. brassicae* pathotypes, this thesis research confirmed their involvement in resistance to the recently evolved pathotypes such as 3A, 2B and 3D. Thus, the knowledge gained from this study can be used in the breeding of clubroot-resistant *B. napus* canola cultivars through MAS as well as for fine-mapping of the QTL regions and map-based cloning of the clubroot resistance genes.

3.6 Tables

Table 3.1 Pedigree information of the 94 *Brassica napus* doubled haploid (DH) lines carrying clubroot resistance of the rutabaga cv. Polycross.

Pedigree	Female parents[1]	Male parents	No. of DH lines
(A05-17NI × Polycross) × A04-73NA	1IA1190.030	A04-73NA	7
(A05-17NI × Polycross) × A06-19NA	1IA1190.030	A06-19NA	14
(A05-17NI × Polycross) × A01-104NA	1IA1190.030	A01-104NA	29
(A05-17NI × Polycross) × A04-73NA	1IA1190.044	A04-73NA	21
(A05-17NI × Polycross) × A01-104NA	1IA1190.044	A01-104NA	6
(A05-17NI × Polycross) × A04-73NA	1IA1190.052	A04-73NA	9
(A05-17NI × Polycross) × A04-73NA	1IA1190.064	A04-73NA	8

[1]The female lines carry clubroot resistance of the rutabaga cv. Polycross, and derived from A04-17NI (canola) × Polycross (rutabaga) cross.

Table 3.2 Resistance to *Plasmodiophora brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H and 3D in a *Brassica napus* doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross. R = resistant (DSI ≤ 20%); MR = moderately resistant (DSI > 20 to ≤ 40%); MS = moderately susceptible (DSI > 40 to ≤ 70%); S= susceptible (DSI > 70%).

Pathotypes	No. of R lines	No. of MR lines	No. of MS lines	No. of S lines
2B	15	13	10	56
3A	34	6	7	47
5x (L-G1)	31	14	6	43
3H	23	4	12	55
3D	20	6	11	56

Table 3.3 SSR markers used for genotyping the *Brassica napus* doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross.

Chromosomes	Genomic regions	No. of SSR markers tested	No. of polymorphic markers used
A03	15-26 Mb	70	17
A04	15-20 Mb	52	8
A08	10-15 Mb	183	16
A09	10-15 Mb	50	4
C01	10-15 Mb & 35-40 Mb	65	8
C04	20-25 Mb	18	3
C05	10-15 Mb	26	6
Total	-	464	62

Table 3.4 List of 27 polymorphic SSR markers used to study their linkage association of clubroot resistance in a *Brassica napus* doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross.

Marker name	Chr.	M13-forward primer (5'-3')	Reverse primer (5'-3')	Marker source
yau376	A03	TGTCACCAGCGCATTATAG	AAGGGAGGGAAGATGGGTTG	Pang et al. (2018)
yau106	A03	GGTCACCAATCGAAGCCTT	GCATGCGGGTATACACATCT	Pang et al. (2018)
sNRA85	A03	GGTGGGTTAGTAGGCGATC	ACCGACTTCCACTTCCCTTT	AAFC[a]
BnGMS417	A03	AATGGAACGACTCAACATAG	GGATCGACTCAAAGTCACAT	Cheng et al. (2009)
sR11644	A03	GCAAACTGGTAAACCCTGGA	GGGTAGACTGGTCCCGAGAT	AAFC
MS7-9	A03	AGAGGCTTTCTCCATCAA	GACATAAGAATCCCACAA	Chu et al. (2014)
A03_12776	A03	TTGGCGAAATTCAGTTGACA	CTCAAAAAGCCATCACCACC	*B. rapa* genome[b]
A03_12778	A03	AGAGCAAGTGGCTTTGGAGA	TGGAAAAGACATCAACCACG	*B. rapa* genome
A03_12779	A03	TGGAACCTCCAAAATCTCTAAAA	CAAGATAAAATTGTCGAAATCAAGTG	*B. rapa* genome
A03_12783	A03	GGCACCTTTCGTCTTTTGTC	TTCAAAACTTTAAGGTGGTCTCAA	*B. rapa* genome
A03_12785	A03	CCTGTTCCAGAAATTCAAATCA	AGTGGGGCTTTGCTTGATAA	*B. rapa* genome
A03_12787	A03	GATTCACGTGCTCGAATGAA	GGGGAATTCTTAAGGTGGGA	*B. rapa* genome
A08_3305	A08	GTCACAAAATGGGGTCTTGAGT	TCAGTGGTTGCACGTATGTTTT	*B. napus* genome
A08_4450	A08	TTGGGTCCTGACTTTGAAGC	TGACTAACCATGTCAAACTGCC	*B. rapa* genome
A08_4477	A08	AGAAGCCAGTATTTGGGGGT	TTTTTGGTGTAGGATTTTGGTT	*B. rapa* genome
A08_3314	A08	GTAGTTCCGCAATCCAAATCTC	CAGACAATCTCAAGAACAAGCG	*B. napus* genome
A08_4603	A08	ATCGATGCAATGACGTGTGT	CAAAGAAAGCCTTTTCACGG	*B. rapa* genome
A08_4614	A08	CCAAAGCTGCAGTCGTAACA	GAGGCATTCAAACACAAGCC	*B. rapa* genome
A08_4654	A08	AATTTGAAAGTCAACATGGACC	GAAACTAAAGACTACGCGCACA	*B. rapa* genome
A08_4735	A08	GCCCAATAAGCTAAGATCCG	TACTCGCGGAAGAAGGAGAG	*B. rapa* genome
A08_4987	A08	TTCTCATCACTTGCATTCATCA	GAGAGTATCGTGCATGTGTCG	*B. rapa* genome
A08_3708	A08	GTGATCCCTTTGGAGCTGTAAG	TATCGGGTTTGAGTTCGAGTTT	*B. rapa* genome
A08_5024	A08	GAACACGAAGCGTGTCTGAA	AAGAAACCATCGGTGTCGAG	*B. rapa* genome
A08_5063	A08	CCAAAAGAAATCCAAAACGG	CTTGTGCCTCAAGTCAACGA	*B. rapa* genome
sR1868	A08	CATGGACACACACAACCAGA	GAGAAACCCAATAAAGTAGAACCAA	AAFC
BnGMS452	A08	TAGATGGTCCTTGACCCATA	AACATGTCTTTGATGAAGCC	Cheng et al. (2009)

[a]Obtained from Agriculture and Agri-Food Canada (AAFC) through a material transfer agreement.

[b]Designed by the Canola Program of the University of Alberta based on the genome sequence information of *Brassica rapa* cultivar Chiifu and *Brassica napus* cultivar Darmor-bzh.

Table 3.5 List of putative QTL for resistance to different *Plasmodiophora brassicae* pathotypes detected on chromosomes A03 and A08 of *Brassica napus* using a doubled haploid (DH) families carrying clubroot resistance of the rutabaga cv. Polycross and following Inclusive Composite Interval Mapping-Additive (ICIM-ADD) methods.

QTL	Chr.	Path.	Flanking marker		Position (cM)[a]	Confidence Interval (cM)	LOD	PVE (%)[b]	ADD[c]
			Left	Right					
qCRa_A03	A03	2B	yau376	yau106	43.90	36.25-48.45	27.62	32.21	-33.73
	A03	3A	yau376	yau106	46.00	37.65-49.85	18.94	32.08	-30.79
	A03	5x	yau376	yau106	41.40	31.95-47.35	14.87	34.87	-29.21
	A03	3H	yau376	yau106	39.70	31.45-45.85	26.48	31.13	-34.99
	A03	3D	yau376	yau106	46.00	39.55-49.15	32.37	33.06	-37.41
qCRb_A03	A03	2B	yau106	sNRA85	52.00	50.15-54.35	25.42	28.77	-32.23
	A03	3H	yau106	sNRA85	52.00	50.15-54.35	24.99	26.91	-33.61
	A03	3D	yau106	sNRA85	51.00	49.95-53.45	30.07	30.72	-36.03
qCRc_A03	A03	3H	A03_12778	A03_12779	214.40	209.95-220.55	2.94	1.92	-7.87
	A03	3D	A03_12778	A03_12779	216.40	213.95-220.55	3.40	1.92	-7.76
qCRa_A08	A08	2B	A08_3305	A08_4450	2.30	0.00-2.35	8.73	7.31	-15.05
	A08	5x	A08_3305	A08_4450	0.00	0.00-2.35	14.55	31.16	-26.38
	A08	3H	A08_3305	A08_4450	1.20	0.00-2.35	12.08	9.88	-18.74
	A08	3D	A08_3305	A08_4450	1.60	0.00-2.35	10.32	6.58	-15.17
qCRb_A08	A08	3A	A08_3314	A08_4603	3.50	2.35-5.55	17.96	17.92	-27.10

[a]The estimated position of the putative QTL in cM on the genetic maps of the chromosomes A03 and A08.
[b]Phenotypic variation explained the QTL.
[c]Additive effect represents the effect of allelic substitutions; a negative value indicates that the allele from the rutabaga cultivar Polycross decrease diseases severity index (DSI).

Table 3.6 Linkage association of the SSR markers associated with resistance to *Plasmodiophora brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D in 94 *Brassica napus* doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross.

Marker name	Chr.	Recombination frequency (%)					Physical position (bp)[a]		Genetic position
		2B	3A	5x (L-G1)	3H	3D	Start	End	(cM)[b]
yau376	A03	37.6	33.3	36.6	34.4	32.3	16046633	16046801	0.00
yau106	A03	9.7	20.4	25.8	9.7	9.7	16091107	16091269	49.91
sNRA85	A03	11.7	-	-	10.6	12.8	16677216	16677321	55.31
A03_12778	A03	-	-	-	41.3	37.0	25463971	25464232	213.95
A03_12779	A03	-	-	-	41.5	37.2	25465308	25465567	220.51
A08_3305	A08	35.2	-	23.1	36.3	36.3	11550186	11550423	0.00
A08_4450	A08	32.2	-	20.0	33.3	33.3	11755518	11755827	2.30
A08_3314	A08	-	20.2	-	-	-	11953416	11953630	2.30
A08_4603	A08	-	21.3	-	-	-	12073238	12073493	3.54

[a]Physical position on the *B. rapa* cv. Chiifu-401-42 version 3.5 genome sequence.

[b]Genetic position on genetic linkage maps of the chromosome A03 and A08 constructed by using a DH population of 94 lines carrying clubroot resistance of the rutabaga cv. Polycross.

Table 3.7 List of molecular markers segregating with clubroot resistance following Single Marker Analysis (SMA) in a doubled haploid (DH) population carrying clubroot resistance of the rutabaga cv. Polycross.

Chr.	Path.	Genetic position (cM)	Marker name	LOD	PVE (%)	ADD
A03	2B	0.00	yau376	2.44	2.61	-12.04
A03	2B	49.91	yau106	16.37	12.78	-30.51
A03	2B	55.31	sNRA85	13.42	11.17	-28.93
A03	2B	88.96	BnGMS417	3.46	3.62	-14.43
A03	2B	119.73	sR11644	1.95	2.11	-10.90
A08	2B	0.00	A08_3305	3.02	3.19	-13.93
A08	2B	2.30	A08_4450	3.52	3.68	-15.18
A08	2B	2.30	A08_4477	3.52	3.68	-15.18
A08	2B	2.30	A08_3314	3.52	3.68	-15.18
A08	2B	3.54	A08_4603	3.00	3.16	-14.19
A08	2B	17.54	A08_4654	2.61	2.78	-13.20
A08	2B	17.54	A08_4735	2.61	2.78	-13.20
A08	2B	18.62	A08_4987	2.63	2.81	-13.37
A08	2B	20.80	A08_3708	2.40	2.57	-12.59
A08	2B	22.93	A08_5024	2.57	2.74	-12.82
A08	2B	26.13	A08_5063	2.61	2.78	-13.20
A08	2b	28.25	sR1868	2.08	2.25	-11.69
A08	2B	37.95	BnGMS452	4.60	4.68	-16.40
A03	3A	0.00	yau376	2.60	1.65	-14.47
A03	3A	49.91	yau106	9.62	5.20	-29.39
A03	3A	55.31	sNRA85	6.32	3.68	-25.10
A03	3A	88.86	BnGMS417	2.95	1.86	-15.64
A08	3A	0.00	A08_3305	9.15	5.00	-26.32
A08	3A	2.30	A08_4450	9.26	5.04	-26.85
A08	3A	2.30	A08_4477	9.26	5.04	-26.85
A08	3A	2.30	A08_3314	9.26	5.04	-26.85
A08	3A	3.54	A08_4603	9.70	5.23	-27.58
A08	3A	9.95	A08_4614	4.88	2.94	-19.84
A08	3A	17.54	A08_4654	8.64	4.77	-26.12
A08	3A	17.54	A08_4735	8.64	4.77	-26.12
A08	3A	18.62	A08_4987	8.20	4.58	-25.80
A08	3A	20.80	A08_3708	7.70	4.35	-24.73
A08	3A	22.93	A08_5024	6.70	3.87	-23.01
A08	3A	26.13	A08_5063	8.64	4.77	-26.12
A08	3A	28.25	sR1868	6.89	3.96	-23.44
A08	3A	37.95	BnGMS452	9.58	5.18	-26.07
A03	5x	0.00	yau376	2.30	1.36	-13.53
A03	5x	49.91	yau106	6.53	3.50	-24.83
A03	5x	55.31	sNRA85	4.45	2.51	-21.31
A08	5x	0.00	A08_3305	9.00	4.56	-25.90
A08	5x	2.30	A08_4450	9.30	4.68	-25.89
A08	5x	2.30	A08_4477	9.30	4.68	-25.89

