Genetics of clubroot disease (*Plasmodiophora brassicae*) resistance in Brassica

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in

Plant Science

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Abstract

Clubroot disease, caused by *Plasmodiophora brassicae*, is a major threat to canola production. Cultivation of resistant cultivars is the key component in managing this disease. Canola is an important oilseed crop in the world; this includes the allopolyploid species *Brassica napus* L. (AACC genome, 2*n* = 38) and *B. juncea* L. (AABB genome, 2*n* = 36) and the diploid species *B. rapa* L. (AA, 2*n* = 20). Oilseed *B. napus* and *B. juncea* lack resistance to clubroot disease; therefore, there is a need to introduce resistance to this disease in canola from diverse sources, including its progenitor species.

In this study, the genetic control of clubroot disease resistance in the polyploid *B. napus* var. *napobrassica* and diploid *B. rapa* vars. *pekinensis* and *rapifera* were investigated for resistance in *B. napus* canola and for the development of clubroot resistant *B. juncea*. Doubled haploid (DH) and recombinant inbred line (RIL) populations developed from *B. napus* var. *napobrassica* cv. Brookfield (rutabaga) × *B. napus* spring canola and *B. napus* spring canola × *B. rapa* var. *pekinensis cv.* Bilko (Chinese cabbage) interspecific crosses and synthetic *B. juncea* lines developed from *B. rapa* var. *rapifera cv.* Gelria (turnip) × *B. nigra* interspecific cross was used in this study. These populations were phenotyped for resistance to several *P. brassicae* pathotypes and genotyped using SSR and SNP-based allele-specific (AS) and KASP (Kompetitive allele-specific PCR) markers to map the clubroot resistance (CR) loci and develop genetic markers. The DH lines were also evaluated in replicated field trials for QTL mapping of flowering and seed quality traits.

Analysis of the DH and RIL populations indicated that a major gene controls clubroot resistance in the rutabaga cv. Brookfield. Genetic analysis using the DH lines identified a genomic region, *qCR_A8*, on chromosome A08 is associated with resistance to *P. brassicae* pathotypes 2, 3, 5, 6, and 8, and a locus, *qCR_A3*, on chromosome A03 associated with resistance to pathotype 3. QTL analysis indicated that the rutabaga cv. Brookfield carries

a high oil QTL, *qOIL_C3*, about 15 cM away from a major erucic acid QTL on chromosome C03, and a major QTL on chromosome A02 affects both vernalization requirement and flowering time variation. The *qOIL_C3* could increase oil content in spring canola by about 0.7%. Further analysis exhibited no correlation of clubroot resistance of rutabaga cv. Brookfield, with agronomic and seed quality traits. Genetic analysis of the canola × Chinese cabbage RIL population demonstrated that introgression of a CR locus from the Chinese cabbage cv. Bilko occurred into chromosome A03 of spring *B. napus* canola. The Bilko-CR locus co-segregated with the AS-markers in the homozygous RIL families. However, recombination between the CR loci and their co-segregating markers indicated that the genetic markers developed in this research might not be located within the CR genes; the allelic variation exhibited by the markers may not be due to the variation in sequence motifs affecting the phenotypic variation.

Analysis of the synthetic *B. juncea* lines using A- and B-genome specific SSR markers and their reaction to *P. brassicae* pathotype 3 indicated that clubroot resistant *B. juncea* line could be developed by exploiting the resistance available in *B. rapa*. However, a loss of resistance in around 5.8% of the resynthesized S2 *B. juncea* plants indicated that the genomic regions carrying the CR could be in a state of genomic change.

Thus, the results from this thesis research provided substantial evidence that rutabaga and the Chinese cabbage gene pool can be used to broaden the genetic base for clubroot resistance in *B. napus* canola. Rutabaga also carries favourable alleles for seed oil content. The clubroot resistance of this rutabaga cultivar does not exert any negative effect on agronomic and seed quality traits; thus, this resistance can safely be used in canola breeding. The results from this Ph.D. thesis research also provide evidence that clubroot-resistant *B. juncea* can be developed by exploiting the resistance available in *B. rapa*.

Preface

Muhammad Jakir Hasan submits this dissertation for the degree of Doctor of Philosophy. Muhammad Jakir Hasan conducted all the experiments, analyzed all the data, prepared the draft of all the chapters of this thesis, and incorporated all comments, suggestions, and editorial revisions provided by Professor Habibur Rahman, his supervisor.

A version of chapter 1 of this dissertation has been published as Hasan, J., Megha, S., and Rahman, H. 2021. Clubroot in Brassica : recent advances in genomics, breeding, and disease management. Genome **64**(8): 735–760. doi:10.1139/gen-2020-0089.

Muhammad Jakir Hasan wrote the first draft of the introduction, management, sources, and genetic basis of resistance and breeding resistance sections. Swati Megha wrote the first draft of the molecular biology of resistance Brassica-Plasmodiophora pathosystem and mechanism of resistance/susceptibility. Professor Habibur Rahman supervised the writing of the complete review and contributed to the improvement, including editing the paper.

A version of chapter 2 of this dissertation has been published as 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**(10): 805–815. doi:10.1139/gen-2016-0034.

Muhammad Jakir Hasan carried out the experiments, developed the mapping population, collected the data, performed analysis, design the figures, and wrote the first draft of the manuscript. Professor Habibur Rahman conceived the original research, supervised the experiments, edited the manuscript, and helped interpret the results.

A version of chapter 3 of this dissertation has been published as Hasan, J., Shaikh, R., Megha, S., Herrmann, D.T., Kebede, B., and Rahman, H. 2021. Mapping of flowering time, seed quality and clubroot resistance in rutabaga × spring canola populations and their association. Euphytica **217**(8): 160. doi:10.1007/s10681-021-02889-7.

Muhammad Jakir Hasan carried out the experiments, collected the data, performed analysis, design the figures, and wrote the first draft of the manuscript. Rubeena Shaikh carried out WGRS analysis, designed SNP-based AS-PCR and KASP markers, and generated genotypic data of these markers. Swati Megha contributed to the design and genotyping of the AS-PCR markers. Rubeena Shaikh and Swati Megha also contributed to writing the materials and methods section. David T Herrmann carried out SNP genotyping of the DH population. Berisso Kebede contributed to data collection and QTL analysis. Professor Habibur Rahman conceived the original research, supervised the experiments, provided critical feedback, and helped shape the research, data analysis, and manuscript.

A version of chapter 4 of this dissertation has been submitted as Hasan, J., Shaikh, R., Basu, U. and Rahman, H. 2021. Mapping clubroot resistance of *Brassica rapa* introgressed into *Brassica napus* and development of molecular markers for the resistance. Crop Science.

Muhammad Jakir Hasan carried out the experiments, WGRS analysis, designed SNP-based AS-PCR markers and generated genotypic data of these markers, performed analysis, design figures, and wrote the first draft of the manuscript. Rubeena Shaikh developed the RIL population. Urmila Basu provided critical feedback in WGRS analysis. Professor Habibur Rahman conceived the original research, supervised the experiments, provided critical feedback, and helped shape the research, data analysis, and manuscript.

A version of chapter 5 of this dissertation has been published as 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:10.1270/jsbbs.18010.

Muhammad Jakir Hasan conceived the original research, carried out the experiments, developed a synthetic population, collected the data, performed analysis, design the figures, and wrote the first draft of the manuscript. Professor Habibur Rahman supervised the experiments, edited the manuscript, and helped interpret the results.

Acknowledgments

I want to thank Professor Habibur Rahman for his support during the Ph.D. program. I also appreciate Professor Stephen E Strelkov, Professor Changxi Li, Dr. Berisso Kebede, Dr. Urmila Basu for their supports.

I also would like to thank Dr. Sadia Munir, my spouse, and my mother, Rawson Akhter, for their unlimited supports. I want to thank my children, Aayed Hasan and Raaina Hasan, for always keeping me cheered up.

I am thankful to Dr. Rubeena Shaikh, Dr. Mehdi Farid, and Dr. Swati Megha for their help in collecting a portion of data, and Ann Vo, Salvador Lopez for assistance in various routine works.

I want to acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) and Alberta Canola Producers Commission (ACPC), Alberta Canola Industry Development Fund (ACIDF), SaskCanola, Manitoba Canola Growers Association, and Canola Council of Canada for financial support to Dr. Habibur Rahman for this research.

I also want to acknowledge the Department of Agriculture, Food and Nutritional Science (AFNS), Queen Elizabeth II, for providing me with valuable scholarships and awards.