A08	5x	2.30	A08_3314	9.30	4.68	-25.89
A08	5x	3.54	A08_4603	8.87	4.51	-26.36
A08	5x	9.95	A08_4614	5.40	2.98	-20.53
A08	5x	17.54	A08_4654	7.83	4.08	-24.85
A08	5x	17.54	A08_4735	7.83	4.08	-24.85
A08	5x	18.62	A08_4987	7.18	3.80	-24.18
A08	5x	20.80	A08_3708	6.92	3.68	-23.42
A08	5x	22.93	A08_5024	6.63	3.55	-22.69
A08	5x	26.13	A08_5063	7.83	4.08	-24.85
A08	5x	28.25	sR1868	6.10	3.31	-22.05
A08	5x	37.95	BnGMS452	7.02	3.73	-22.76
A03	3H	0.00	yau376	2.90	2.77	-13.30
A03	3H	49.91	yau106	15.08	10.95	-32.53
A03	3H	55.31	sNRA85	11.87	9.24	-30.32
A03	3H	88.96	BnGMS417	4.07	3.79	-17.01
A03	3H	119.73	sR11644	1.89	1.86	-11.80
A08	3H	0.00	A08_3305	3.69	3.47	-16.73
A08	3H	2.30	A08_4450	4.26	3.95	-18.13
A08	3H	2.30	A08_4477	4.26	3.95	-18.13
A08	3H	2.30	A08_3314	4.26	3.95	-18.13
A08	3H	3.54	A08_4603	3.73	3.50	-17.21
A08	3H	17.54	A08_4654	3.30	3.13	-16.13
A08	3H	17.54	A08_4735	3.30	3.13	-16.3
A08	3H	18.62	A08_4987	3.69	3.47	-17.13
A08	3H	20.80	A08_3708	2.92	2.80	-15.14
A08	3H	22.93	A08_5024	3.05	2.91	-15.23
A08	3H	26.13	A08_5063	3.2958	3.13	-16.13
A08	3H	28.25	sR1868	2.571	2.48	-14.15
A08	3H	37.95	BnGMS452	4.991	4.55	-18.63
A03	3D	0.00	yau376	2.6715	3.34	-13.29
A03	3D	49.91	yau106	20.09	14.46	-34.87
A03	3D	55.31	sNRA85	14.03	12.27	-31.51
A03	3D	88.96	BnGMS417	4.54	4.50	-17.57
A03	3D	119.73	sR11644	3.00	3.00	-14.32
A08	3D	0.00	A08_3305	2.35	2.69	-13.29
A08	3D	2.30	A08_4450	2.82	3.19	-14.69
A08	3D	2.30	A08_4477	2.82	3.19	-14.69
A08	3D	2.30	A08_3314	2.82	3.19	-14.69
A08	3D	3.54	A08_4603	2.73	3.10	-14.60
A08	3D	17.54	A08_4654	2.39	2.73	-13.59
A08	3D	17.54	A08_4735	2.39	2.73	-13.59
A08	3D	18.62	A08_4987	2.65	3.01	-14.39
A08	3D	20.80	A08_3708	2.17	2.73	-12.88
A08	3D	26.13	A08_5063	2.39	2.73	-13.59
A08	3D	28.25	sR1868	1.88	2.18	-11.96
A08	3D	37.95	BnGMS452	4.62	5.01	-17.63

Table 3.8 List of *Arabidopsis thaliana* proteins showing sequence similarity with the predicted protein coding sequences of A03 chromosome carrying clubroot resistance. The genomic sequence was flanked by the SSR marker yau376 and yau106 and located between 16083342-16089681 bp region of chromosome A03 of the *Brassica rapa* cultivar Chiifu-401-42 version 3.01 genome sequence.

Protein	Function	Score	E-value
AT5G46270	Disease resistance protein (TIR-NBS-LRR class) family	1186	0
AT5G46260	Disease resistance protein (TIR-NBS-LRR class) family	1149	0
AT5G46450	Disease resistance protein (TIR-NBS-LRR class) family	1132	0
AT1G31540	Disease resistance protein (TIR-NBS-LRR class) family	1114	0
AT5G46520	Disease resistance protein (TIR-NBS-LRR class) family	1113	0
AT5G46510	Disease resistance protein (TIR-NBS-LRR class) family	1110	0
AT5G46470	RPS6 | Disease resistance protein (TIR-NBS-LRR class) family	1106	0
AT4G08450	Disease resistance protein (TIR-NBS-LRR class) family	1048	0
AT5G40060	Disease resistance protein (TIR-NBS-LRR class) family	930	0
AT1G31540	Disease resistance protein (TIR-NBS-LRR class) family	903	0
AT5G22690	Disease resistance protein (TIR-NBS-LRR class) family	880	0
AT5G46490	Disease resistance protein (TIR-NBS-LRR class) family	860	0
AT5G51630	Disease resistance protein (TIR-NBS-LRR class) family	798	0
AT4G16890	SNC1, BAL | Disease resistance protein (TIR-NBS-LRR class) family	758	0
AT5G51630	Disease resistance protein (TIR-NBS-LRR class) family	728	0
AT2G14080	Disease resistance protein (TIR-NBS-LRR class) family	710	0
AT5G18360	Disease resistance protein (TIR-NBS-LRR class) family	705	0
AT5G17970	Disease resistance protein (TIR-NBS-LRR class) family	695	0
AT5G44510	TAO1 | Target of AVRB operation1	686	0
AT5G41750	Disease resistance protein (TIR-NBS-LRR class) family	684	0
AT5G41750	Disease resistance protein (TIR-NBS-LRR class) family	684	0
AT5G41550	Disease resistance protein (TIR-NBS-LRR class) family	683	0
AT3G44480	RPP1, cog1 | Disease resistance protein (TIR-NBS-LRR class) family	678	0
AT4G16950	RPP5 | Disease resistance protein (TIR-NBS-LRR class) family	674	0
AT5G49140	Disease resistance protein (TIR-NBS-LRR class) family	673	0
AT4G16950	RPP5 | Disease resistance protein (TIR-NBS-LRR class) family	673	0
AT3G44670	Disease resistance protein (TIR-NBS-LRR class) family	672	0
AT3G44670	Disease resistance protein (TIR-NBS-LRR class) family	672	0
AT5G11250	Disease resistance protein (TIR-NBS-LRR class) family	668	0
AT5G38340	Disease resistance protein (TIR-NBS-LRR class) family	662	0
AT4G11170	Disease resistance protein (TIR-NBS-LRR class) family	659	0
AT5G41540	Disease resistance protein (TIR-NBS-LRR class) family	657	0
AT5G38850	Disease resistance protein (TIR-NBS-LRR class) family	653	0
AT5G58120	Disease resistance protein (TIR-NBS-LRR class) family	652	0
AT3G44630	Disease resistance protein (TIR-NBS-LRR class) family	648	0
AT4G16900	Disease resistance protein (TIR-NBS-LRR class) family	647	0
AT3G44630	Disease resistance protein (TIR-NBS-LRR class) family	647	0
AT3G44630	Disease resistance protein (TIR-NBS-LRR class) family	647	0
AT5G18370	Disease resistance protein (TIR-NBS-LRR class) family	647	0
AT5G40910	Disease resistance protein (TIR-NBS-LRR class) family	647	0
AT1G65850	Disease resistance protein (TIR-NBS-LRR class) family	641	0
AT1G65850	Disease resistance protein (TIR-NBS-LRR class) family	641	0
AT4G16960	Disease resistance protein (TIR-NBS-LRR class) family	640	0
AT4G16920	Disease resistance protein (TIR-NBS-LRR class) family	630	0
AT2G16870	Disease resistance protein (TIR-NBS-LRR class) family	629	0
AT5G41740	Disease resistance protein (TIR-NBS-LRR class) family	626	0
AT5G41740	Disease resistance protein (TIR-NBS-LRR class) family	626	0
AT4G14370	Disease resistance protein (TIR-NBS-LRR class) family	622	0

AT4G16940	Disease resistance protein (TIR-NBS-LRR class) family	620	0
AT5G18350	Disease resistance protein (TIR-NBS-LRR class) family	620	0
AT1G64070	RLM1 | Disease resistance protein (TIR-NBS-LRR class) family	617	0
AT1G63870	Disease resistance protein (TIR-NBS-LRR class) family	616	0
AT4G16860	RPP4 | Disease resistance protein (TIR-NBS-LRR class) family	615	0
AT3G25510	Disease resistance protein (TIR-NBS-LRR class) family	610	0
AT1G63880	Disease resistance protein (TIR-NBS-LRR class) family	610	0
AT1G56510	WRR4, ADR2 | Disease resistance protein (TIR-NBS-LRR class) family	608	0
AT1G56540	Disease resistance protein (TIR-NBS-LRR class) family	607	0
AT3G04220	Disease resistance protein (TIR-NBS-LRR class) family	603	0
AT1G63730	Disease resistance protein (TIR-NBS-LRR class) family	601	0
AT1G69550	Disease resistance protein (TIR-NBS-LRR class) family	599	0
AT1G63750	Disease resistance protein (TIR-NBS-LRR class) family	593	0
AT1G56520	Disease resistance protein (TIR-NBS-LRR class) family	586	0
AT1G56520	Disease resistance protein (TIR-NBS-LRR class) family	586	0
AT3G44400	Disease resistance protein (TIR-NBS-LRR class) family	583	0
AT3G44400	Disease resistance protein (TIR-NBS-LRR class) family	583	0
AT5G17680	Disease resistance protein (TIR-NBS-LRR class) family	579	0
AT1G63860	Disease resistance protein (TIR-NBS-LRR class) family	563	0
AT1G63860	Disease resistance protein (TIR-NBS-LRR class) family	563	1.00E-180
AT1G63740	Disease resistance protein (TIR-NBS-LRR class) family	549	2.00E-175
AT5G51630	Disease resistance protein (TIR-NBS-LRR class) family	547	2.00E-172
AT5G38350	Disease resistance protein (TIR-NBS-LRR class) family	499	3.00E-158
AT5G46490	Disease resistance protein (TIR-NBS-LRR class) family	440	1.00E-142
AT1G63750	Disease resistance protein (TIR-NBS-LRR class) family	449	4.00E-138
AT1G63750	Disease resistance protein (TIR-NBS-LRR class) family	449	4.00E-138
AT4G12010	Disease resistance protein (TIR-NBS-LRR class) family	407	1.00E-120
AT4G16990	RLM3 | Disease resistance protein (TIR-NBS-LRR class) family	385	5.00E-118
AT4G19500	Nucleoside-triphosphatase/transmembrane receptor/nucleotide binding/ATP binding protein	399	3.00E-117
AT4G16990	RLM3 | Disease resistance protein (TIR-NBS-LRR class) family	384	2.00E-116
AT1G72840	Disease resistance protein (TIR-NBS-LRR class) family	382	4.00E-113
AT5G48770	Disease resistance protein (TIR-NBS-LRR class) family	385	5.00E-113
AT3G04210	Disease resistance protein (TIR-NBS-LRR class) family	365	1.00E-112
AT1G72840	Disease resistance protein (TIR-NBS-LRR class) family	383	2.00E-112
AT1G17600	Disease resistance protein (TIR-NBS-LRR class) family	366	2.00E-107
AT1G66090	Disease resistance protein (TIR-NBS-LRR class) family	332	5.00E-102
AT4G19510	Disease resistance protein (TIR-NBS-LRR class) family	343	3.00E-99
AT3G05960	ATSTP6, STP6 | Sugar transporter 6	327	4.00E-99
AT5G36930	Disease resistance protein (TIR-NBS-LRR class) family	332	1.00E-94
AT5G36930	Disease resistance protein (TIR-NBS-LRR class) family	332	2.00E-94
AT5G40100	Disease resistance protein (TIR-NBS-LRR class) family	328	2.00E-94
AT4G36140	Disease resistance protein (TIR-NBS-LRR class) family	332	5.00E-93
AT5G45200	Disease resistance protein (TIR-NBS-LRR class) family	325	6.00E-92
AT3G51560	Disease resistance protein (TIR-NBS-LRR class) family	322	5.00E-91
AT1G72860	Disease resistance protein (TIR-NBS-LRR class) family	320	8.00E-91
AT4G19510	Disease resistance protein (TIR-NBS-LRR class) family	316	4.00E-89
AT1G27170	Transmembrane receptors; ATP binding	318	6.00E-89
AT5G26250	Major facilitator superfamily protein	298	1.00E-88
AT1G27180	Disease resistance protein (TIR-NBS-LRR class) family	318	1.00E-88
AT4G19520	Disease resistance protein (TIR-NBS-LRR class) family	316	1.00E-87
AT5G17880	CSA1 |Disease resistance protein (TIR-NBS-LRR class) family	303	7.00E-85
AT5G45050	TTR1, ATWRKY16, WRKY16 |Disease resistance protein (TIR-NBS-LRR class) family	301	9.00E-84