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List of Symbols and Abbreviations

±	Plus/minus
=	Equal
>	Greater than
<	Less than
%	Percent
~	About
°C	Degree Celsius
n	Haploid number of chromosomes
2*n*	Diploid number of chromosomes
DH	Doubled haploid
µmol/g	Micromoles per gram
QTL	Quantitative trait locus
BLAST	Basic local alignment
Mb	Million base pair
ECD	European clubroot differentials
CCD	Canadian clubroot differentials
ETI	Effector-triggered immunity
TIR	Toll-interleukin-1
NBS	Nucleotide-binding site
LRR	Leucine-rich repeats
TNL	TIR-NBS-LRR
PTI	Pattern-triggered immunity
PRRs	Pattern recognition receptors
ROS	Reactive oxygen species
PAMP	Pathogen-associated molecular patterns
Flg	Flagellin
EF-Tu	Elongation factor Tu
PGN	Peptidoglycan
EIX	Ethylene-induced xylanase
CBEL	Cellulose-binding elicitor lectin
PR	Pathogenesis-related

DEGs	Differentially expressed genes
IAA	Indole-3-acetic-acid
SA	Salicylic Acid
JA	Jasmonic acid
iTRAQ	Isobaric tags for relative and absolute quantitation
ET	Ethylene transferase
MST	Mono-Saccharide Transporter
*SWEET*s	Sugars Will Eventually be Exported Transporters
lncRNAs	long non-coding RNAs
miRNAs	microRNAs
RNA	Ribonucleic acid
DNA	Deoxyribonucleic acid
mRNA	Messenger RNA
TIR1	Transport Inhibitor Response 1
PCR	Polymerase Chain Reaction
e-PCR	Electronic PCR
RT-PCR	Real-Time Polymerase Chain Reaction
dai	Days after inoculation
hai	Hours after inoculation
BC_1	First backcross generation
F_1	First generation
BC_1F_x	X^{th} generation of BC1-derived population
RIL	Recombinant Inbred Line
NLN	Nitsch & Nitsch
pmol/L	Picomole per milli liter
mmole/L	Millimole per milli liter
SNP	Single nucleotide polymorphism
SSR	Simple Sequence Repeat
SCAR	Sequence Characterized Amplified Region
WGRS	Whole-genome resequencing
BSA	Bulked-segregant analysis
LOD	Logarithm of the Odds
ICIM	Inclusive Composite Interval Mapping

cM	Centi-Morgan
KASP	Kompetitive allele-specific PCR
AS-PCR	Allele-Specific PCR
SSI	Single-spore isolate
DSI	Disease Severity Index
AAFC	Agriculture Agri-Food Canada
MBGP	Multinational *Brassica* Genome Project
BRAD	*Brassica* Database
TAIR	The Arabidopsis Information Resource
C.I.	Confidence Interval
DTF	Days-to-flowering
Vern	Vernalization
H^2	Broad-sense heritability
σ_g^2	Genetic variance
σ_e^2	Error variance
σ_{ge}^2	Interaction variance between genotypes and environments
df	Degrees of Freedom
χ^2	Chi-square

1. Chapter 1: Literature review[1]

1.1. Introduction

Brassica is an economically important genus of the tribe *Brassiceae* (Al-Shehbaz 2012) and includes 39 species (Warwick et al. 2006). Enormous morphological diversity exists among these species, making them suitable for use as sources of oil, vegetable, condiment, and fodder. In the world's context, Brassicas ranks second as a source of vegetable oil and third as a source of vegetables (FAO 2020).

Among various threats to the production of Brassica crops, the clubroot disease caused by *Plasmodiophora brassicae* Woronin is one of the most important (for review, see Dixon 2009). According to Agrios (2005), the infected roots (clubbed roots) use much of the nutrients typically available for the plant's growth and development. The clubbed roots also interfere with the absorption and translocation of mineral nutrients and water through the root system and result in gradual stunting and wilting of the plant's above-ground parts, causing a severe crop yield reduction. Clubroot disease has been reported in more than 60 countries worldwide, causing about 10-15% yield loss globally (for review, see Dixon 2009). Aside from yield loss, this disease can also adversely affect the quality of the crop. For example, about a 2–6% decrease in seed oil content (Engqvist 1994; Pageau et al. 2006) and a 50% increase in chlorophyll content in oil (Engqvist 1994) is associated with clubroot infection.

The pathogen *P. brassicae* is an obligate, intracellular plant-parasitic lower fungus, classified as Protista under the phylum Plasmodiophoromycota (Barr 1992). Its form is

[1] A version of this chapter has been published as

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.

plasmodium in nature (Karling 1969), which can overwinter in the soil as a resting spore. The half-life (time required for a quantity to fall to half of its value as measured at the beginning of the period) of this spore is about 3.6 years; however, it can persist more than 17 years in an infested field (Wallenhammar 1996). In the presence of host species, the resting spores germinate in early spring and produce primary zoospores. The primary zoospore injects its cellular content into root hair (Aist and Williams 1971) and produces primary plasmodium, which develops into zoosporangia. Secondary zoospores, produced in the zoosporangium (Ingram and Tommerup 1972; Kageyama and Asano 2009), are released in the rhizosphere (rhizosphere is the region of soil around the roots of a plant whose property is influenced by the chemicals released from the root and is inhabited by microorganisms), where they infect either young root tissue through direct penetration or older thickened root and even underground stem by penetration through wounds and produce secondary plasmodium (Agrios 2005; Kageyama and Asano 2009). The primary zoospores can also directly cause secondary infection when the host is already under primary infection (Feng et al. 2013*a*) and produce secondary plasmodium. The secondary zoospores can cause both primary and secondary infections (McDonald et al. 2014). The secondary plasmodium undergoes synchronous mitotic division and becomes multinucleate (Figure 1.1). The multinucleate plasmodia further spread to cortical cells and then into cambium through direct penetration. Once inside, plasmodium spreads in every direction in the cambium, such as outward into the cortex and inward towards the xylem, and into the medullary rays (Cook and Schwartz 1930). As the plasmodia pass through the cells, they establish themselves in some of the cells and activate the auxin (Jahn et al. 2013) and cytokinin (Lan et al. 2019), and possibly the brassinosteroid signalling pathways (Schuller et al. 2014). This altered cellular physiology results in the allocation of a higher amount of photosynthates from leaves to the roots (Ludwig-Müller et al. 2009), promotes

abnormal development of the cells in masses and formation of characteristic galls, known as the clubbed root, the size of which is a few times greater than the healthy roots (Figure 1.2).

The field population of *P. brassicae* generally exhibits diversity for virulence (Holtz et al. 2018; Sedaghatkish et al. 2019). The population structure of this pathogen can also change in the infested field in a short time. For example, in the early 2000s, only five *P. brassicae* pathotypes (a pathotype is a group of organisms of the same species that exhibit the same pathogenicity on a specified host), *viz*., 2, 3, 5, 6, and 8, classified as per William's (1966) system, were reported in canola fields in Alberta, Canada (Strelkov et al. 2006, 2007; Xue et al. 2008). Pathotype 3 was the most prevalent and virulent among the five pathotypes (Strelkov et al. 2006). However, only after a decade, several new and more virulent pathotypes, such as A, B, D, and X, have been found to occur predominantly in some of the canola fields in Alberta (Strelkov et al. 2016b). Repeated cultivation of cultivars carrying the same resistance gene(s) opened the door for the rare pathotypes to multiply rapidly, resulting in a shift in pathogen population structure where the rare pathotypes have become predominant (Sedaghatkish et al. 2019; Cao et al. 2020). Strelkov et al. (2018) used Canadian Clubroot Differential (CCD) set and reported a total of 17 pathotypes, *viz*. pathotypes A, B, C, D, E, F, G, H, I, J, K, L, M, N, O, P and X, from canola fields in Canada. The differential hosts for a pathogen are a set of plant cultivars used to define pathotypes of the pathogen based on their known resistant or susceptible reactions. The newly evolved pathotypes are genetically distinct from the initial pathotypes (Sedaghatkish et al. 2019). Thus, the ability of the *P. brassicae* to persist in soil without a host for several years (Wallenhammar 1996) and to change its virulence in a short period (Strelkov et al. 2016b) makes this pathogen a significant threat to Brassica crop production.

1.2. Management of clubroot disease

At present, there is no cultural method for the eradication of *P. brassicae* from an infested field. Soil amendment with lime, such as calcium cyanamide ($CaCN_2$), calcium carbonate ($CaCO_3$) (Murakami et al. 2002; Tremblay et al. 2005; Hwang et al. 2011*c*), dolomite [$CaMg(CO_3)_2$] (Murakami et al. 2002), wood ash (Hwang et al. 2011*c*) and boron (Deora et al. 2011) minimizes the impact of clubroot on cruciferous vegetables and canola. Liming elevates soil pH to alkalinity and reduces clubroot disease severity (Tremblay et al. 2005). Besides increasing soil pH, calcium cyanamide releases intermediate anion (CN^{-2}), which is toxic to fungi (Venter 1970). However, the effectiveness of liming depends on their chemical form and particle size (reviewed by Donald and Porter 2009); finely ground lime is more effective in reducing clubroot incidence than the coarser limes (Dobson et al. 1983; Tremblay et al. 2005). The effectiveness of lime in reducing soil acidity depends on its neutralizing value (NV) - a higher NV indicates the greater ability of the lime to neutralize soil acidity (Campbell and Grethead 1989). For example, the NV of pure calcium carbonate ($CaCO_3$) is 100%, while the NV of dolomite is about 80-90%. The effectiveness of lime for the control of clubroot disease also depends on the type of soil (Myers et al. 1981) and the time of its application (Tremblay et al. 2005).

Boron mitigates the impact of clubroot disease by inhibiting pathogens' development during primary and secondary infection (Webster and Dixon 1991; Deora et al. 2011). However, this compound cause phytotoxicity to canola seedlings under field conditions at an application rate of $\geq$8 kg boron ha^{-1} (Deora et al. 2011). Although some of the chemicals are suitable to manage clubroot disease in vegetable Brassicas, which are high-value crops and produced on limited acres of land, the use of these chemicals is not economically feasible in canola cropping systems (Hwang et al. 2014*b*). A few pentachloronitrobenzene (PCNB) and cyazofamid type fungicides (Hwang et al. 2011*c*) and sodium N-methyldithiocarbamate

(Vapam) (Hwang et al. 2014*a*) and dazomet (Hwang et al. 2017*a*) type fumigants reduce clubroot disease severity. However, they are suitable for the containment of small and localized areas in commercial canola fields (Hwang et al. 2014*b*). For the efficacy of Vapam, soil moisture in the range of 10-30% (v/v) is required (Hwang et al. 2014*a*). An inadequate interval between the application of dazomet and seeding time can cause phytotoxicity in the crop (Hwang et al. 2017*a*). Some cultural management practices, such as manipulation of seeding date (Gossen et al. 2009; Hwang et al. 2012*b*), crop rotation with non-host species (Hwang et al. 2015; Peng et al. 2015), or growing resistant cultivars (Hwang et al. 2015) can reduce the severity of this disease to some extent. Alberta Clubroot Management Committee (2010) recommended farm machinery and equipment sanitation to prevent disseminating the pathogen between fields.

The severity of clubroot disease correlates well (r^2 = 0.87 - 0.95) with the concentration of resting spores in soil (Wallenhammar et al. 1999; Hwang et al. 2011*a*, 2011*b*, 2017*b*); therefore, reduction of spore density in the soil is a vital strategy for the management of this disease. As mentioned above, *P. brassicae* resting spores can survive in the soil for more than 17 years (Wallenhammar 1996). However, a break from growing susceptible cultivars for ≥2 years can reduce spore density by about 90% (Peng et al. 2015). Therefore, it is imperative to avoid growing susceptible cultivars and use longer crop rotation to reduce inoculum concentration in soil (Peng et al. 2015; Hwang et al. 2019). Cultivation of resistant cultivars every year in the clubroot-infested fields can result in resistance breakdown due to the emergence of new virulent pathotypes, as has been reported in Canada (Strelkov et al. 2016b; Cao et al. 2020). Furthermore, when a cultivar is exposed to a mixture of virulent and avirulent pathotypes, even with a low concentration of virulent type, the virulent type can facilitate subsequent infection by the avirulent type and compromise cultivar resistance (Jiang et al. 2020). Therefore, cultivating resistant cultivars in a minimum of two or more years crop-

rotation scheme or susceptible cultivars in a four-year crop-rotation scheme with non-host species is recommended in Canada (Alberta Clubroot Management Committee 2010; Hwang et al. 2019). The growing of cultivars carrying multiple resistance genes and adoption of appropriate crop rotation can provide a better safeguard against the growth of the *P. brassicae* population, and thus would provide the growers with a relatively cheap and reliable measure for the control of this disease (Donald and Porter 2009; Hwang et al. 2012*b*; Peng et al. 2015). The presence of susceptible host plant species as weeds can also increase inoculum load in soil (Hwang et al. 2012*a*). Therefore, the cultivation of resistant cultivars in a minimum of two or more year crop-rotation scheme with non-host species coupled with farm sanitation practices are needed to manage clubroot disease in Brassica crops (Peng et al. 2014*b*; Ernst et al. 2019; Hwang et al. 2019).

1.3. Sources of clubroot resistance (CR)

The negative effect of clubroot disease on yield and quality of Brassica vegetables, fodder, and oilseed crop (reviewed by Dixon 2009) and the difficulty of managing this disease by cultural practices underlined the need for the development of clubroot resistant cultivars. The first step towards this would be identifying and choosing the resistance genes for use in breeding. Today, hybrid cultivars have captured most of the Brassica crops market (Morrison et al. 2016); therefore, using a dominant resistance gene is preferred for developing an F_1 hybrid cultivar. In this case, one of the hybrid parents carrying the resistance gene in the homozygous condition is expected to confer complete resistance in the hybrid.

Several Brassica germplasm collections of the 'triangle of U' (U 1935), such as *B. rapa* (AA, $2n = 20$), *B. oleracea* (CC, $2n = 18$), *B. nigra* (BB, $2n = 16$), *B. napus* (AACC, $2n = 38$), *B. juncea* (AABB, $2n = 36$) and *B. carinata* (BBCC, $2n = 34$), have been evaluated by different research groups for resistance to *P. brassicae* (Ayers and Lelacheur 1972; Buczacki et al. 1975; Hasan et al. 2012; Peng et al. 2014*a*; Ramzi et al. 2018; Fredua-Agyeman et al. 2019; Farid et

al. 2020). Among these, complete resistance to clubroot disease are reported in the diploid *B. rapa* and *B. nigra* (Hasan et al. 2012; Fredua-Agyeman et al. 2019) and amphidiploid *B. napus* var. *napobrassica* (swede/rutabaga) and *B. napus* var. *pabularia* (forage rape) (Karling 1969; Johnston 1970; Ayers and Lelacheur 1972; Buczacki et al. 1975; Hasan et al. 2012; Peng et al. 2014*a*; Laperche et al. 2017; Fredua-Agyeman et al. 2019). In the case of *B. oleracea*, the important vegetable *Brassica* species and one of the parental species of *B. napus*, Crisp et al. (1989) evaluated about a thousand accessions of cabbage, cauliflower, broccoli, kale, and Brussels sprout and found resistance in some of the north and west European cabbage and kale accessions. Ramzi et al. (2018) evaluated 223 accessions from the Netherlands and Canada, where only 13 were carrying resistance; among these, three accessions (CGN11150, CGN14078, CGN15227) exhibited broad-spectrum resistance to six pathotypes. Peng et al. (2014a) reported that the cabbage cultivars 'Kilaherb' and 'Tekila' carry resistance to multiple *P. brassicae* pathotypes from Canada. Thus, resistance in *B. oleracea* seems to be more frequent in kale (*B. oleracea* var. *acephala*) and cabbage (*B. oleracea* var. *capitata*) as compared to other varieties of this species (Hasan et al. 2012; Peng et al. 2014*a*; Ramzi et al. 2018; Fredua-Agyeman et al. 2019; Farid et al. 2020).

1.4. The genetic basis of resistance

Researchers have studied the genetic basis of clubroot resistance in some of the resistant germplasm and reported race-specific (race is a genetically variable group of related populations, grouped based on how strongly they affect particular host plants) (Toxopeus and Janssen 1975; Voorrips and Visser 1993; Hirai et al. 2004; Hirani et al. 2018) and broad-spectrum resistance to *P. brassicae* pathotypes (Johnston 1970; Diederichsen and Sacristan 1996; Hasan and Rahman 2016). The A-genome resistance is frequently reported to be controlled by a major dominant gene (Ayers and Lelacheur 1972; Piao et al. 2004; Yu et al. 2017), while the C genome resistance is often controlled by several quantitative trait loci (QTL)

(Manzanares-Dauleux et al. 2000; Werner et al. 2008; Piao et al. 2009; Hejna et al. 2019; Farid et al. 2020). In the following sections, this review discusses the genetic basis of resistance in six Brassica species. The occurrence of resistance in all three diploid species, *B. rapa*, *B. oleracea,* and *B. nigra*, suggests that these resistances can be used in the breeding of the amphidiploid species.

1.4.1. The genetic basis of resistance in *Brassica rapa*

Among the different forms of *B. rapa*, the European turnip (*B. rapa* ssp. *rapifera*) has been widely reported to carry resistance to clubroot disease (Karling 1969; Buczacki et al. 1975; Crute et al. 1983; Hasan et al. 2012; Peng et al. 2014*a*). Several cultivars and lines of this form of *B. rapa*, such as Gelria R, Siloga, Debra, Milan White, and ECD-04, have been used in the breeding of clubroot-resistant Chinese cabbage (*B. rapa* ssp. *chinensis*/*pekinensis*) (Kuginuki et al. 1997; Hirai et al. 2004), oilseed *B. napus* (Diederichsen and Sacristan 1996) and swede or rutabaga (Lammerink 1970; Bradshaw et al. 1997) cultivars, as well as for the development of clubroot resistant *B. juncea* lines (Hasan and Rahman 2018). A total of 24 clubroot resistance loci have been reported from seven A-genome chromosomes of *B. rapa*, viz. A01, A02, A03, A05, A06, A07 and A08. About half (11 of 24) of these loci have been reported from A03, five from A08, and the remaining chromosomes are reported to carry one or two loci (Table 1.1). Basic local alignment (BLASTn) of the markers sequences, flanking putative CR genes or co-segregating with resistance, to the whole-genome assembly of *B. rapa* cv. Chiifu-401 v3.0 indicated the presence of two major clusters of CR genes on A03 — one at about 15.5 - 16.3 Mb region harbouring three CR loci, *CRd, CRk,* and *Crr3*, and the second cluster at about 23.8 - 26.8 Mb region harbouring seven CR loci, *CRa, CRb, CRq, Rcr1, Rcr2, Rcr4* and *Rcr5* (Table 1.1). Different researchers have reported these 24 CR loci by using different pathotypes/races of *P. brassicae*. Therefore, some of these may be the same locus designated by different researchers. Indeed, recent studies have shown that *CRa* and *CRb* are the same locus (Kato et

al. 2013; Hatakeyama et al. 2017) and *Rcr1* (≈*Rpb1*)/*Rcr2* are located in the same genomic region of *CRa* (Huang et al. 2017). Molecular characterization of *CRa* of A03 (Ueno et al. 2012) and *Crr1a* of A08 (Hatakeyama et al. 2013) revealed that they encode TIR-NBS-LRR (TNL) proteins. Yu et al. (2017) reported a total of 441 genes at 23.8 - 26.8 Mb region of A03 of the *B. rapa* reference genome; among these, six genes, viz. *Bra012541*, *Bra019413*, *Bra019412*, *Bra019410*, *Bra019409,* and *Bra019273*, encode TNL-class of disease resistance proteins.

A combined effect or interaction of the genes has also been reported for CR. For example, Suwabe et al. (2003, 2006) mapped the CR gene *Crr1* and a modifier gene *Crr2* on chromosomes A08 and A01, respectively, of *B. rapa* ssp. *rapifera* cv. Siloga. The *Crr1* confer resistance to *P. brassicae* race 2, but not to a more virulent race 4. The *Crr2* did not exert any effect against these two races; however, *Crr1* and *Crr2* together in homozygous conditions showed resistance to race 4.