3.7 Figures

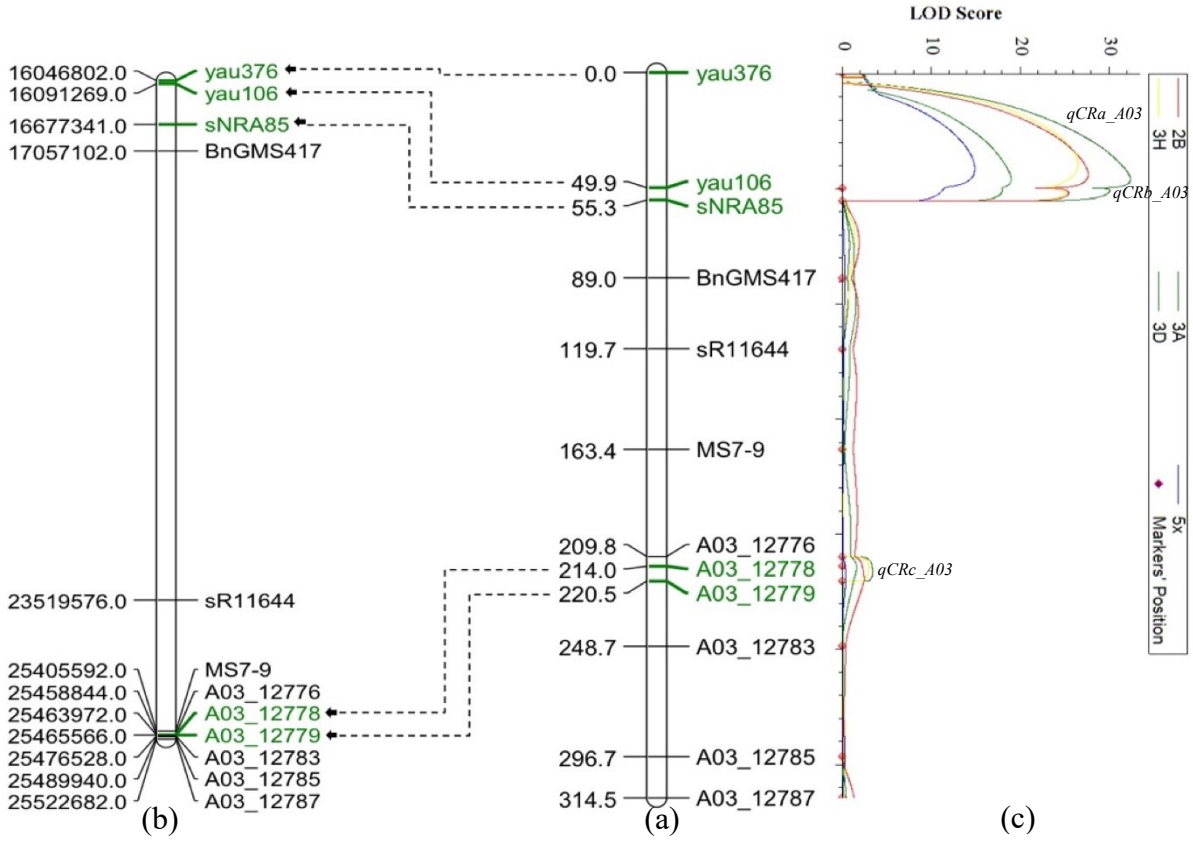

Figure 3.1 (a) A genetic linkage map of A03 constructed by using 12 SSR markers, (b) a partial physical map of chromosome A03 based on the positions of 12 SSR markers on *Brassica rapa* cv. Chiifu-401-42 whole-genome assembly version 3.5, and (c) likelihood profile of the QTL associated with clubroot resistance where the x and y axes represent the LOD value and map distance, respectively. Marker names are shown on the right side of both physical and linkage maps. The physical (bp) and genetic (cM) positions of the maps are presented on the left side of the maps. *qCRa_A03*, *qCRb_A03* and *qCRc_A03* are the QTL detected in this study.

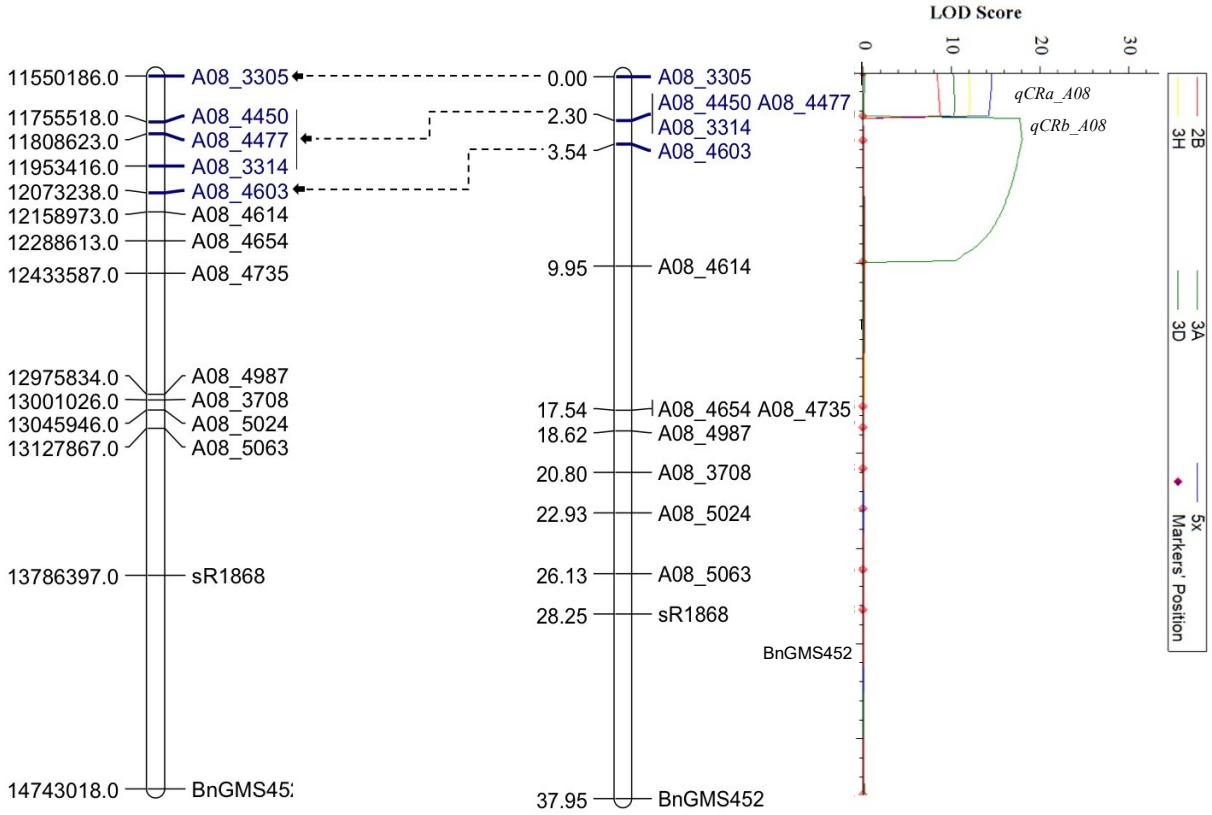

Figure 3.2 (a) A genetic linkage map of chromosome A08 constructed based on the genetic distance of 14 SSR markers identified in this study, (b) a partial physical map constructed by using the physical locations of 14 SSR markers on the chromosome A08 of *Brassica rapa* cultivar Chiifu-401-42 whole-genome assembly version 3.5, and (c) a QTL likelihood profile illustrated by QTL IciMappig. Marker names are shown on the right side of both physical and linkage maps; the physical location (bp) and genetic distance (cM) are indicated on the left side of the physical map and linkage map, respectively; *qCRa_A08* and *qCRb_A08* are the clubroot resistance loci identified in this study; the LOD score and map distance are shown on the x and y axes, respectively.

4 Chapter 4: General discussion and conclusions

4.1 General discussion

Clubroot disease, caused by *Plasmodiophora brassicae*, is one of the most serious threats to *Brassica napus* canola production in Canada (Braselton 1995; Pageau et al. 2006; for review, see Howard et al. 2010). The spreading of clubroot disease from field to field occurs rapidly. Since the first identification of clubroot in canola fields in central Alberta in 2003 (Tewari et al. 2005), this disease has been reported in additional canola fields in Alberta (Strelkov et al. 2007, 2021), Saskatchewan (Dokken-Bounchard et al. 2012), Manitoba (McLaren et al. 2014), and Ontario (Al-Daoud et al. 2018). In Canada, clubroot disease causes 30-100% yield loss in canola under moderate to heavy infestation conditions (Tewari et al. 2005; Pageau et al. 2006). *Plasmodiophora brassicae* is a fungus-like, soil-borne pathogen, which can survive in the soil and remain virulent as long-lasting resting spores for more than 17 years (Wallenhammar 1996). The traditional management methods such as cultural practices, as well as chemical and biological treatments are either not efficient or expensive for controlling clubroot disease in commercial canola fields. Therefore, growing of clubroot-resistant cultivars together with appropriate cultural practices, such as crop rotation and sanitation of field equipment are required for sustainable management of this disease (for review, see Hasan et al. 2021a).

To date, more than 20 major clubroot resistance loci and several quantitative trait loci (QTL) have been identified on the chromosomes of *B. napus*, *B. rapa*, *B. oleracea*, and *B. nigra* (for review, see Hasan et al 2021a). However, due to the emergence of new virulent pathotypes of *P. brassicae*, the available genetic resistances may become ineffective in a short period of time (Strelkov et al. 2016, 2021; Askarian et al. 2021; Hollman et al. 2021). Accumulation of multiple clubroot resistance genes through marker-assisted selection (MAS) has been reported to improve

resistance to multiple pathotypes in *B. napus* (Shah et al. 2019). Therefore, more efforts needed to identify additional clubroot resistance genes in different *Brassica* resources for broadening the genetic base of clubroot resistance genes in *B. napus* canola.

The European fodder turnips (*B. rapa* subsp. *rapifera*) and rutabaga (*B. napus* var. *napobrassica*) have been reported to carry excellent resistance to different pathotypes of *P. brassicae* (Ayers and Lelacheur 1972; Hasan et al. 2012; Peng et al. 2014). Among these, the clubroot resistance of rutabaga can be introgressed into *B. napus* canola without much difficulty as both belong to the same species. This thesis research was carried out to investigate the clubroot resistance in two advanced-generation breeding populations of spring *B. napus* canola carrying resistance of the *B. rapa* European fodder turnip accession ECD 01 (cv. Debra) and rutabaga cv. Polycross. The *B. napus* population clubroot resistance of the rutabaga cv. Polycross was further used to map the clubroot resistance loci and identify molecular markers for clubroot resistance.

B. rapa (AA), one of the diploid progenitor species of the allopolyploid species *B. napus* (AACC), is a valuable source for broadening the genetic base of the A-genome of *B. napus* through *B. napus* × *B. rapa* interspecific hybridization (Leflon et al. 2006; Allender and King 2010; Chu et al. 2014; Attri and Rahman 2018; Hasan et al. 2021b). Previous studies have identified at least 24 major clubroot resistance loci in different forms of *B. rapa*, including the European fodder turnips and Chinese cabbage (*B. rapa* ssp. *chinensis* and *pekinensis*) (for review, see Hasan et al. 2021a). Hirani et al. (2018) mapped two independent dominant loci on A03 and one locus on A08 in the European clubroot differential (ECD) set, where ECD 01 carrying two of the three loci showed resistance to multiple field isolates of *P. brassicae*. Two major loci *Rcr10* ^{ECD01} and *Rcr9* ^{ECD01} were subsequently identified and mapped to chromosomes A03 and A08, respectively, by Yu et al. (2022). In this study, a pathotype 3H-resistant BC_1F_8 *B. napus* population derived from

B. napus × *B. rapa* turnip ECD 01 (cv. Debra) interspecific cross was accessed for resistance to *P. brassicae* pathotypes 2B, 3A, 5x (L-G1) and 3H found in canola fields in Canada. Amongst the 68 BC_1F_8 *B. napus* lines, 10 (14.7%) were resistant to all four pathotypes and 29 (42.6%) were resistant to pathotype 3H but susceptible to the rest three pathotypes (Figure 2.1D). The clubroot resistance test results indicated that resistance to multiple *P. brassicae* pathotypes such as 2B, 3A, 5x (L-G1), and 3H can be introgressed from ECD 01 into spring *B. napus* canola through *B. napus* × *B. rapa* interspecific hybridization. Furthermore, the results from PCA and correlation analysis of the BC_1F_8 lines demonstrated that the same gene or multiple genes from the same genomic region might be involved in the control of resistance to pathotypes 2B, 3A, and 5x, while resistance to pathotype 3H could be under a different genetic control. However, Yu et al. (2022) reported that both *Rcr10* ^{ECD01} and *Rcr9* ^{ECD01} were responsible for resistance to pathotypes 3A, 3H, and 3D, while *Rcr9* ^{ECD01} conferred resistance to pathotype 5x. Additional research will be needed to understand and identify the genetic control of clubroot resistance in *B. napus* introgressed from *B. rapa* turnip ECD 01 for resistance to multiple *P. brassicae* pathotypes.