1.4.2. The genetic basis of resistance in *Brassica oleracea*

Most genetic studies indicate that clubroot resistance in *B. oleracea* is controlled by multiple loci (Yoshikawa 1993), where these loci exert either major or minor effects (Table 1.2). Some of the loci exhibit dominance effect (Laurens and Thomas 1993), while Chiang and Crête (1970) and Voorrips and Visser (1993) reported multiple recessive genes in the control of this trait in cabbage. Voorrips et al. (1997) identified major and minor QTL in cabbage (*B. oleracea* var. *capitata*), conferring resistance to *P. brassicae* pathotype ECD 16/3/30. However, later studies by different researchers using different pathotypes identified QTL from almost every C genome chromosome (Table 1.2). For example, Rocherieux et al. (2004) reported nine QTL conferring isolate-specific to broad-spectrum resistance to five isolates from seven of the nine chromosomes, except C06 and C07, of kale. However, Nagaoka et al. (2010), Dakouri et al. (2018), and Farid et al. (2020) reported CR QTL from C06 and C07. Thus, it is

apparent that QTL for clubroot resistance can be found in all C genome chromosomes; however, these QTL may exhibit resistance to different pathotypes or isolates.

1.4.3. The genetic basis of resistance in *Brassica nigra*

Information on the genetic control of clubroot resistance in the Brassica B-genome is scarce. Chang et al. (2019) reported a major locus, *Rcr6*, conferring resistance to *P. brassicae* pathotype 3 on chromosome B3 of *B. nigra* line PI 219576. The genomic region of *Rcr6* is homoeologous to chromosome A08 of the A-genome of *B. rapa*.

1.4.4. The genetic basis of resistance in *Brassica napus*

Manzanares-Dauleux et al. (2000) reported a major QTL *Pb-Bn1* and two small-effect QTL from two A- and C-genome chromosomes of a winter-type dwarf *B. napus* line (Table 1.3). The *Pb-Bn1* confers resistance to *P. brassicae* isolate PB137-522, while the two small effect QTL confer resistance to isolate K92-16. By using 245 diverse accessions of *B. napus,* Hejna et al. (2019) identified two major QTL on chromosomes A02 and A03, conferring resistance to pathotype ECD 17/31/31.

Clubroot resistance of the parental species has been utilized in the breeding of clubroot resistant *B. napus* cultivars. For example, the first clubroot-resistant winter canola cv. Mendel (Frauen 1999) was introduced in Europe in the early 2000s to combat the clubroot disease in affected areas of Great Britain and Germany (reviewed by Friedt and Snowdon 2010). This cultivar possesses a race-specific gene originating from a resistant *B. napus* line (Diederichsen et al. 2006). The resistant *B. napus* line was synthesized from a clubroot resistant turnip (*B. rapa* ssp. *rapifera* cv. ECD-04) and partially resistant cabbage (*B. oleracea* ssp. *capitata* cv. ECD-15) line (Diederichsen and Sacristan 1996). Diederichsen et al. (2006) reported one dominant and two recessive genes in the resynthesized *B. napus* line, and these genes conferred resistance to *P. brassicae* single spore isolate-1 from France. However, Werner et al. (2008) reported 19 QTL from eight chromosomes in a mapping population (Table 1.3) developed by

using the above-mentioned resynthesized *B. napus* line developed by Diederichsen and Sacristan (1996). These QTL conferred resistance to seven *P. brassicae* field isolates. By using two spring canola lines carrying resistance introgressed from cv. Mendel, Fredua-Agyeman and Rahman (2016) mapped a single dominant gene on A03, conferring resistance to pathotype 3. Similarly, by using a spring *B. napus* line with resistance derived from the resynthesized *B. napus* line developed by Diederichsen and Sacristan (1996), Zhang et al. (2016*a*) mapped the major locus on A03 and named this as *CRa*.

Swedes or rutabaga (*B. napus* var. *napobrassica*) and forage rape (*B. napus* var. *pabularia*) are frequently reported to carry clubroot resistance (Walker 1939; Lammerink 1967; Karling 1969; Johnston 1970; Ayers and Lelacheur 1972; Buczacki et al. 1975; Crute et al. 1983; Gustafsson and Falt 1986; Yu 2019; Fredua-Agyeman et al. 2019). Many of these resistances may have been derived from the European turnips (Bradshaw et al. 1997). Lammerink (1967) reported two dominant genes controlling resistance to race B and race C in New Zealand giant rape (forage rape) and Wilhelmsburger swede. Johnston (1970) reported a single dominant gene conferring resistance to race N4 in New Zealand giant rape and Wilhelmsburger. Ayers and Lelacheur (1972) reported that cv. Wilhelmsburger carries one gene for resistance to race 3 and two genes for resistance to race 2. In the case of rutabaga cv. York, a single dominant gene confers resistance to both races (races 2 and 3). Thus, it is apparent that more than one major clubroot resistance locus can be found in rutabaga, and these loci can confer resistance to more than one pathotype. Indeed, mapping of resistance by using rutabaga cv. Brookfield, Hasan and Rahman (2016) identified a genomic region of chromosome A08 conferring resistance to pathotypes 2, 3, 5, 6, and 8.

1.4.5. The genetic basis of resistance in *Brassica juncea*

Despite resistance being found in the diploid parental species of *B. juncea*, *i.e.*, in *B. rapa* (Buczacki et al. 1975; Crute et al. 1983; Hasan et al. 2012; Peng et al. 2014*a*; Fredua-Agyeman

et al. 2019) and *B. nigra* (Hasan et al. 2012; Peng et al. 2014*a*; Fredua-Agyeman et al. 2019), clubroot resistance has rarely been reported in natural *B. juncea* (Hasan et al. 2012; Peng et al. 2014*a*; Zhang et al. 2015). Liu et al. (2018) reported a single *B. juncea* accession '1012' carrying resistance to *P. brassicae* pathotype 4. Hasan and Rahman (2018) synthesized a clubroot resistant *B. juncea* line from an interspecific cross between clubroot resistant *B. rapa* ssp. *rapifera* cv. Gelria and clubroot susceptible *B. nigra* accession 'CR2137'.

1.4.6. The genetic basis of resistance in *Brassica carinata*

As mentioned above, clubroot resistant germplasm was reported in the diploid species *B. nigra* and *B. oleracea* (Hasan et al. 2012; Peng et al. 2014*a*; Fredua-Agyeman et al. 2019), yet not in the amphidiploid species *B. carinata*. However, by using clubroot resistant accessions of *B. nigra* and *B. oleracea*, a clubroot resistant *B. carinata* line can be resynthesized as has been done to develop a clubroot resistant *B. juncea* (Hasan and Rahman 2018).

1.5. Comparative genetic analysis of the CR loci

The clubroot resistance genes might have originated from a conserved Major Resistance Clusters (MRCs) of the ancestral genome (Suwabe et al. 2006, 2012). The MRCs are a cluster of *R*-genes, where individual genes evolved through duplication events, and their sequences are highly homologous (Michelmore and Meyers 1998; Leister 2004). Comparative analysis of the diploid *Brassica* species and their close relatives indicated that the diploid *Brassica* species evolved from a common hexaploid ancestor (Lagercrantz and Lydiate 1996; Lysak et al. 2005, 2007; Parkin et al. 2005; Cheng et al. 2013) through triplication of the ancestral genome followed by the reshuffling of the genomic blocks and change in chromosome number (Blanc et al. 2000; Vision et al. 2000; Cheng et al. 2013). Micro-synteny analysis indicated that for each genomic fragment of *Arabidopsis thaliana,* three syntenic copies are present in *B. rapa* (Wang et al. 2011). Rana et al. (2004) studied aspects of gene conservation and micro-synteny of *B. napus* genome segments with its diploid relatives using the *Arabidopsis* genome

sequence. Rana and coworkers reported highly conserved collinearity of the conserved genes with their orthologues across the genome. This indicates that upon polyploidization, the conserved segments of the Brassica A and C genomes did not undergo a burst of evolution to be discernible at the microstructure scale.

Synteny analysis by using molecular markers showed that the genomic region of C02 of *B. oleracea* carrying the CR QTL *pb-Bo(Anju)1* and *pb-Bo(Anju)2* (Nagaoka et al. 2010) and *QTL-GN_1* (Lee et al. 2016) are collinear with the genomic region of A02 of *B. rapa* harbouring *CRc*. The distal end of chromosome C03 carrying the QTL *pb-Bo(Anju)3* is collinear with a genomic region of chromosome A03 (Nagaoka et al. 2010). Chromosome A03 has been reported to carry the CR loci *Crr3, CRk,* and *CRd,* and this genomic region is collinear with the genomic region of C03 carrying the QTL *CRQTL-GN_2* and *CRQTL-YC*. On the other hand, the genomic region of C07 carrying *pb-Bo(Anju)4* is homoeologous to a part of A03 where *CRb* has been mapped (Nagaoka et al. 2010). Dakouri et al. (2018) reported two genes, *Bo7g108760* and *Bo7g109000*, encoding TNL (TIR-NBS-LRR) disease resistance proteins in a genomic region of C07 housing *Rcr7*. These two genes are homoeologous to the *B. rapa* TNL genes *Bra019305* and *Bra019277*, respectively, located on chromosome A03. The approximately 25 Mb region of A03 (as per *B. rapa* cv. Chiifu-401 whole-genome assembly v.3.0) houses the genetic markers B4732 and B1005 linked to *CRa*/*CRb* (Kato et al. 2013; Lee et al. 2016). The marker sequences of the CR locus of the *B. napus* cv. Mendel reported by Fredua-Agyeman and Rahman (2016) positioned between 25,522,010 and 25,835,881 bp region of A03 (as per *B. rapa* cv. Chiifu-401 whole-genome assembly v.3.0); this further support the existence of sequence synteny between the genomic region of C07 of *B. oleracea* housing *Rcr7* with the genomic region of A03 housing the *CRa/CRb* locus.