Rutabaga carries the same genome as *B. napus* canola; this is an excellent genetically diverse material for genetic improvement of clubroot resistance, agronomic, as well as seed quality traits in *B. napus* canola (Shiranifar et al. 2020, 2021; Hasan et al. 2021b). Resistance to different isolates of *P. brassicae* has been reported in several rutabaga cultivars (Spaner 2002; Hasan et al. 2012; Peng et al. 2014; Fredua-Agyeman et al. 2020b). The rutabaga cv. Brookfield has been reported to carry a minor QTL on A03 for resistance to pathotype 3H and a major locus on A08 for resistance to pathotypes 2 or 2F, 3 or 3H, 5 or 5I, 6 or 6M, and 8 or 8N (Hasan and Rahman 2016; Hasan et al. 2021b). Resistance to these pathotypes has also been reported in the rutabaga cv. Polycross (Hasan et al. 2012). In this thesis research, a doubled-haploid (DH) *B. napus*

population derived from the crosses involving rutabaga cv. Polycross in their pedigree was evaluated for resistance to *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D. Of the 106 DH *B. napus* lines, 14 (13.2%) were resistant to all five pathotypes and 37 (34.9%) were resistant to one to four pathotypes (Figure 2.4A). Resistance to all five pathotypes was positively correlated. Thus, the clubroot resistance of the rutabaga cv. Polycross can be used to broaden the genetic base of *B. napus* canola for clubroot resistance.

Simple sequence repeat (SSR) markers associated with clubroot resistance are excellent tools for mapping clubroot resistance in *B. napus* (Fredua-Agyeman and Rahman 2016; Hasan and Rahman 2016; Hasan et al. 2021b). In this thesis research, I used the *B. napus* DH population of 94 lines carrying clubroot resistance of the rutabaga cv. Polycross to map the major clubroot resistance loci through the construction of genetic linkage maps of the chromosomes. Three genomic regions (*qCRa_A03*, *qCRb_A03*, and *qCRc_A03*) were mapped on A03 by using 12 SSR markers, and two genomic regions (*qCRa_A08* and *qCRb_A08*) were mapped on A08 by using 14 SSR markers. Five SSR markers (yau 376, yau106, sNRA85, A03_12778, and A03_12779) from chromosome A03 and three markers (A08_3305, A08_4450, A08_3314, and A08_4603) from A08 were found to be co-segregating with clubroot resistance in this DH population. The loci *qCRa_A03* and *qCRb_A03*, located at about 16 Mb of A03 of *B. rapa* cv. Chiifu-401-42 whole-genome assembly version 3.5 (Zhang et al. 2022), could be a single major genomic region conferring resistance to *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D; this genomic region most likely corresponds the previously identified locus *CRd* reported by Pang et al. (2018) using *P. brassicae* race 4 or *Crr3* reported by Hirai et al. (2004) and Saito et al. (2006) using *P. brassicae* pathotype 2. The minor locus *qCRc_A03* which conferred resistance to pathotype 3H and 3D and positioned at about 25 Mb region of A03 is most likely the locus *Rcr2* reported by

Huang et al. (2017) using *P. brassicae* pathotype 3. In case of the A08 map, the locus *qCRa_A08* conferred resistance to pathotypes 2B, 5x (L-G1), 3H, and 3D, while *qCRa_A08* was involved in resistance to pathotype 3A; these two QTL were mapped at 11.3-12.7 Mb region of A08 of *B. rapa* cv. Chiifu-401-42 whole-genome assembly version 3.5 (Zhang et al. 2022); this region corresponds to *CRs* reported by Laila et al. (2019) using *P. brassicae* pathotype 4 or *Rcr3* reported by Karim et al. (2020) using *P. brassicae* pathotype 3 or *Crr1a* reported by Suwabe et al. (2003, 2006) and Hatakeyama et al. (2013) using *P. brassicae* pathotype 2 or *Rcr9* reported by Yu et al. (2017) and Karim et al. (2020) using *P. brassicae* pathotype 5x. These major clubroot resistance loci were identified by using different types of experimental materials, molecular markers and *P. brassicae* pathotypes; therefore, some of them could be a single locus reported by different researchers using different names. A comparative study using the above-mentioned experimental materials and the same set of single spore derived *P. brassicae* pathotypes as well as fine mapping of these resistances will be needed to determine the actual number of clubroot resistance loci present in these genomic regions and their roles in resistance to a specific pathotype.

4.2 Conclusions

The main conclusions drawn from this MSc thesis research include:

- Clubroot resistance of the *B. rapa* European fodder turnip accession ECD 01 (cv. Debra) can be used for the improvement of resistance to *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), and 3H in *B. napus* canola.
- The clubroot resistance of *B. napus* rutabaga cv. Polycross can be used to broaden the genetic base of *B. napus* canola for resistance to *P. brassicae* pathotypes 2B, 3A, 5x (L-G1), 3H, and 3D.

- The chromosomes A03 and A08 of the rutabaga cv. Polycross carry clubroot resistance loci.
- SSR markers co-segregating with clubroot resistance identified in this study can be used in MAS; however, recombination between the markers and resistance needs to be considered.

4.3 Future research

- Genetic analysis and molecular mapping need to be carried out using the *B. napus* BC_1F_8 population of *B. napus* × *B. rapa* interspecific cross to understand the genetic control of resistance which has been introgressed from *B. rapa* (turnip), and to identify molecular markers for resistance for use in the breeding of clubroot-resistant *B. napus* canola cultivars.
- Fine mapping of the A03 and A08 QTL regions of the rutabaga cv. Polycross introgressed into oilseed *B. napus* will be needed using a larger population to develop tightly linked markers for use in MAS as well as map-based cloning of the clubroot resistance genes.
- Transcriptomics and other 'omics' studies need to be conducted to identify the candidate genes in the QTL regions conferring resistance to different *P. brassicae* pathotypes and develop functional markers for MAS of clubroot-resistant *B. napus* canola cultivars.

Reference

Ahmed, H.U., Hwang, S.F., Strelkov, S.E., Gossen, B.D., Peng, G., Howard, R.J., and et al. 2011. Assessment of bait crops to reduce inoculum of clubroot (*Plasmodiophora brassicae*) of canola. Can. J. Plant Sci. **91**(3):545-551. doi:10.4141/cjps10200.

Akira, S., and Hemmi, H. 2003. Recognition of pathogen-associated molecular patterns by TLR Family. Immunol. Lett. **85**:85-95. doi:10.1016/S0165-2478(02)00228-6.

Alamery, S., Tirnaz, S., Bayer, P., Tollenaere, R., Chaloub, B., Edwards, D., and Batley, J. 2018. Genome-wide identification and comparative analysis of NBS-LRR resistance genes in *Brassica napus*. Crop Pasture Sci. **69**:72-93. doi:10.1071/CP17214.

Al-Daoud, F., Moran, M., Gossen, B., and Mcdonald, M.R. 2018. First report of clubroot (*Plasmodiophora brassicae*) on canola in Ontario. Can. J. Plant Pathol. **40**:96-99. doi:10.1080/07060661.2017.1393696.

Allender, C.J., and King, G.J. 2010. Origins of the amphiploid species *Brassica napus* L. investigated by chloroplast and nuclear molecular markers. BMC Plant Biol. **10**:54. doi:10.1186/1471-2229-10-54.

Asaduzzaman, M., Pratley, J.E., Luckett, D., Lemerle, D., and Wu, H. 2020. Weed management in canola (*Brassica napus* L): A review of current constraints and future strategies for Australia. Arch. Agron. Soil Sci. **66**(4):427-444. doi:10.1080/03650340.2019.1624726.

Askarian, H., Akhavan, A., Manolii, V.P., Cao, T., Hwang, S.F., and Strelkov, S.E. 2021. Virulence spectrum of single-spore and field isolates of *Plasmodiophora brassicae* able to overcome resistance in canola (*Brassica napus*). Plant Dis. **105**(1):43-52. doi:10.1094/PDIS-03-20-0471-RE.

Attri, R., and Rahman, H. 2018. Introgression of allelic diversity from genetically distinct variants of *Brassica rapa* into *Brassica napus* canola and inheritance of the *B. rapa* alleles. Crop Pasture Sci. **69**(1):94-106. doi:10.1071/cp17193.

Ayers, G.W. 1944. Studies on the life history of the club root organism, *Plasmodiophora brassicae*. Can. J. Res. **22**(4):143-149. doi:10.1139/cjr44c-012.

Ayers, G.W., and Lelacheur, K.E. 1972. Genetics of resistance in rutabaga to two races of *Plasmodiophora brassicae*. Can. J. Plant Sci. **52**:897-900. doi:10.4141/cjps72-155.

Barthet, V.J. 2016. Canola: Overview. *In* Reference Module in Food Science. Elsevier. 237-241. doi:10.1016/B978-0-08-100596-5.00029-9.

Benesty, J., Chen, J., Huang, Y., and Cohen, P.I. 2009. Pearson correlation coefficient. In Noise Reduction in Speech Processing. Springer, Heidelbeg, Berlin. 1-4. doi:10.1007/978-3-642-00296-0_5.

Bonnardeaux, J. 2007. Uses for canola meal. Department of Primary Industries and Regional Development, Western Australia, Perth. Report. Available from https://researchlibrary.agric.wa.gov.au/pubns/29/ [accessed 2 May, 2020].

Braselton, J.P. 1995. Current status of Plasmodiophorids. Critic. Rev. Microbiol. **21**(4):263-275. doi:10.3109/10408419509113543.

Bro, R., and Smilde, A.K. 2014. Principal component analysis. Anal. Methods. **9**:2812-2831. doi:10.1039/c3ay41907j.

Buczacki, S.T., Toxopeus, H., Mattusch, P., Johnston, T.D., Dixon, G.R., and Hobolth, L.A. 1975. Study of physiologic specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. Trans. Brit. Mycol. Soc. **65**(2):295-303. doi:10.1016/S0007-1536(75)80013-1.

Burton, W., Salisbury, P., Males, D., and Potts, D. 2019. "Dune" - the first canola quality *Brassica juncea* (*Juncea* canola) cultivar and future *Juncea* canola research priorities for Australia. Paper presented at the 15th Australian research assembly on *Brassicas*, Geralton, Western Australia.10-14.

Bus, A., Körber, N., Snowdon, R.J., and Stich B. 2011. Patterns of molecular variation in a species-wide germplasm set of Brassica napus. Theor. Appl. Genet. **123**:1413-1423. doi:10.1007/s00122-011-1676-7.

CABI/EPPO. 2011. *Plasmodiophora brassicae*. [Distribution map]. *In* Distribution Maps of Plant Diseases, 4th edition. CABI Head Office, Wallingford, UK. doi:10.1079/DMPD/20113091531.

Canola Encyclopedia. 2021. Clubroot Disease. Available from https://www.canolacouncil.org/canola-encyclopedia/diseases/clubroot/#manage-clubroot-disease [accessed 4 June, 2022]

Cao, T., Manolii, V.P., Strelkov, S.E., Hwang, S.F., and Howard R.J. 2009. Virulence and spread of *Plasmodiophora brassicae* [clubroot] in Alberta, Canada. Can. J. Plant Pathol. **31**(3):321-329. doi:10.1080/07060660909507606.

Ce, F., Mei, J., He, H., Zhao, Y., Hu, W., Yu, F., and et al. 2021. Identification of candidate genes for clubroot-resistance in *Brassica oleracea* using quantitative trait loci-sequencing. Front. Plant Sci. **12**:703520. doi:10.3389/fpls.2021.703520.

Chalhoub B., Denoeud F., Liu S., Parkin, I.A.P., Tang, H., Wang, X., and et al. 2014. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Sci. **345**(6199):950-953. doi:10.1126/science.1253435.

Chang, A., Lamara, M., Wei, Y., Hu, H., Parkin, I.A.P., Gossen, B.D., and et al. 2019. Clubroot resistance gene *Rcr6* in *Brassica nigra* resides in a genomic region homologous to chromosome A08 in *B. rapa*. BMC Plant Biol. **19**:224. doi:10.1186/s12870-019-1844-5.

Chen, H., and Boutros, P.C. 2011. VennDiagram: A package for the generation of highly-customizable Venn and Euler diagrams in R. BMC Bioinf. **12**(1):35. doi:10.1186/1471-2105-12-35.

Chen, J., Jing, J., Zhan, Z., Zhang, T., Zhang, C., and Piao, Z. 2013. Identification of novel QTLs for isolate-specific partial resistance to *Plasmodiophora brassicae* in *Brassica rapa*. PLoS One. **8**(12):e85307. doi:10.1371/journal.pone.0085307.

Cheng, F., Liu, S., Wu, J., Fang, L., Sun, S., Liu, B., et al. 2011. BRAD, the genetics and genomics database for *Brassica* plants. BMC Plant Biol. **11**:136. doi:10.1186/1471-2229-11-136.

Cheng, X., Xu, J., Xia, S., Gu, J., Yang, Y., Fu, J., and et al. 2009. Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor. Appl. Genet. **118**:1121-1131. doi:10.1007/s00122-009-0967-8.

Choi, S.R., Oh, S.H., Chhapekar, S.S., Dhandapani, V., Lee, C.Y., Rameneni, J.J., and et al. 2020. Quantitative trait locus mapping of clubroot resistance and *Plasmodiophora brassicae* pathotype Banglim-specific marker development in *Brassica rapa*. Int. J. Mol. Sci. **21**(11):4157. doi:10.3390/ijms21114157.

Chu, M., Yu, F., Falk, K.C., Liu, X., Zhang, X., Chang, A., and et al. 2014. Identification of the clubroot resistance gene *Rpb1* and introgression of the resistance gene into canola breeding lines using a marker-assisted selection approach. Acta Hortic. **1005**:599-605. doi:10.17660/ActaHortic.2013.1005.74.

Churchill, G.A., and Doerge, R.W. 1994. Empirical threshold values for quantitative trait mapping. Genetics. **138**(3):963-71. doi:10.1093/genetics/138.3.963.

Dakouri, A., Zhang, X., Peng, G., Falk, K.C., Gossen, B.D., Strelkov, S.E., and et al. 2018. Analysis of genome-wide variants through bulked segregant RNA sequencing reveals a major gene for resistance to *Plasmodiophora brassicae* in *Brassica oleracea*. Sci. Rep. **8**:17657. doi:10.1038/s41598-018-36187-5.

Dangl, J.L., and Jones, J.D. 2001. Plant pathogens and integrated defence responses to infection. Nature. **411**(6839):826-33. doi:10.1038/35081161.

Daun, J., and Unger, E. 2016. Canola: Processing. In Reference Module in Food Science. Elsevier. 497-502. doi:10.1016/B978-0-12-394437-5.00236-9.

Diederichsen, E., Frauen, M., Linders, E.G.A., Hatakeyama, K., and Hirai, M. 2009. Status and perspectives of clubroot resistance breeding in crucifer crops. J. Plant Growth Regul. **28**:265-281. doi:10.1007/s00344-009-9100-0.

Dixon, G.R. 2009. The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. J. Plant Growth Regul. **28**:194-202. doi:10.1007/s00344-009-9090-y.

Dokken-Bouchard, F.L., Anderson, K., Bassendowski, K.A., Bouchard, A., Brown, B., Cranston, R., and et al. 2012. Survey of canola diseases in Saskatchewan, 2011. *In* 2012 The Canadian Phytopathological Society Canadian Plant Disease Survey: Disease Hightlights. Can. J. Plant Pathol. **92**(1):125-129.

Dolatabadian, A., Cornelsen, J., Huang, S., Zou, Z., and Fernando, W.G.D. 2021. Sustainability on the farm: breeding for resistance and management of major canola diseases in Canada contributing towards an IPM approach. Can. J. Plant Pathol. doi:10.1080/07060661.2021.1991480.

Downey K. 2021. The Canola Story. Available from https://www.saskcanola.com/about/story.php [accessed 1 December, 2021].

Dupont, J., White, P.J., Johnston, K.M., Heggtveit, H.A., McDonald, B.E., Grundy, S.M., and et al. 1989. Food safety and health effects of canola oil. J. Am. Co. Nutr. **8**(5):360-375. doi:10.1080/07315724.1989.10720311.

El-Esawi, M.A., Germaine, K., Bourke, P., and Malone, R. 2016. Genetic diversity and population structure of *Brassica oleracea* germplasm in Ireland using SSR markers. C. R. Biol. **339**(3-4):133-140. doi:10.1016/j.crvi.2016.02.002.

Farid, M., Yang, R.C., Kebede, B., and Rahman, H. 2020. Evaluation of *Brassica oleracea* accessions for resistance to *Plasmodiophora brassicae* and identification of genomic regions associated with resistance. Genome. **63**:91-101. doi:10.1139/gen-2019-0098.

Fei, W., Feng, J., Rong, S., Strelkov, S.E., Gao, Z., and Hwang, S.F. 2016. Infection and gene expression of the clubroot pathogen *Plasmodiophora brassicae* in resistant and susceptible canola cultivars. Plant Dis. **100**(4):824-828. doi:10.1094/PDIS-11-15-1255-RE.

Fredua-Agyeman, R., and Rahman, H. 2016. Mapping of the clubroot disease resistance in spring *Brassica napus* canola introgressed from European winter canola cv. 'Mendel'. Euphytica. **211**:201-213. doi:10.1007/s10681-016-1730-2.

Fredua-Agyeman, R., Jiang, J., Hwang, S.F., and Strelkov, S.E. 2020a. QTL mapping and inheritance of clubroot resistance genes derived from *Brassica rapa* subsp. *rapifera* (ECD 02) reveals resistance loci and distorted segregation ratios in two F2 populations of different crosses. Front. Plant Sci. **11**:899. doi:10.3389%2Ffpls.2020.00899.

Fredua-Agyeman, R., Yu, Z., Hwang, S.F., and Strelkov, S.E. 2020b. Genome-wide mapping of loci associated with resistance to clubroot in *Brassica napus* ssp. *napobrassica* (rutabaga) accessions from Nordic countries. Front. Plant Sci. **11**:742. doi:10.3389/fpls.2020.00742.

Friberg, H., Lagerlöf, J., and Rämert, B. 2005. Germination of *Plasmodiophora brassicae* resting spores stimulated by a non-host plant. Eur. J. Plant Pathol. **113**:275-281. doi:10.1007/s10658-005-2797-0.

Froese, R.D., Derksen, H., Gou, X., and McLaren, D.L. 2019. Monitoring and occurrence of clubroot in Manitoba in 2018. *In* The Canadian Phytopathological Society Canadian Survey: Disease Highlights 2019. Can. J. Plant Pathol. **42**:179. doi:10.1080/07060661.2020.1752524.

Fu, P., Piao, Y., Zhan, Z., Zhao, Y., Pang, W., Li, X., and Piao, Z. 2019a. Transcriptome arofile of *Brassica rapa* L. reveals the involvement of jasmonic acid, ethylene, and brassinosteroid signaling pathways in clubroot resistance. Agronomy. **9**(10):589. doi:10.3390/agronomy9100589.

Fu, Y., Zhang, Y., Mason, A. S., Lin, B., Zhang, D., Yu, H., and et al. 2019b. NBS-encoding genes in *Brassica napus* evolved rapidly after allopolyploidization and co-localize with known disease resistance loci. Front. Plant Sci. **10**:26. doi:10.3389/fpls.2019.00026.

Gavloski, J., Cárcamo, H., and Dosdall, L. 2011. Insects of canola, mustard, and flax in Canadian grasslands. *In* Arthropods of Canadian Grasslands: Inhabitants of a Changing Landscape. *Edited by* K.D. Floate. Biological Survey of Canada. **2**:181-214.

Ge, J.C., Kim, H.Y., Yoon, S.K., and Choi, N.J. 2018. Reducing volatile organic compound emissions from diesel engines using canola oil biodiesel fuel and blends. Fuel. **218**:266-274. doi:10.1016/j.fuel.2018.01.045.

Ge, J.C., Yoon, S.K., and Choi, N.J. 2017. Using canola oil biodiesel as an alternative fuel in diesel engines: A review. Appl. Sci. **7**(9):881. doi:10.3390/app7090881.

Ghobadi, S., Hassanzadeh-Rostami, Z., Mohammadian, F., Zare, M., and Faghih, S. 2018. Effects of canola oil consumption on lipid profile: A systematic review and meta-analysis of randomized controlled clinical trials. J. Am. Co. Nutr. **38**(2):185-196. doi:10.1080/07315724.2018.1475270.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. **43**:205-27. doi:10.1146/annurev.phyto.43.040204.135923.

Gómez-Campo, C., and Prakash, S. 1999. Origin and domestication. *In* Biology of *Brassica* Coenospecies. *Edited by* C. Gomez-Campo. Elsevier, Amsterdam. 33-58.

Gossen, B.D., Strelkov, S.E., Manolii, V.P., Rennie, D.C., Cao, T., Hwang, S.F., and et al. 2015. Spread of *Plasmodiophora brassicae* on canola in Canada, 2003-2014: Old pathogen, new home. Can. J. Plant Pathol. **37**(4):403-413. doi:10.1080/07060661.2015.1105871.

Hasan, M., Seyis, F., Badani, A.G., Pons-Kühnemann, J., Friedt, W., Lühs, W., and et al. 2006. Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet. Resour. Crop Evol. **53**:793-802. doi:10.1007/s10722-004-5541-2.

Hasan, M.J., Megha, S., and Rahman, H. 2021a. Clubroot in *Brassica*: recent advances in genomics, breeding, and disease management. Genome. **64**(8):735-760. doi:10.1139/gen-2020-0089.

Hasan, M.J., and Rahman, H. 2016. Genetics and molecular mapping of resistance to *Plasmodiophora brassicae* pathotypes 2, 3, 5, 6, and 8 in rutabaga (*Brassica napus* var. *napobrassica*). Genome. **59**:805-815. doi:10.1139/gen-2016-0034.

Hasan, M.J., and Rahman, H. 2018. Resynthesis of *Brassica juncea* for resistance to *Plasmodiophora brassicae* pathotype 3. Breed. Sci. **68**(3):385-391. doi:10.1139%2Fgen-2020-0089.

Hasan, M.J., Shaikh, R., Basu, U., and Rahman, H. 2021b. Mapping clubroot resistance of *Brassica rapa* introgressed into *Brassica napus* and development of molecular markers for the resistance. Crop Sci. **6**:4112-4127. doi:10.1002/csc2.20626.

Hasan, J., Shaikh, R., Megha, S., Herrmann, D. T., Kebede, B., and Rahman, H. 2021c. Mapping of flowering time, seed quality and clubroot resistance in rutabaga × spring canola populations and their association. Euphytica. **217**:106. doi:10.1007/s10681-021-02889-7.

Hasan, M.J., Strelkov, S.E., Howard, R.J., and Rahman, H. 2012. Screening of *Brassica* germplasm for resistance to *Plasmodiophora brassicae* pathotypes prevalent in Canada for broadening diversity in clubroot resistance. Can. J. Plant Sci. **92**:501-515. doi:10.4141/cjps2010-006.

Hatakeyama, K., Niwa, T., Kato, T., Ohara, T., Kakizaki, T., and Matsumoto, S. 2017. The tandem repeated organization of NB-LRR genes in the clubroot-resistant *CRb* locus in *Brassica rapa* L. Mol. Genet. Genomics. **292**(2):397-405. doi:10.1007/s00438-016-1281-1.

Hatakeyama, K., Suwabe, K., Tomita, R.N., Kato, T., Nunome, T., Fukuoka, H., and et al. 2013. Identification and characterization of *Crr1a*, a gene for resistance to clubroot disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. PLoS One. **8**(1):e54745. doi:10.1371/journal.pone.0054745.

Hayashida, N., Takabatake, Y., Nakazawa, N., Aruga, D., Nakanishi, H., Taguchi, G., and et al. 2008. Construction of a practical SCAR marker linked to clubroot resistance in Chinese

cabbage, with intensive analysis of *HC352b* genes. J. Jpn. Soc. Hortic. Sci. **77**:150-154. doi:10.2503/JJSHS1.77.150.

Hejna, O., Havlickova, L., He, Z., Bancroft, I., and Curn, V. 2019. Analysing the genetic architecture of clubroot resistance variation in *Brassica napus* by associative transcriptomics. Mol. Breeding. **39**:112. doi:10.1007/s11032-019-1021-4.

Hirai, M., Harada, T., Kubo, N., Tsukada, M., Suwabe, K., and Matsumoto, S. 2004. A novel locus for clubroot resistance in *Brassica rapa* and its linkage markers. Theor. Appl. Genet. **108**(4):639-643. doi:10.1007/s00122-003-1475-x.

Hirani, A.H., Gao, F., Liu, J., Fu, G., Wu, C., McVetty, P.B.E., and et al. 2018. Combinations of independent dominant loci conferring clubroot resistance in all four turnip accessions (*Brassica rapa*) from the European clubroot differential set. Front. Plant Sci. **9**:1628. doi:10.3389/fpls.2018.01628.