As reviewed above, chromosome A03 carries several CR loci (Table 1.1). The CR loci, *Rcr1* (Chu et al. 2014), and *Rcr2* (Huang et al. 2017) were mapped at an interval of 24,574,673

and 25,715,499 bp of A03 of *B. rapa* cv. Chiifu-401 whole-genome assembly v.3.0. This genomic region is known to carry the clubroot resistance locus *CRa/CRb* and houses six TNL genes, *Bra012541, Bra019406, Bra019409, Bra019410, Bra019412,* and *Bra019413,* encoding NBS-LRR class disease resistance proteins. However, a BLASTn search indicated that the *CRa* was homologous to the TNL gene *Bra019409*, however, not to *Rcr1* (Yu et al. 2016). A QTL for clubroot resistance, *Rcr4* (Yu et al. 2017), and a major locus, *Rcr5* (Huang et al. 2019), have also been mapped to the *Rcr1*/*Rcr2*/*CRa* region. The locus *CRd* was mapped upstream of *Crr3* to a 60 kb (1 cM) region between the markers yau389 and yau376 located on A03, and a total of four genes, *Bra001160*, *Bra001161*, *Bra001162*, and *Bra001175*, which encode TNL protein were identified in this gene region (Pang et al. 2018).

The locus *CRs* is located at intervals between 11,344,437 and 12,169,364 bp of chromosome A08 of *B. rapa* cv. Chiifu-401 whole-genome assembly v3.0 (Laila et al. 2019). Hasan and Rahman (2016) also mapped a CR locus in the same genomic region (12,116,024 bp - 13,046,214 bp per *B. rapa* cv. Chiifu-401 whole-genome assembly v3.0) of A08 of *B. napus*. This region houses 201 genes, including the TNL genes, *Bra020918,* and *Bra020876* (Hasan and Rahman 2016).

1.6. Molecular biology of clubroot resistance in Brassica

1.6.1. Brassica-*Plasmodiophora brassicae* pathosystem

The plant relies on its innate immunity to defend itself from pathogen attack (reviewed by Maekawa et al. 2011). The innate immune system relies on detecting pathogenic molecules followed by activation of disease resistance responses (reviewed by Andersen et al. 2018). Once the pathogen overcomes the mechanical barrier of the plants, such as bark and waxy cuticles, pattern recognition receptors (PRRs), located in the plasma membrane, recognize the conserved pathogen-associated molecular patterns (PAMPs) and activate the pattern-triggered immunity (PTI) (reviewed by Jones and Dangl 2006; De Lorenzo et al. 2011; Zipfel 2014)

(Figure 1.3). PTI is the first level of the immune response providing a broad-spectrum resistance to a pathogen (reviewed by Boller and Felix 2009). The PAMPs are highly conserved microbe-specific molecules, which includes flagellin (Flg) (Felix et al. 1999), elongation factor Tu (EF-Tu) (Kunze et al. 2004), peptidoglycan (PGN) (Gust et al. 2007), lipopolysaccharides (LPS) (Newman et al. 1995), ß-glucans elicitor (Umemoto et al. 1997; Shimizu et al. 2010), ethylene-induced xylanase (EIX) (Ron and Avni 2004), and cellulose-binding elicitor lectin (CBEL) (Gaulin et al. 2006; Newman et al. 2013). Virulent pathogens can overcome the PTI by delivering effector molecules inside the host cells (reviewed by Rafiqi et al. 2012). The nucleotide-binding (NBS) domain and leucine-rich repeats (LRR), commonly known as the NBS-LRR family of intracellular receptor proteins, encoded by *R*-gene (Dangl and Jones 2001), detects the presence of effectors (reviewed by Eitas and Dangl 2010) and results in effector-triggered immunity (ETI) (reviewed by Jones and Dangl 2006). The ETI is an accelerated and amplified form of PTI response (Hou et al. 2011), resulting in hypersensitive cell death (HR) type resistance at the infection site (reviewed by Ting et al. 2008). The precise mechanism of the activation of NB-LRR protein and its role in ETI yet to be clarified in the Brassica-*P. brassicae* pathosystem.

Research on clubroot disease and *P. brassicae* have been conducted for more than a century; however, very little is known about *P. brassicae* effectors and their role in the infection process (Pérez-López et al. 2018). This section discusses the advances in identifying *P. brassicae* genes and effector proteins required for infection of host plants. Feng et al. (2013*b*) observed an up-regulated expression of 58 genes and down-regulated expression of 55 genes in secondary zoospores compared to primary zoospores of *P. brassicae,* indicating that the primary and secondary zoospores utilize different mechanisms for infection. Fei et al. (2016) also found upregulation of two *P. brassicae* genes for primary infection and three genes for secondary infection and upregulation of seven other genes during gall development. They

identified a single gene, *Cr811,* in *P. brassicae* pathotype 5 and other newly emerged virulent pathotypes and concluded that *Cr811* plays a major role in the interaction between host resistance and pathogenicity of *P. brassicae* pathotype 5.

Before the advent of high throughput sequencing technologies, only a few genes have been reported by different researchers to be involved in the pathogenicity of *P. brassicae* (Ito et al. 1999; Brodmann et al. 2002; Siemens et al. 2009; FENG et al. 2010). For instance, using Northern and Southern blot analysis, Ito et al. (1999) identified a transcript of *Y10* of the vegetative stage of *P. brassicae* growing *in planta* of a susceptible Chinese cabbage. Similarly, Siemens et al. (2009) found a strong expression of two *P. brassicae* genes, *PbBrip9* and *PbCC249*, in *Arabidopsis* root galls at the sporulation stage of plasmodia (14, 21, and 28 dai). In particular, *PbBrip9* expression was high at 21 dai; this stage coincides with the occurrence of plasmodia inside the galls, *i.e*., the time of switching from vegetative to sporulation stage. Feng et al. (2010) reported a gene encoding a serine protease (*PRO1*) involved in clubroot pathogenesis that stimulates resting spores' germination by its proteolytic activity.

Recently, transcriptome analysis has been employed to identify the effector proteins secreted by *P. brassicae* during primary and secondary infection stages (Chen et al. 2019; Pérez-López et al. 2020). Chen et al. (2019) identified 33 secretory proteins expressed during primary infection (3 dai) of a susceptible oilseed *B. napus*, two proteins, PBCN_002550 PBCN_005499, induced cell death in *Nicotiana benthamiana* leaves, and the protein PBCN_002550 induced cell death in Chinese cabbage hypocotyls. By using the RNA-Seq approach, Pérez-López et al. (2020) identified a total of 32 highly expressed small secreted *P. brassicae* proteins (SSPbPs) during secondary infection (17, 20, and 24 dai) of susceptible *Arabidopsis*. Thirty-one of these SSPbPs were found to contain signal peptides such as RxLR (arginine–any amino acid–leucine–arginine) motif, DEER (aspartate-glutamate-glutamate-arginine) motif, Pexel motif, ankyrin domains, and cysteine-rich domains responsible for the

secretion of these effector proteins. Expression profiling of 21 selected genes, encoding the SSPbP proteins, by Real Time-PCR (RT-PCR) over a time course experiment (0, 2, 5, 7, 14, 21, and 28 dai) verified their involvement in a secondary infection. While a Canadian pathotype P3 (Pb3) was used to generate RNA-Seq data by Pérez-López and coworkers, another research group used single spore isolates of two *P. brassicae* pathotypes (Pb3, 16/2/12) for transcriptome analysis during infection of clubroot-susceptible *B. napus* and *Arabidopsis* (Rolfe et al. 2016). Differential expression of a total of 617 Pb3 genes, involved in carbohydrate processing, amino-sugar and nucleotide sugar metabolism, motor proteins and flagella-associated proteins, was identified during the 6-week course of infection of *B. napus.* Out of these 617 DE genes, 41 genes were predicted to be secreted proteins, with 14 of them being SSPbPs. A predicted chitin-binding domain (PbPT3Sc00048_S_5.266_1, CBM18) was found to be highly expressed during primary infection (7 dai), indicating its putative role in germination and formation of spores. As an obligate parasite, *P. brassicae* lost its capacity to synthesize several amino acids, as evident from the absence of the genes involved in the biosynthesis of amino acids such as histidine and valine/leucine/isoleucine in its genome or incomplete biosynthetic capacity to synthesize threonine, methionine, arginine and lysine (Rolfe et al. 2016). However, transcriptome analysis showed that the pathotype Pb3 contained ten putative amino acid transporters; therefore, it can be assumed that these transporters acquire amino acids from the host plants for the growth and development of the pathogen. Nonetheless, the annotated sequence data reported by Rolfe et al. (2016) lay the foundation for understanding the role of the genes of *P. brassicae* in the development of clubroot disease symptoms.