Hirani, A.H., and Li, G. 2015. Understanding the genetics of clubroot resistance for effectively controlling this disease in *Brassica* Species. *In* Plants for the Future. *Edited by* H. El-Shemy. doi:10.5772/60936.

Hollman, K.B., Hwang, S.F., Manolii, V.P., and Strelkov, S.E. 2021. Pathotypes of *Plasmodiophora brassicae* collected from clubroot resistant canola (*Brassica napus* L.) cultivars in western Canada in 2017-2018. Can. J. Plant Pathol. **43**(4):622-630. doi:10.1080/07060661.2020.1851893.

Horiuchi, S., and Hori, M. 1980. A simple greenhouse technique for obtaining high levels of clubroot incidence. Bull. Chugoku Natl. Agric. Exp. Stn. E. **17**:33-55.

Howard, R.J., Strelkov, S.E., and Harding, M.W. 2010. Clubroot of cruciferous crops – new perspectives on an old disease. Can. J. Plant Pathol. **32**(1):43-57. doi:10.1080/07060661003621761.

Huang, Z., Peng, G., Gossen, B.D., and Yu, F. 2019. Fine mapping of a clubroot resistance gene from turnip using SNP markers identified from bulked segregant RNA-Seq. Mol. Breeding. **39**:131. doi:10.1007/s11032-019-1038-8.

Huang, Z., Peng, G., Liu, X., Deora, A., Falk, K.C., Gossen, B.D., and et al. 2017. Fine mapping of a clubroot resistance gene in Chinese cabbage using SNP markers identified from bulked segregant RNA sequencing. Front. Plant Sci. **8**:1448. doi:10.3389/fpls.2017.01448.

Huhtanen, P., Hetta, M., and Swensson, C. 2011. Evaluation of canola meal as a protein supplement for dairy cows: A review and a meta-analysis. Can. J. Anim. Sci. **91**:529-543. doi:10.4141/cjas2011-029.

Hwang, S.F., Ahmed, H.U., Strelkov, S.E., Zhou, Q., Gossen, B.D., McDonald, M.R., and et al. 2017. Suppression of clubroot by dazomet fumigant. Can. J. Plant Sci. **98**(1):172-182. doi:10.1139/cjps-2017-0099.

Hwang, S.F., Ahmed, H.U., Zhou, Q., Fu, H., Turnbull, G.D., Fredua-Agyeman, R., Strelkov, S.E., Gossen, B.D., and Peng, G. 2019. Influence of resistant cultivars and crop intervals on clubroot of canola. Can. J. Plant Sci. **99**(6):862-872. doi:10.1139/cjps-2019-0018.

Hwang, S.F., Ahmed, H.U., Zhou, Q., Turnbulla, G.D., Strelkov, S.E., Gossen, B.D., and et al. 2013. Effect of susceptible and resistant canola plants on *Plasmodiophora brassicae* resting spore populations in the soil. Plant Pathol. **64**:1198–1206. doi:10.1111/ppa.12347.

Hwang, S.F., Howard, R.J., Strelkov, S.E., Gossen, B.D., and Peng, G. 2014. Management of clubroot (*Plasmodiophora brassicae*) on canola (*Brassica napus*) in western Canada. Can. J. Plant Pathol. **36**:49-65. doi:10.1080/07060661.2013.863806.

Hwang S.F., Strelkov, S.E., Feng J., Gossen, B.D., and Howard, R.J. 2011a. *Plasmodiophora brassicae*: A review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop. Mol. Plant Pathol. **13**(2):105-113. doi:10.1111/j.1364-3703.2011.00729.x

Hwang, S.F., Strelkov, S.E., Gossen, B.D., Turnbull, G.D., Ahmed, H.U., and Manolii, V.P. 2011b. Soil treatments and amendments for amelioration of clubroot of canola. Can. J. Plant Sci. **6**:999-1010. doi:10.4141/cjps2011-028.

Iftikhar, R., Wang, X. and Rahman, H. 2018. Broadening the genetic base of *Brassica napus* canola by interspecific crosses with different variants of *B. oleracea*. Euphytica. **214**:133. doi:10.1007/s10681-018-2213-4.

Jabeen, N. 2020. Agricultural, Economic and Societal Importance of Brassicaceae Plants. *In* The Plant Family Brassicaceae. *Edited by* M. Hasanuzzaman. Springer, Singapore. doi:10.1007/978-981-15-6345-4_2.

Jahreis, G., and Schäfer, U. 2011. Rapeseed (*Brassica napus*) oil and its benefits for human health. *In* Nuts and Seeds in Health and Disease Prevention. *Edited by* V.R. Preedy, R.R. Watson and V.B. Patel. 967-974. doi:10.1016/B978-0-12-375688-6.10114-8.

Kageyama, K., and Asano, T. 2009. Life cycle of *Plasmodiophora brassicae*. J. Plant Growth Regul. **28**(3):203-211. doi:10.1007/s00344-009-9101-z.

Kalia, R.K., Rai, M.K., Kalia, S., Singh, R., and Dhawan, A.K. 2011. Microsatellite markers: An overview of the recent progress in plants. Euphytica. **177**:309-334. doi:10.1007/s10681-010-0286-9.

Karim, M.M., Dakouri, A., Zhang, Y., Chen, Q., Peng, G., Strelkov, S.E., and et al. 2020. Two clubroot-resistance genes, *Rcr3* and *Rcr9*^{wa}, mapped in *Brassica rapa* using bulk segregant RNA sequencing. Int. J. Mol. Sci. **21**(14):5033. doi:10.3390/ijms21145033.

Kato, T., Hatakeyama, K., Fukino, N., and Matsumoto, S. 2013. Fine mapping of the clubroot resistance gene *CRb* and development of a useful selectable marker in *Brassica rapa*. Breed. Sci. **63**(1):116-124. doi:10.1270/jsbbs.63.116.

Knutsen, H.K., Alexander J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., and et al. 2016. Erucic acid in feed and food. EFSA J. **14**(11):e04593. doi:10.2903/j.efsa.2016.4593.

Kosambi, D.D. 1944. The estimation of map distances from recombination values. Ann. Eugen. **12**:172-175. doi:10.1111/j.1469-1809.1943.tb02321.x.

Kuginuki, Y., Ajisaka, H., Yui, M., Hida, K., and Hirai, M. 1997. RAPD markers linked to a clubroot-resistance locus in *Brassica rapa* L. Euphytica. **98**:149-154. doi:10.1023/A:1003147815692.

Kutcher, H.R., Brandt, S.A., Smith, E.G., Ulrich, D., Malhi, S.S., and Johnston, A.M. 2013. Blackleg disease of canola mitigated by resistant cultivars and four-year crop rotations in western Canada. Can. J. Plant Pathol. **35**(2):209-221. doi:10.1080/07060661.2013.775600.

Kutcher, H.R., Warland, J.S., and Brandt, S.A. 2010. Temperature and precipitation effects on canola yields in Saskatchewan, Canada. Agric. For Meteorol. **150**(2):161-165. doi:10.1016/j.agrformet.2009.09.011.

Laila, R., Park, J.I., Robin, A.H.K., Natarajan, S., Vijayakumar, H., Shirasawa, K., and et al. 2019. Mapping of a novel clubroot resistance QTL using ddRAD-seq in Chinese cabbage (*Brassica rapa* L.). BMC Plant Biol. **19**(1):13. doi:10.1186/s12870-018-1615-8.

Lander, E., and Kruglyak, L. 1995. Genetic dissection of complex traits: Guidelines for interpreting and reporting linkage results. Nat. Genet. **11**:241-247. doi:10.1038/ng1195-241.

Lee, G.P., Baek, N.K., and Park, K.W. 2002. Genetic mapping of resistant genes in *Brassica pekinensis* against *Plasmodiophora brassicae* race 6. Plant Pathol. J. **18**(5):266-270. doi:10.5423/ppj.2002.18.5.266.

Lee, B.T., Barber, G.P., Benet-Pagès, A., Casper, J., Clawson, H., Diekhans, M., and et al. 2022. The UCSC Genome Browser database: 2022 update. Nucleic Acids Res. **50**(D1):D1115-D1122. doi:10.1093/nar/gkab959.

Lee, J., Izzah, N.K., Choi, B., Joh, H.J., Lee, S., Perumal, S., and et al. 2016. Genotyping-by-sequencing map permits identification of clubroot resistance QTLs and revision of the reference genome assembly in cabbage (*Brassica oleracea* L.), DNA Res. **23**(1):29-41. doi:10.1093/dnares/dsv034.

Leeson, S., Atteh, J.O., and Summers, J.D. 1987. The replacement value of canola meal for soybean meal in poultry diets. Can. J. Anim. Sci. **67**(1):151-158. doi:10.4141/cjas87-017.

Leflon, M., Eber, F., Letanneur, J.C., Chelysheva, L., Coriton, O., Huteau, V., and et al. 2006. Pairing and recombination at meiosis of *Brassica rapa* (AA) × *Brassica napus* (AACC) hybrids. Theor. Appl. Genet. **113**:1467-1480. doi:10.1007/s00122-006-0393-0.

Liao, J., Luo, L., Zhang, L., Wang, L., Shi, X., Yang, H., and et al. 2021. Comparison of the effects of three fungicides on clubroot disease of tumorous stem mustard and soil bacterial community. J. Soils Sediments. **22**: 256-271. doi:10.1007/s11368-021-03073-z.

Lin, L., Allemekinders, H., Dansby, A., Campbell, L., Durance-Tod, S., Berger, A., and et al. 2013. Evidence of health benefits of canola oil. Nutr. Rev. **71**(6):370-85. doi:10.1111/nure.12033.

Liu, L., Qin, L., Cheng, X., Zhang Y, Xu, L., Liu, F., and et al. 2020. Comparing the infection biology of *Plasmodiophora brassicae* in clubroot susceptible and resistant hosts and non-hosts. Front. Microbiol. **11**:507036. doi:10.3389/fmicb.2020.507036.

Liu, L., Qin, L., Zhou, Z., Hendriks, W.G.H.M., Liu, S., and Wei, Y. 2020. Refining the life cycle of *Plasmodiophora brassicae*. Phytopathology. **110**:1704-1712. doi:10.1094/phyto-02-20-0029-r.

Liu, X., Kris-Etherton, P.M., West, S.G., Lamarche, B., Jenkins, D.J., Fleming, J.A., and et al. 2016. Effects of canola and high-oleic-acid canola oils on abdominal fat mass in individuals with central obesity. Obesity. **24**(11):2261-2268. doi:10.1002/oby.21584.

Liu, Y., Xu, A., Liang, F., Yao, X., Wang, Y., Liu, X., and et al. 2018. Screening of clubroot-resistant varieties and transfer of clubroot resistance genes to *Brassica napus* using distant hybridization. Breed Sci. **68**:258-267. doi:10.1270/jsbbs.17125.

LMC International. 2020. The Economic Impact of Canola on the Canadian Economy: 2020 Update. Available from https://www.canolacouncil.org/download/131/economic-impact/17818/economic-impact-report-canada_december-2020 [accessed 2 October 2021].

Lu, K., Wei, L., Li, X., Wang, Y., Wu, J., Liu, M., and et al. 2019. Whole-genome resequencing reveals *Brassica napus* origin and genetic loci involved in its improvement. Nat. Commun. **10**:1154. doi:10.1038/s41467-019-09134-9.

Manichaikul, A., Moon, J.Y., Sen, S., Yandell, B.S., and Broman, K.W. 2009. A model selection approach for the identification of quantitative trait loci in experimental crosses, allowing epistasis. Genetics. **181**(3):1077-1086. doi:10.1534/genetics.108.094565.

Manzanares-Dauleux, M., Delourme, R., Baron, F., and Thomas, G. 2000. Mapping of one major gene and of QTLs involved in resistance to clubroot in *Brassica napus*. Theor. Appl. Genet. **101**:885-891. doi:10.1007/s001220051557.

Matsumoto, E., Ueno, H., Aruga, D., Sakamoto, K., and Hayashida, N. 2012. Accumulation of three clubroot resistance genes through marker-assisted selection in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). J. Japanese Society Hort. Sci. **2**:184-190. doi:10.2503/jjshs1.81.184.

McGrann, G.R.D., Gladders, P., Smith, J.A., and Burnett, F.J. 2016. Control of clubroot (*Plasmodiophora brassicae*) in oilseed rape using varietal resistance and soil amendments. Field Crops Res. **186**:146-156. doi:10.1016/j.fcr.2015.11.013.

McHale, L., Tan, X., Koehl, P., and Michelmore, R.W. 2006. Plant NBS-LRR proteins: adaptable guards. Genome Biol. **7**:212. doi:10.1186/gb-2006-7-4-212.

McKeon, T.A., Hayes, D.G., Hildebrand, D.F., and Weselake, R.J. 2016. Introduction to Industrial Oil Crops. *In* Industrial Oil Crops. Edited by T.A. McKeon, D.G. Hayes, D.F. Hildebrand and R.J. Weselake. 1-13. doi:10.1016/B978-1-893997-98-1.00001-4.