1.6.2. Utilization of -omics to understand the molecular mechanism for clubroot resistance.

Susceptible, partially susceptible, partially resistant, and even completely resistant Brassica plants are prone to infection by primary zoospores (Kroll et al. 1983; Deora et al. 2013; Wagner et al. 2019; Summanwar et al. 2019); however, completely resistant plants

exhibit resistance during the initial stage of secondary infection (Deora et al. 2012; Hatakeyama et al. 2013; Wagner et al. 2019). Proteomic analysis indicated that the *R*-gene encodes TIR-NBS-LRR protein in stele and cortex hypocotyl, *i.e*., at the site of secondary infection, but not in the root hairs where primary infection occurs (Hatakeyama et al. 2013). The NBS-LRR (NLR) is the largest group of plant *R* proteins, with about 167 genes in *A. thaliana* (Yu et al. 2014) and about 508 genes in rice (Li et al. 2010*a*). Alamery et al. (2018) reported 641 NLR genes in *B. napus*, 249 in *B. rapa,* and 443 in *B. oleracea*. Based on the coding sequence of the N-terminal domain, these genes can be grouped into three subclasses comprising an N-terminal toll-interleukin-1 (TIR) domain (TIR-NBS-LRR) (Akira and Hemmi 2003), a coiled-coil (CC) domain (CC-NBS-LRR) (Meyers et al. 2003) and an N-terminal domain of the as yet unknown function (X-NBS-LRR) (Monosi et al. 2004). Genes encoding TIR-NBS-LRR class proteins have been reported to be involved in clubroot resistance in *B. rapa* (Ueno et al. 2012; Hatakeyama et al. 2013; Zhang et al. 2014; Yuan et al. 2015; Huang et al. 2017; Yu et al. 2017; Chang et al. 2019) and *B. oleracea* (Dakouri et al. 2018). The LRR domain of the *TIR-NBS-LRR* gene plays a role in recognition specificity (Collier and Moffett 2009), while the TIR domain plays a signalling role in the induction of defence response (Swiderski et al. 2009). Hatakeyama et al. (2013) observed complete susceptibility to clubroot disease in *B. rapa* due to the lack of about half of the TIR domain, while Swiderski et al. (2009) observed complete loss-of-function of the *TNL* (*TIR-NBS-LRR*) gene due to a mutation at its TIR domain induced by site-directed mutagenesis.

In addition to understanding the genetic control of clubroot resistance, including molecular mapping of this trait in Brassica crops, researchers have begun to utilize various 'omics' techniques to obtain a better insight into molecular mechanisms involved in hosts in response to *P. brassicae* infection and for identification of candidate resistance genes. Several 'omics' studies, including proteomics (Devos et al. 2006; Cao et al. 2008; Ji et al. 2018; Lan et al. 2019)

and transcriptomics (Table 4), have been conducted to understand the molecular mechanism involved in infection by *P. brassicae* and resistance in Brassica crops. Devos et al. (2006) identified 35 up-regulated and 11 down-regulated proteins, which were mainly associated with defence, cell differentiation, cell metabolism, and active oxygen activity in roots of clubroot susceptible *Arabidopsis* at four dai. Changes in the expression of 20 proteins, including those involved in the synthesis of lignin, cytokinin, glycolysis, and detoxification of ROS, at 12, 24, 48 and 72 hours after inoculation (hai) has also been reported in a susceptible *B. napus* cultivar by Cao et al. (2008). High-throughput profiling of protein species using the isobaric tags for relative and absolute quantitation (iTRAQ)-based quantitative proteomics analysis identified 5,003 proteins in resistant and susceptible Chinese cabbage lines after infection by *P. brassicae* (Lan et al. 2019). Among these, 487 proteins (13.4%) were up-or down-regulated, indicating that infection by *P. brassicae* strongly influences the physiology of the plant. This is also evident from the upregulation of the early nodulin protein in susceptible plants in response to infection, which can change hormone balance in roots and thus result in hypertrophy and gall formation ─ the typical clubroot disease symptom (Lan et al. 2019).

With the advances in next-generation sequencing technologies, RNA-seq has become a powerful and widely used approach for profiling transcriptomes (Wang et al. 2009b). Several researchers have used this technique to study interactions between *P. brassicae* and their *Brassica* hosts to detect candidate genes involved in host resistance (Table 1.4). Upregulation of several pathogenesis-related (*PR*) and differentially expressed genes (*DEGs*) with TIR-NB-LRR and NBS-LRR domains have been reported in clubroot resistant *B. rapa* inoculated with *P. brassicae* (Chen et al. 2016; Jia et al. 2017; Fu et al. 2019). The following sections will review the changes in different metabolic pathways in host plants in response to infection by *P. brassicae* (Figure 1.3).

1.6.2.1. ***Cell wall modifications***

Upon infection of the host root hair by *P. brassicae*, marked morphological changes, such as gall formation, occur in the roots due to abnormal cell division and enlargement. Of the different parts of a plant cell, the cell walls are very dynamic, which are modulated and remodelled during normal developmental processes, such as cell growth and elongation, and in response to pathogen attack (Underwood 2012). The plant cell wall comprises polysaccharides, cellulose, hemicelluloses, pectins, lignin, and glycol proteins. Proteins and enzymes which can modify and degrade the cell wall components include expansins, polygalacturonases (PGs), xyloglucan endotransglycosylases/hyrdolases (XTHs), pectate lyases (PLs) and endo-β-1, 4 glucanases (EGases). All these play an essential role in maintaining cell wall dynamics, e.g., cell wall expansion and deposition of newly synthesized cell wall components (for review, see Sasidharan et al. 2011). These enzymes are also necessary for infection and the establishment of plant pathogens (for review, see Sasidharan et al. 2011). Siemens et al. (2006) reported that some of the genes involved in cell division and expansion were upregulated at 10 and 23 dai in susceptible *Arabidopsis*.

The expression of caffeoyl-CoA O-methyltransferase (*CCoAOMT*), which plays a vital role in synthesizing guaiacyl lignin units, was down-regulated in susceptible canola line 'Westar' following infection by *P. brassicae* (Cao et al. 2008). In another study, the expression of three genes, 4-coumarate-CoA ligase (*4CL1*), cinnamyl coenzyme A reductase (*CCR1*) and cinnamyl alcohol dehydrogenase (*CAD5*), involved in lignin biosynthesis has been found to be downregulated in susceptible *Arabidopsis* at four dai (Agarwal et al. 2011). Similarly, expression of *PAL* (phenylalanine ammonia lyase), *C4H* (cinnamate 4-hydroxylase), *4CL* (4-coumarate: CoA ligase), *CCoAOMT* and *CCR1* were found downregulated in Brassica plants after infection (Badstöber et al. 2020). In contrast, an upregulation in the expression of *CCR1* and *CCoAOMT* has been reported by Zhao et al. (2017) in *Arabidopsis* at 24 hai; however, this

research group found downregulation of *CAD5*. While working with *B. oleracea,* Zhang et al. (2016*b*) found an increased expression of six genes associated with the phenylpropanoid biosynthesis pathway (involved in the biosynthesis of guaiacyl and syringyl lignin – the crucial components of the cell wall) in clubroot resistant plants as compared to susceptible ones. Lignification of cell wall provides a defence to the host against fungal infection (for review, see Miedes et al. 2014); in this regard, a decreased expression of the genes involved in lignin biosynthesis can result in a decreased content of lignin in the cell wall, and thereby allow a greater attack by *P. brassicae* resulting in an increased formation of galls in clubroot susceptible plants. Several other genes involved in cell wall formation and stability, such as one isoform of GAUT (a-1,4-galacturonosyltransferase) enzyme, which are essential for the biosynthesis of plant cell wall component pectic polysaccharide homogalacturonan (Zhang et al. 2016*b*), the pectin methylesterases, which changes the pectin pattern and thus are involved in regulating the permeability and stability of cell walls (Wang et al. 2019), and the pectin esterase inhibitor protein, which could inhibit cell wall formation and facilitate root hypertrophy through the formation of the vacuole (Lan et al. 2019), were found to be upregulated in different susceptible Chinese cabbage cultivars. On the other hand, the expression of transcripts involved in the synthesis of pectins, such as rhamnose I and homogalacturonan (Badstöber et al. 2020), as well as many cell wall degradation genes such as pectinases, glucanses and cellullase, were found to be downregulated (Irani et al. 2018) in clubroot infected roots of Brassica and *Arabidopsis*. The down-regulation of hydrolytic enzymes in *Arabidopsis* suggests their possible role in cell wall modifications during the infection process (Irani et al. 2018). The depolymerization of pectin during *P. brassicae* infection serves three purposes: (i) reduce the rigidity of the plant cell wall, (ii) the depolymerized pectin serves as a carbon source for the pathogen, and (iii) increase the accessibility of the cell wall components to cell wall degrading enzymes (CWDEs) (Bauer et

al. 1977; reviewed in Jia et al. 2009). *Plasmodiophora brassicae* infection decreases the host root cell walls' rigidity and stability by downregulating the biosynthesis of cell wall components like pectin, lignin, cellulose and enlarges host cells by upregulating CWDEs that destabilize cell wall structure. Based on the literature reviewed above, it is apparent that lignification, pectin deposition and reinforcement of cell walls are the critical processes in plants in response to infection by *P. brassicae*.

1.6.2.2. ***Hormonal changes after Plasmodiophora brassicae infection***

During the development of root galls, changes in the levels of plant growth hormones, such as auxins, cytokinins, salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), play an essential role (Ludwig-Müller 2009*a*). For a detailed review of auxin and cytokinin biosynthesis and metabolism, see Ljung (2013) and Mok and Mok (2001), and for their role in clubroot disease, see Ludwig-Müller (2014) and Malinowski et al. (2016). According to Siemens et al. (2006), cytokinin plays a vital role during the formation of root galls. Interconverting cytokinin ribosides regulate active cytokinin levels in the plant to corresponding nucleotides (Vo et al. 1998). Devos et al. (2006) observed *P. brassicae* infection to downregulate enzyme adenylate kinase *ADK* in susceptible *Arabidopsis*, indicating its role in maintaining a high level of active cytokinins during disease development. Siemens et al. (2006) reported downregulation of two cytokinin biosynthesis genes and cytokinin oxidases/dehydrogenases in *P. brassicae* infected clubroot susceptible *Arabidopsis* through microarray analysis. Inhibition of these cytokinin-related genes during *P. brassicae* infection might increase the content of cytokinins at the site of infection (Figure 1.3).

Auxins have also been considered essential pathogenicity factors during infection by *P. brassicae* (for review, see Ludwig-Müller 2014). This growth hormone has been reported to increase during clubroot disease development at various time points in *Arabidopsis* (Devos et al. 2006). Differential regulation of the genes involved in the synthesis of indole-3-acetic-acid

(IAA) has been found in infected *Arabidopsis* through microarray analysis by Siemens et al. (2006). The enzyme nitrilase is known to catalyze IAA biosynthesis from indole acetonitrile (Park et al. 2003). Increased expression of two isoforms of the nitrilase gene, *NIT1* and *NIT2*, was found in susceptible *Arabidopsis* due to *P. brassicae* infection (Grsic-Rausch et al. 2000; Siemens et al. 2006). Similarly, increased nitrilase activity and increased IAA content have been observed in clubroot susceptible cultivars of turnip and Chinese cabbage during this disease development (Ugajin et al. 2003; Ando et al. 2008). In a comparative transcriptomic study, Jia et al. (2017) found a total of 18 DEGs associated with IAA and cytokinin signalling pathways. These 18 DEGs were exclusively upregulated in a clubroot susceptible *B. rapa*, while no change in their expression could be detected in the resistant line infected by *P. brassicae*. Expression of the genes encoding auxin-responsive proteins has also been found to be downregulated in clubroot-resistant canola after infection by *P. brassicae* (Li et al. 2020). Similar transcriptome analysis of *Arabidopsis* at early stages of infection (24-48 dai) identified upregulation of up to 24 genes involved in the biosynthesis of IAA and up to three genes involved in the cytokinin pathway (Zhao et al. 2017). Thus, the upregulation of auxin and cytokinin-related genes in susceptible lines due to infection by *P. brassicae* indicates their involvement in uncontrolled cell division and root swelling during the development of clubroot disease symptoms. This is further supported by the upregulation of the negative regulators of auxin synthesis and transport in clubroot-resistant *B. oleracea* (Ning et al. 2019); upregulation of such genes might result in a decreased content of IAA and, thus, prevent the enlargement of root galls in the resistant lines. This corroborates the result reported by Summanwar et al. (2019) that *P. brassicae* infection occurs at an early growth stage in both resistant and susceptible *B. napus* plants; however, disease progression is repressed in the resistant plant two weeks after inoculation.

Among the different plant secondary metabolites, increased abundance of some of the glucosinolates, such as indole glucosinolates, during clubroot disease development in susceptible plants, and the role of aliphatic glucosinolates in defence against *P. brassicae* have been discussed in detail by Ludwig-Müller (2009*b*). Flavonoids are another class of secondary metabolites reported to be involved in auxin transport and breakdown (Mathesius 2001; Peer and Murphy 2007). This group of compounds has also been reported to be accumulated in root galls of clubroot susceptible *Arabidopsis* due to infection by *P. brassicae,* and this increase has been corroborated with an upregulation of the genes involved in flavonoid biosynthesis (Päsold et al. 2010; Zhao et al. 2017)

In plants SA, JA and ET are involved in mediating major disease signalling pathways. SA is engaged in a plant's response to biotrophs/hemibiotrophs, such as powdery mildew. Simultaneously, JA and ET-dependent defences are associated with necrotrophs, such as Botrytis (for review, see Li et al. 2019). Exogenous application of SA and JA application significantly decreases root galls' formation in *B. oleracea* and *Arabidopsis* due to infection by *P. brassicae* (Lovelock et al. 2013; Lemarié et al. 2015). Biosynthesis of SA in plants largely relies on the genes *NPR1* (nonexpressor of PR gene 1), a master regulator in SA signalling, and *ICS1* (isochorismate synthase 1), a key enzyme required for the biosynthesis of this phenolic acid plant hormone (Glazebrook 2005). Chen et al. (2016) reported an upregulation of *NIMIN2* in clubroot-resistant *B. rapa,* which interacts with *NPR1*, *PR1* and *PR2* genes. Ning et al. (2019) found increased *NPR1* transcripts in a resistant *B. oleracea* after infection by *P. brassicae*, indicating the induction of the SA signalling pathway in the resistant plants. In contrast, Wang et al. (2019) found no significant difference in the expression of *NPR1* and *ICS1* while working with a different resistant cabbage accession. The difference between the genes involved in the biosynthesis of SA and their relationship with clubroot resistance might be due to the difference in time points at which the samples were collected and used for

molecular analyses. For instance, root tissue was collected at 0, 12, 72, and 96 hai by Chen et al. (2016), while Ning et al. (2019) conducted transcriptome analysis at 7 and 28 dai, and Wang et al. (2019) collected root tissue at 3 dai.

In the case of JA, an accumulation of this plant hormone in developing galls (Gravot et al. 2012) and the induction of expression of several JA-responsive genes have been reported in infected root tissues of clubroot-susceptible *Arabidopsis* (Siemens et al. 2006; Gravot et al. 2012). Similarly, a higher accumulation of JA was found in susceptible *B. napus* plants than resistant plants at 14-28 dai (Xu et al. 2018). Ning et al. (2019) found the expression of seven JAZs (Jasmonate ZIM domain-containing protein) upregulated in a clubroot resistant *B. oleracea* but down-regulated in the susceptible plants. Jasmonate ZIM domain-containing protein acts as JA co-receptors and transcriptional repressors in the JA signal pathway. Eight genes related to JA biosynthesis, signalling, and jasmonate-zim-domain protein have also been found to be downregulated in a resistant *B. rapa* after infection (Chen et al. 2016). The importance of JA in clubroot disease development has also been demonstrated by Gravot et al. (2012) by using mutant *Arabidopsis* lines, where the jasmonate resistant 1 (*jar1*) mutant lines with impaired JA-Ile accumulation showed a greater disease symptom as compared to the wild type. Thus, it is apparent that plant hormones auxin and cytokinin play a critical role in disease development and SA in host resistance. In contrast, JA plays a role in the compatibility interaction of the host with *P. brassicae*.

1.6.2.3. ***Role of sugars and transporters in Plasmodiophora brassicae infection***

Sugars act as the primary energy source; they are synthesized in leaves and translocated to sink organs via phloem sap (for review, see Lemoine et al. 2013). Sucrose transporter, a Mono-Saccharide Transporter (MST) (Toyofuku et al. 2000) and Sugars Will Eventually be Exported Transporters (*SWEET*s) (Chen et al. 2012) mediate sugar transport in plants (for review, see Julius et al. 2017). Several investigations have reported that an active carbohydrate sink

develops during gall development, preceded by an increased accumulation of cytokinin and local stimulation of extracellular cell wall invertase (cwINV) (Walters and McRoberts 2006; Siemens et al. 2011). A functional approach utilizing two invertase inhibitors ((with high homology to vacuolar (AtC/VIF1) and cell wall (AtC/VIF2) invertase inhibitors from tobacco)) showed a reduced clubroot symptom in transgenic *Arabidopsis,* indicating invertases' vital role in gall development (Siemens et al. 2011). In the case of the *SWEET*s, Li and coworkers (2018) identified a total of 32 *SWEET* genes forming four different clades in *B. rapa*, and Zhang et al. (2019*a*) identified 30 *SWEET* genes forming four different clades in *B. oleracea*. The expression level of the several *BrSWEETs* belonging to Clade I and III and six *BoSWEET* genes belonging to clade III was upregulated in clubroot susceptible lines compared to the control plants. None of these *BrSWEETs* and *BoSWEET* genes has been reported to be upregulated in clubroot resistant plants after infection by *P. brassicae*, indicating that these *SWEET* genes play a role in the transportation of the sugars to the sink roots of susceptible plants (Li et al. 2018; Zhang et al. 2019*a*). To further confirm the role of the *SWEET* genes in clubroot disease development, Li et al. (2018) tested *Arabidopsis sweet11* mutants inoculated with *P. brassicae* and found a significantly lower disease symptom in the mutant line as compared to the wild type. Similar results about the involvement of the *SWEET* genes in plant-pathogen interaction have also been reported by Walerowski et al. (2018) using an *Arabidopsis* double mutant line (*sweet 11;12*). This research group also found a significant increase in glucose and fructose in the roots of clubroot susceptible plants compared to the resistant plants. Similar results have also been reported in clubroot susceptible *Arabidopsis* during root gall development (Brodmann et al. 2002), suggesting that sucrose transport and rapid metabolism from leaves to clubbed roots occur in the susceptible plants. Recently, a total of 22 *Sugar Transporter Protein* (*STP)* genes, with a conserved sugar transporter domain, have been identified in the *B. oleracea* genome (Zhang et al. 2019*b*). The role of these *STP* genes in the partitioning of sugar during

the development of clubroot galls has been demonstrated by Zhang et al. (2019*b*) through the expression analysis of two members of this family*, BoSTP4b* and *BoSTP12*, in roots of a clubroot-susceptible cabbage inoculated with *P. brassicae*. Several other transcriptomics studies with *Arabidopsis*, *B. oleracea,* and *B. napus* roots infected by *P. brassicae* have also revealed an upregulation of the genes involved in synthesizing the sucrose-degrading enzymes and biosynthesis of hexose sugars, sugar transporters, and sugar metabolism (Irani et al. 2018; Zhang et al. 2019*b*; Li et al. 2020). Thus, the studies mentioned above suggest that infection by *P. brassicae* probably triggers an active translocation and partitioning of sugar between the source and the clubbed root tissues, and the genes involved in the metabolism and transport of sugar influence plant-*P. brassicae* interactions.