McLaren, D.L., Platford, R.G., Kubinec, A., Derksen, H., Bargen, E., Brackenreed, and et al. 2014. Survey of canola diseases in Manitoba in 2013. *In* 2014 The Canadian Phytopathological Society Canadian Plant Disease Survey: Disease Highlights. Can. J. Plant Pathol. **94**(1):180-183.

McVetty, P.B.E., and Duncan, R.W. 2015. Canola, rapeseed, and mustard: For biofuels and bioproducts. *In* Handbook of Plant Breeding. *Edited by* V.M.V. Cruz and D.A. Dierig. Springer, New York, New York. **9**:133-156. doi:10.1007/978-1-4939-1447-0_7.

McVetty, P., and Duncan, R. 2016. Canola/rapeseed: Genetics and breeding. *In* Reference Module in Food Science. Elsevier. 311-316. doi:10.1016/B978-0-08-100596-5.00209-2.

Mejicanos, G., Sanjayan, N., Kim, I.H., and Nyachoti, C.M. 2016. Recent advances in canola meal utilization in swine nutrition. J. Anim. Sci. Technol. **58**:7. doi:10.1186/s40781-016-0085-5.

Mendham, N. J., and Robertson, M. J. 2016. Canola: Agronomy. *In* Encyclopedia of Food Grains, 2nd edition. *Edited by* C. Wrigley, H. Corke, and J. Faubion. Elsevier. **4**:207-215. doi:10.1016/B978-0-12-394437-5.00235-7.

Meng, L., Li, H., Zhang, L., and Wang, J. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. Crop J. **3**(3):269-283. doi:10.1016/j.cj.2015.01.001.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H., and Michelmore, R.W. 2003. Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. Plant cell. **15**(4):809-834. doi:10.1105/tpc.009308.

Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., and et al. 2018. DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. Biotechnol. Biotechnol. Equip. **32**(2):261-285. doi:10.1080/13102818.2017.1400401.

Nagaoka, T., Doullah, M.A.U., Matsumoto, S. Kawasaki, S., Ishikawa, T., Hori, H., and et al. 2010. Identification of QTLs that control clubroot resistance in *Brassica oleracea* and comparative analysis of clubroot resistance genes between *B. rapa* and *B. oleracea*. Theor. Appl. Genet. **120**:1335-1346. doi:10.1007/s00122-010-1259-z.

Nguyen, M.L., Monakhos, G.F., Komakhin, R.A., and Monakhos, S.G. 2018. The new clubroot resistance locus is located on chromosome A05 in Chinese cabbage (*Brassica rapa* L.). Russ. J. Genet. **54**(3):296-304. doi:10.1134/S1022795418030080.

Nikzad, A., Kebede, B., Pinzon, J., Bhavikkumar, J., Wang, X., Yang, R. C., and et al. 2020. Potential of the C genome of the different variants of *Brassica oleracea* for heterosis in spring *B. napus* Canola. Front. Plant Sci. **10**:1691. doi:10.3389/fpls.2019.01691.

Pageau, D., Lajeunesse, J., and Lafond, J. 2006. Impact de l'hernie des crucifères [*Plasmodiophora brassicae*] sur la productivité et la qualité du canola. Can. J. Plant Pathol. **28**(1):137-143. doi:10.1080/07060660609507280.

Pang, W., Fu, P., Li, X., Zhan, Z., Yu, S., and Piao, Z. 2018. Identification and mapping of the clubroot resistance gene *CRd* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Front. Plant Sci. doi:10.3389/fpls.2018.00653.

Peng, G., Falk, K.C., Gugel, R.K., Franke, C., Yu, F., James, B., and et al. 2014a. Sources of resistance to *Plasmodiophora brassicae* (clubroot) pathotypes virulent on canola. Can J. Plant Pathol. **36**(1):89-99. doi:10.1080/07060661.2013.863805.

Peng, G., Lahlali, R., Hwang, S.F., Pageau, D., Hynes, R.K., Mcdonald, M.R., and et al. 2014b. Crop rotation, cultivar resistance, and fungicides/biofungicides for managing clubroot (*Plasmodiophora brassicae*) on canola. Can. J. Plant Pathol. **36**:99-112. doi:10.1080/07060661.2013.860398.

Peng G., McGregor L., Lahlali R., Gossen, B.D., Hwang, S.F., Adhikari, K.K., and et al. 2011. Potential biological control of clubroot on canola and crucifer vegetable crops. Plant Pathol. **60**:566-574. doi:10.1111/j.1365-3059.2010.02400.x.

Peng, L., Zhou, L., Li, Q., Wei, D., Ren, X., Song, H., and et al. 2018. Identification of quantitative trait loci for clubroot resistance in *Brassica oleracea* with the use of *Brassica* SNP microarray. Front. Plant Sci. **9**:822. doi:10.3389/fpls.2018.00822.

Piao, Z.Y., Deng, Y.Q., Choi, S. R., Park, Y.J., and Lim, Y.P. 2004. SCAR and CAPS mapping of CRb, a gene conferring resistance to *Plasmodiophora brassicae* in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Theor. Appl. Genet. **108**(8):1458-1465. doi:10.1007/s00122-003-1577-5.

Piao, Z., Ramchiary, N., and Lim, Y.P. 2009. Genetics of clubroot resistance in *Brassica* species. J. Plant Growth Regul. **28**:252-264. doi:10.1007/s0034-009-9093-8.

Powell, W., Machray, G.C., and Provan J. 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Sci. **1**(7):215-222. doi:10.1016/1360-1385(96)86898-1.

Prieto, M.A., López, C.J., and Simal-Gandara, J. 2019. Glucosinolates: Molecular structure, breakdown, genetic, bioavailability, properties and healthy and adverse effects. Adv. Food Nutr. **90**:305-350. doi:10.1016/bs.afnr.2019.02.008.

Pullens, J.W.M., Sharif, B., Trnka, M., Balek, J., Semenov, M.A., Olesen, J.E. 2019. Risk factors for European winter oilseed rape production under climate change. *In* Agricultural and Forest Meteorology. Elsevier. **272–273**:30-39. doi:10.1016/j.agrformet.2019.03.023.

Qian, B., Jing, Q., Bélanger, G., Shang, J., Huffman, T., Liu, J., and et al. 2018. Simulated canola yield responses to climate change and adaptation in Canada. Agron. J. **110**:133-146. doi:10.2134/agronj2017.02.0076.

Rahman, H., 2013. Review: Breeding spring canola (*Brassica napus* L.) by the use exotic germplasm. Can. J. Plant Sci. **93**:363-373. doi:10.4141/CJPS2012-074.

Rahman, H., Bennett, R.A., and Séguin-Swartz, G. 2015. Broadening genetic diversity in *Brassica napus* canola: Development of canola-quality spring *B. napus* from *B. napus* × *B. oleracea* var. *alboglabra* interspecific crosses. Can. J. Plant Sci. **95**(1):29-41. doi:10.4141/cjps-2014-017.

Rahman, H., Peng, G., Yu, F., Falk, K.C., Kulkarni, M., and Selvaraj, G. 2014. Genetics and breeding for clubroot resistance in Canadian spring canola (*Brassica napus* L.). Can. J. Plant Pathol. **36**:122-134. doi:10.1080/07060661.2013.862571.

Rahman, H., Shakir, A., and Hasan, M.J. 2011. Breeding for clubroot resistant spring canola (*Brassica napus* L.) for the Canadian prairies: Can the European winter canola cv. Mendel be used as a source of resistance? Can. J. Plant Sci. **91**(3):447-458. doi:10.4141/cjps10073.

Rakow, G. 2004. Species origin and economic importance of *Brassica*. *In* Biotechnology in Agriculture and Forestry. *Edited by* E.C. Pua and C.J. Dougla. Springer, Berlin, Heidelberg. **54**:3-11. doi:10.1007/978-3-662-06164-0_1.

Rashid, A., Ahmed, H.U., Xiao, Q., Hwang, S.F., and Strelkov, S.E. 2013. Effects of root exudates and pH on *Plasmodiophora brassicae* resting spore germination and infection of canola (*Brassica napus* L.) root hairs. Crop Prot. **48**:16-23. doi:10.1016/j.cropro.2012.11.025.

Raza, A., Mehmood, S.S., Ashraf, F., and Khan, R.S.A. 2019. Genetic diversity analysis of *Brassica* species using PCR-based SSR markers. Gesunde Pflanzen. **71**:1-7. doi:10.1007/s10343-018-0435-y.

Rennie, D.C., Holtz, M.D., Turkington, T.K., Leboldus, J.M., Hwang, S.F., Howard, R.J., and Strelkov, S.E. 2015. Movement of *Plasmodiophora brassicae* resting spores in windblown dust. Can.J. Plant Pathol. **37**(2):188-196. doi:10.1080/07060661.2015.1036362.

Robin, A.H.K., Hossain, M.R., Kim, H.T., Nou, I.S., and Park, J.I. 2019. Role of cytokinins in clubroot disease development. Plant Breed. Biotechnol. **7**(2):73-82. doi:10.9787/pbb.2019.7.2.73.

Rousseau-Gueutin, M., Belser, C., Silva, C.Da., Richard, G., Istace, B., Cruaud, C., and et al. 2020. Long-read assembly of the *Brassica napus* reference genome Darmor-bzh. Gigascience. **9**(12):137. doi:10.1093/gigascience/giaa137.

Saito, M., Kubo, N., Matsumoto, S., Suwabe, K., Tsukada, M., and Hirai, M. 2006. Fine mapping of the clubroot resistance gene, *Crr3*, in *Brassica rapa*. Theor. Appl. Genet. **114**(1):81-91. doi:10.1007/s00122-006-0412-1.

Sakamoto, K., Saito, A., Hayashida, N., Taguchi, G., and Matsumoto, E. 2008. Mapping of isolate-specific QTLs for clubroot resistance in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Theor. Appl. Genet. **117**(5):759-767. doi:10.1007/s00122-008-0817-0.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. **18**:233-234. doi:10.1038/72708.

Schwelm A., Fogelqvist J., Knaust A., Jülke, S., Lilja, T., Bonilla-Rosso, G., and et al. 2015. The *Plasmodiophora brassicae* genome reveals insights in its life cycle and ancestry of chitin synthases. Sci. Rep. **5**:11153. doi:10.1038/srep11153.

Shah, N., Sun, J., Yu, S., Wang, Z., Huang, F., Dun, B., and et al. 2019. Genetic variation analysis of field isolates of clubroot and their responses to *Brassica napus* lines containing resistant genes *CRb* and *PbBa8.1* and their combination in homozygous and heterozygous state. Mol. Breeding. **39**:153. doi:10.1007/s11032-019-1075-3.

Sharma K., Gossen, B.D., and McDonald, M.R. 2011. Effect of temperature on primary infection by *Plasmodiophora brassicae* and initiation of clubroot symptoms. Plant Pathol. **60**(5):830-38. doi:10.1111/j.1365-3059.2011.02458.x.

Shattuck, V.I., and Proudfoot, K.G. 1990. Rutabaga breeding. Plant Breed. Rev. **8**:217-248. doi:10.1002/9781118061053.ch710.1002/9781118061053.ch7.

Shiranifar, B., Hobson, N., Kebede, B., Yang, R., and Rahman, H. 2020. Potential of rutabaga (*Brassica napus* var. *napobrassica*) gene pool for use in the breeding of *B. napus* canola. Crop Sci. **60**(1):157-171. doi:10.1002/csc2.20074.

Shiranifar, B., Hobson, N., Kebede, B., Yang, R.C, and Rahman, H. 2021. Potential of rutabaga (*Brassica napus* var. *napobrassica*) gene pool for use in the breeding of hybrid spring *Brassica napus* canola. Plant Breed. **140**:305-319. doi:10.1111/pbr.12895.

Some, A., Manzanares, M.J., Laurens, F., Baron, F., Thomas, G., and Rouxel, F. 1996. Variation for virulence on *Brassica napus* L. amongst *Plasmodiophora brassicae* collections from France and derived single-spore isolates. Plant Pathol. **45**:432-439. doi:10.1046/j.1365-3059.1996.d01-155.x.

Spaner, D. 2002. Agronomic and horticultural characters of rutabaga in eastern Canada. Can. J. Plant Sci. **82**(1): 221-224. doi:10.4141/P01-086.

Stanke, M., and Waack, S. 2003. Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics. **19**(2):27. doi:10.1093/bioinformatics/btg1080.

Statistics Canada. 2021a. Canadian International Merchandise Trade Web Application – Exports. Available from https://www150.statcan.gc.ca/n1/pub/71-607-x/2021004/exp-eng.htm [accessed 10 January 2022].

Statistics Canada. 2021b. Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units. doi:10.25318/3210035901-eng. Available from https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210035901 [accessed 10 January 2022].

Statistics Canada. 2019. Production of principal field crops. Available from https://www150.statcan.gc.ca/n1/daily-quotidien/191206/dq191206b-eng.htm [accessed 11 September 2020].

Stefansson, B.R., and Kondra, Z.P. 1975. Tower summer rape. Can. J. Plant Sci. **55**:343-344. doi:10.4141/cjps75-053.

Strelkov, S.E., and Hwang, S.F. 2014. Clubroot in the Canadian canola crop: 10 years into the outbreak. Can. J.Plant Pathol. **36**(1):27-36. doi:10.1080/07060661.2013.863807.

Strelkov, S.E., Hwang, S.F., Manolii, V.P., Cao, T., and Feindel, D. 2016. Emergence of new virulence phenotypes of *Plasmodiophora brassicae* on canola (*Brassica napus*) in Alberta, Canada. Eur. J. Plant Pathol. **145**:517-529. doi:10.1007/s10658-016-0888-8.

Strelkov S.E., Hwang S.F., Manolii V.P., Cao, T., Fredua-Agyeman, R., Harding, M.W., and et al. 2018. Virulence and pathotype classification of *Plasmodiophora brassicae* populations collected from clubroot resistant canola (*Brassica napus*) in Canada. Can. J. Plant Pathol. **40**(2):284-298. doi:10.1080/07060661.2018.1459851.

Strelkov, S.E., Hwang, S.F., Manolii, V.P., Turnbull, G., Fredua-Agyeman, R., Hollman K., and et al. 2021. Characterization of clubroot (*Plasmodiophora brassicae*) from canola (*Brassica napus*) in the Peace Country of Alberta, Canada. Can. J. Plant Pathol, **43**(1):155-161. doi:10.1080/07060661.2020.1776931.

Strelkov, S.E., Manolii, V.P., Cao, T., Xue, S., and Hwang, S.F. 2007. Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta. Canada. J. Phytopathol. **155**: 706-712. doi:10.1111/j.1439-0434.2007.01303.x.

Strelkov, S.E., Manolii, V.P., Lageyre J., Hwang, S.F., Harding, M.W., and Daniels, G.C. 2019. Occurrence and spread of clubroot on canola in Alberta in 2018. *In* The Canadian Phytopathological Society Canadian Plant Disease Survey: Disease Highlights 2019. Can. J. Plant Pathol. **42**:129-131. doi:10.1080/07060661.2020.1752524.

Strelkov, S.E., Tewari, J.P., and Smith-Degenhardt, E. 2006. Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. Can. J. Plant Pathol. **28**(3):467-474. doi:10.1080/07060660609507321.

Summanwar, A., Farid, M., Basu, U., Kav, N., and Rahman, H. 2021. Comparative transcriptome analysis of canola carrying clubroot resistance from 'Mendel' or Rutabaga and the development of molecular markers. Physiol. Mol. Plant Pathol. **114**:101640. doi:10.1016/j.pmpp.2021.101640.

Sun, F., Fan G., Hu Q., Zhou, Y., Guan, M., Tong, C., and et al. 2017. The high-quality genome of *Brassica napus* cultivar 'ZS11' reveals the introgression history in semi-winter morphotype. Plant J. **92**(3):452-468. doi:10.1111/tpj.13669.

Sun, L., and Zhang, J. 2020. Regulatory role of receptor-like cytoplasmic kinases in early immune signaling events in plants. FEMS Microbiol Rev. **44**(6):845-856. doi:10.1093/femsre/fuaa035.

Suwabe, K., Iketani, H., Nunome, T., Kage, T., and Hirai, M. 2002. Isolation and characterization of microsatellites in *Brassica rapa* L. Theor. Appl. Genet. **104**:1092-1098. doi:10.1007/s00122-002-0875-7.

Suwabe, K., Tsukazaki, H., Iketani, H., Hatakeyama, K., Fujimura, M., Nunome, T., and et al. 2003. Identification of two loci for resistance to clubroot (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. Theor. Appl. Genet. **107**(6):997-1002. doi:10.1007/s00122-003-1309-x.

Suwabe, K., Tsukazaki, H., Iketani, H., Hatakeyama, K., Kondo, M., Fujimura, M., and et al. 2006. Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: The genetic origin of clubroot resistance. Genetics. **173**(1):309-319. doi:10.1534/genetics.104.038968.

Tamura, K., Nishioka, M., Hayashi, M., Zhang, Z., Lian, C., Hougetsu, T., and et al. 2005. Development of microsatellite markers by ISSR-suppression-PCR method in *Brassica rapa*. Breed. Sci. **55**(2):247-252. doi:10.1270/jsbbs.55.247.

Tan, S.H., Mailer, R.J., Blanchard, C.L., and Agboola, S.O. 2011. Canola proteins for human consumption: Extraction, profile, and functional properties. J. Food Sci. **76**(1):R16-R28. doi:10.1111/j.1750-3841.2010.01930.x.

Tang, D., Wang, G., and Zhou, J.M. 2017. Receptor kinases in plant-pathogen interactions: more than pattern recognition. Plant Cell. **29**(4):618-637. doi:10.1105/tpc.16.00891.

Tewari, J.P., Strelkov, S.E., Orchard, D., Hartman, M., Lange, R.M., and Turkington, T.K. 2005. Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Can. J. Plant Pathol. **27**:143-144. doi:10.1080/07060660509507206.

Thakur, A.K., Singh, K.H., Singh, L., Nanjundan, J., Khan, Y.J., and Singh, D. 2018. SSR marker variations in *Brassica* species provide insight into the origin and evolution of *Brassica* amphidiploids. Hereditas. **155**:6. doi:10.1186/s41065-017-0041-5.

Tomita, H., Shimizu, M., Doullah, M.A., Fujimoto, R., and Okazaki, K. 2013. Accumulation of quantitative trait loci conferring broad-spectrum clubroot resistance in *Brassica oleracea*. Mol. Breeding. **32**:889-900. doi:10.1007/s11032-013-9918-9.

Tommerup, I.C., and Ingram, D.S. 1971. The life-cycle of *Plasmodiophora brassicae* Woron. In *Brassica* tissue cultures and in intact roots. New Phytol. **70**(2): 327-332. doi:10.1111/j.1469-8137.1971.tb02531.

Tripathi, M.K., and Mishra A.S. 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. **132**(1-2):1-27. doi:10.1016/j.anifeedsci.2006.03.003.

Ueno, H., Matsumoto, E., Aruga, D., Kitagawa, S., Matsumura, H., and Hayashida, N. 2012. Molecular characterization of the *CRa* gene conferring clubroot resistance in *Brassica rapa*. Plant Mol. Biol. **80**(6):621-629. doi:10.1007/s11103-012-9971-5.

USDA-FAS. 2021. Oilseeds: world markets and trade. Available from https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf [accessed 10 December, 2021].

Vinu, V., Singh, N., Vasudev, S., Yadava, D.K., Kumar, S., Naresh, S., Bhat, S.R., and Prabhu, K.V. 2013. Assessment of genetic diversity in *Brassica juncea* (Brassicaceae) genotypes using phenotypic differences and SSR markers. Rev. Biol. Trop. **61**(4):1919-1934.

Voorrips, R.E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. J. Hered. **93**(1):77-78. doi:10.1093/jhered/93.1.77.

Voorrips, R.E., Jongerius, M.C., and Kanne, H.J. 1997. Mapping of two genes for resistance to clubroot (*Plasmodiophora brassicae*) in a population of doubled haploid lines of *Brassica oleracea* by means of RFLP and AFLP markers. Theor. Appl. Genet. **94**:75-82. doi:10.1007/s001220050384.

Wallenhammar, A.C. 1996. Prevalence of *Plasmodiophora brassicae* in a spring oilseed rape growing area in central Sweden and factors influencing soil infestation levels. Plant Pathol. **45**(4):710-719. doi:10.1046/j.1365-3059.1996.d01-173.x.

Warwick, S., Francis, A., and Mulligan, G. 2013. Brassicaceae of Canada. Available from http://www.cbif.gc.ca/eng/species-bank/brassicaceae-of-canada/?id=1370403267260#cruciferae [accessed 2 March 2021].

Werner, S., Diederichsen, E., Frauen, M., Schondelmaier, J., and Jung, C. 2008. Genetic mapping of clubroot resistance genes in oilseed rape. Theor. Appl. Genet. **116**(3):363-72. doi:10.1007/s00122-007-0674-2.

Wickham, H. 2016. ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York. Available from https://ggplot2.tidyverse.org [accessed 20 December 2021].

Williams, P.H. 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology. **56**(6):624-626.

Xue, S., Cao T., Howard R.J., Hwang, S.F., and Strelkov, S.E. 2008. Isolation and variation in virulence of single-spore isolates of *Plasmodiophora brassicae* from Canada. Plant Dis. **92**:456- 462. doi:10.1094/PDIS-92-3-0456.

Yoshikawa, H. 1981. Breeding for clubroot resistance in Chinese cabbage. *In* Chinese cabbage. *Edited by* N.S., Talekar, and T.D., Griggs. Tsukuba. 405-413.

Yu, F., Zhang, X., Huang, Z., Chu, M., Song, T., Falk, K.C., and et al. 2016. Identification of genome-wide variants and discovery of variants associated with *Brassica rapa* clubroot resistance gene *Rcr1* through bulked segregant RNA sequencing. PLOS ONE. **11**(4):e0153218. doi:10.1371/journal.pone.0153218.

Yu, F., Zhang, X., Peng, G., Falk, K.C., Strelkov, S.E., and Gossen, B.D. 2017. Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*. Sci. Rep. **7**:4516. doi:10.1038/s41598-017-04903-2.

Yu, F., Zhang, Y., Wang, J., Chen, Q., Karim, M.M., Gossen, B.D., and Peng, G. 2022. Identification of two major QTLs in *Brassica napus* lines with introgressed clubroot resistance from turnip cultivar ECD01. Front. Plant Sci. **12**:785989. doi:10.3389/fpls.2021.785989.

Yuan, Y., Wei, X., Zhang, Q., Zhao, Y., Jiang, W., and Yao, Q. 2015. BSA-Seq technologies identify a major QTL for clubroot resistance in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). In KSM Spring Meeting & KSM-ICWG-GSP Joint Clubroot Symposium. 41.

Yun, H.M., Lei, X.J., Lee, S.I., and Kim, I.H. 2018. Rapeseed meal and canola meal can partially replace soybean meal as a protein source in finishing pigs. J. Appl. Anim. Res. **46**(1):195-199. doi:10.1080/09712119.2017.1284076.

Zambiazi, R.C., Przybylski, R., Zambiazi, M.W., and Mendonça, C.B. 2007. Fatty acid composition of vegetable oils and fats. Bol. Cent. Pesqui. Process. Aliment. **25**(1):111-120. doi:10.5380/cep.v25i1.8399.

Zhang, H., Feng, J., Zhang, S., Zhang, S., Li, F., Strelkov, S.E., and et al. 2015. Resistance to *Plasmodiophora brassicae* in *Brassica rapa* and *Brassica juncea* genotypes from China. Plant Dis. **99**(6):776-779. doi:10.1094/PDIS-08-14-0863-RE.

Zhang, H., Ma, X., Liu, X., Zhang, S., Li, F., Li, G., and et al. 2022a. Identification and fine-mapping of clubroot (*Plasmodiophora brassicae*) resistant QTL in *Brassica rapa*. Hortic. **8**(1):66. doi:10.3390/horticulturae8010066.

Zhang, T., Wu, D., Zhao, Z., Wang, Z., and Piao, Z. 2012. Development of near isogenic lines for clubroot resistance in Chinese cabbage and their assessment. Mol. Plant Breed. **6**:722-730.

Zhang, Z., Guo, J., Cai, X., Li, Y., Xi, X., Lin, R., and et al. 2022b. Improved reference genome annotation of *Brassica rapa* by Pacific Biosciences RNA sequencing. Front. Plant Sci. 13:. doi:10.3389/fpls.2022.841618.

Zhou, L., Zhang, L., He, Y., Liu, F., Li, M., Wang, Z., and et al. 2014. Isolation and characterization of bacterial isolates for biological control of clubroot on Chinese cabbage. Eur. J. Plant Pathol. **140**:159-168. doi:10.1007/s10658-014-0451-4.

Zhu, H., Zhai, W., Li, X., and Zhu, Y. 2019. Two QTLs controlling clubroot resistance identified from bulked segregant sequencing in Pakchoi (*Brassica campestris* ssp. *chinensis* Makino). Sci. Rep. **9**:9228. doi:10.1038/s41598-019-44724-z.

Zhu, M., He, Y., Li, Y., Ren, T., Liu, H., Huang, J., and et al. 2020. Two new biocontrol agents against clubroot caused by *Plasmodiophora brassicae*. Front. Microbiol. **10**:3099. doi:10.3389/fmicb.2019.03099.

Ziesman, B., Avila, R., Ursu, K., Kwasnicki, J., Roszell, L., Baraniecki, C., and et al. 2019. 2018 clubroot survey and known occurrence of clubroot in Saskatchewan. *In* The Canadian Phytopathological Society Canadian Plant Disease Survey: Disease Highlights 2019. Can.J. Plant Pathol. **42**:159-161. doi:10.1080/07060661.2020.1752524.