1.6.2.4. ***Non-coding RNAs and their role in clubroot development***

Emerging evidence suggests that non-coding RNAs such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) play an essential role in regulating gene expression in response to abiotic and biotic stresses (Nejat and Mantri 2018; for review see, Shriram et al. 2016). The most well-known class of short non-coding RNAs (18–22 nt) is miRNAs; many miRNA families are reported to be highly conserved across major plant lineages (Axtell and Meyers 2018; for review see, Bartel 2004) and regulate the expression of mRNAs through two main mechanisms: (i) mRNA cleavage and (ii) translational inhibition (Rhoades et al. 2002). The degree of complementarity between the miRNA and its binding site within the target mRNA decides its mode of action; a high complementarity between miRNA and its binding site within the target implies miRNA mediated cleavage of the target, while low complementarity mediates translational inhibition (Iwakawa and Tomari 2015). Although several studies have been reported for the role of miRNAs in regulating plant response to various biotic stresses such as infection by bacteria, fungi, and viruses (for review, see Song et al. 2019), little information is available about the expression pattern of miRNAs in Brassica

after infection by *P. brassicae*. Verma et al. (2014) studied the changes in miRNA profile in canola after infection by *P. brassicae* at 10 and 20 dai using a miRNA-based microarray and identified 10 miRNAs showing differential expression at 10 dai and 34 miRNAs at 20 dai. These differentially expressed (DE) miRNAs included the miRNAs which have been reported to be responsive to infection by powdery mildew in wheat (miR396) (Xin et al. 2010), rust fungus in pine (miR160) (Lu et al. 2007), and pathogen attack in *Arabidopsis* (miR169) (Li et al. 2010*b*). Downregulation of miR396 during infection by *P. brassicae* was speculated to impact clubroot disease development as TIR1 (Transport Inhibitor Response 1 protein), a known regulator of auxin signalling in response to biotic and abiotic stress, was found to be one of the targets of this miRNA (Verma et al. 2014). Wei et al. (2016) identified 14 known, and 10 novel differentially expressed miRNAs in Chinese cabbage infected by *P. brassicae*. Among these, seven miRNAs exhibited up-regulated expression while the others, including miR164a, were down-regulated due to this infection. Guo et al. (2005) reported miR164a to be involved in auxin homeostasis and lateral root development and, thus, miR164a may play a role in clubroot disease development (Wei et al. 2016).

Long non-coding RNAs (lncRNAs) are a group of poorly conserved RNA molecules with a sequence length of more than 200 nucleotides. lncRNAs are known to regulate protein modification, chromatin remodelling, protein functional activity, and RNA metabolism *in vivo* through cis- or trans-activation at the transcriptional, post-transcriptional, and epigenetic levels (for review, see Peschansky and Wahlestedt 2014). Zhu et al. (2019*a*) employed the RNA-seq approach and identified DE 5,193 mRNAs and 114 lncRNAs from the roots of Chinese cabbage infected by *P. brassicae*. Comparative analysis of the position of the lncRNAs and the loci associated with clubroot resistance located eight lncRNAs in six regions; for instance, the lncRNA TCONS_00007793 near the locus *Anju1* of the chromosome A02, two lncRNAs (TCONS_00007004, TCONS_00007046) near *Rcr8* of A02, the lncRNA TCONS_00014032

near *CRd* of A03, and the lncRNA TCONS_00038153 near *CRs* of A08 (Zhu et al., 2019*a*). In another study, a strand-specific lncRNA-Seq approach was employed to identify the lncRNAs associated with clubroot resistance in *B. napus* canola lines carrying resistance introgressed from rutabaga (A08) (Summanwar et al. 2019). This research group identified 530 DE lncRNAs, of which 170 were upregulated and 337 downregulated in the resistant plants. Among these, 15 lncRNAs were detected only in the resistant lines and eight on chromosome A08. Chromosome A08 carries a genomic region conferring resistance to five *P. brassicae* pathotypes (Hasan and Rahman 2016). The target genes of these eight lncRNAs belong to plant defence pathways (defensin, pathogen-related protein, and disease resistance protein). This research group also identified eight lncRNAs as possible precursors of miRNAs. Thus, these studies identified several non-coding RNAs to be involved, in coordination with transcriptional and post-transcriptional regulation of the genes, in resistance to clubroot disease; however, the biological functions of these lncRNAs and their target genes are yet to be elucidated.

1.7. Breeding for clubroot resistance

Resistance to disease in plants can be under mono-, oligo- or polygenic control. Mono- and oligogenic resistances are also called *R*-gene or major gene resistance. The *R*-genes play an essential role in recognizing the pathogen, which makes the host and pathogen incompatible, or the host may respond with a hypersensitive reaction and inhibit pathogen reproduction (for review, see Balint-Kurti 2019). The *R*-gene can provide complete resistance to infection; however, the resistance is often effective only against a specific pathotype or race of the pathogen. On the other hand, polygenic resistance is race non-specific and quantitatively inherited, where resistance results from the combined effect of many genes (Lindhout 2002). Quantitative resistance slows down infection development in host plants (Leclerc et al. 2019) rather than protecting them from becoming infected (as reviewed by Niks et al. 2015). Quantitative resistance is considered more durable than *R*-gene resistance (reviewed by Pilet-

Nayel et al. 2017); this type of resistance is often found in the Brassica C-genome. The major resistance genes from A-genome have been extensively used in the breeding of oilseed *B. napus* (for review, see Rahman et al. 2014) and vegetable *B. rapa* (for review, see Hirai et al. 2004); however, not much effort has been made to use C-genome resistance in the breeding of *B. napus*. This might be due to different constraints, including the difficulty of producing *B. napus* × *B. oleracea* interspecific hybrids and recovering a euploid *B. napus* canola plant (for review, see Rahman 2013). However, with the advances in our knowledge of the use of *B. oleracea* in the breeding of *B. napus* (Bennett et al. 2008; Rahman et al. 2015; Nikzad et al. 2019), it is expected that the clubroot resistance of *B. oleracea* will be used in the breeding of *B. napus* canola.

Pyramiding of multiple *R*-genes or major genes conferring resistance to various pathotypes (Boskovic et al. 2001; Werner et al. 2005; Feechan et al. 2015) or in combination with genes with minor effect (Ellis et al. 2014; Fukuoka et al. 2015) has been hypothesized to increase the durability of resistance (Mundt 1990; Li 2016; Pilet-Nayel et al. 2017). Pyramiding is a strategy of accumulation of multiple resistance genes into a single cultivar. The accumulated genes may impart resistance to either multiple pathotypes of a pathogen (Matsumoto et al. 2012; Feechan et al. 2015; Shah et al. 2019) or provide high-level resistance to a single pathotype (Tomita et al. 2013; Feechan et al. 2015). Matsumoto et al. (2012) found that pyramiding of three major clubroot resistance genes, *CRa, CRk,* and *CRc,* in a Chinese cabbage line gave resistance to six isolates of *P. brassicae*. Similarly, a pyramiding of a major gene and a minor gene has been reported to increase resistance to multiple pathotypes in *B. napus* (Shah et al. 2019) and *B. oleracea* (Tomita et al. 2013). The approach of gene pyramiding for resistance to multiple pathotypes has also been used in other crops, such as for rust (leaf, stem, and strip) and fusarium head blight resistance in wheat (Boskovic et al. 2001; Laroche et al. 2019), barley stem rust (Sharma Poudel et al. 2018) and barley yellow mosaic virus complex resistance (Werner et al.

2005), and blast resistance in rice (Fukuoka et al. 2015; Jiang et al. 2019). The success of gene pyramiding depends on the number of genes to be incorporated and the number of lines/cultivars to be used (Joshi and Nayak 2010). Verification of the number of resistance genes to be pyramided in a single line or cultivar is challenging. In essence, a plant carrying a single resistance gene can exhibit the same resistance phenotype as a plant carrying multiple resistance genes. In this regard, the development of gene-specific molecular markers that co-segregate with the resistance genes (Ruane and Sonnino 2007) and use them in marker-assisted selection (MAS) can resolve this constraint. Typically, DNA markers with a genetic distance of <1 cM from the gene of interest considered tightly linked and suitable for use in MAS. Also, validation of the markers in different breeding populations is essential for reliable use of the markers in breeding programs.

1.8. Conclusion and Future Perspectives

The difficulty of managing the clubroot disease in canola by cultural practices, as described in this review, brings the cultivation of resistant cultivars to the front line. Among the different resistance sources, *B. rapa* or the A-genome remains a good source for major gene resistance and *B. oleracea* as the source of quantitative resistance. The advancement of high-throughput sequencing technologies and bioinformatics approaches have facilitated our understanding of the CR genes and QTL and their relationships within and between the Brassica genomes. This review summarized the major CR genes/loci and QTL information so far been reported in Brassica; however, only a few major-gene resistances have been used in *B. napus* and vegetable *B. rapa* breeding. The naming of the CR loci has been done independently by different researchers, and many of these genes are reported from the same genomic region; therefore, different names given for some of these loci cannot be ruled out. Precise designation of these loci would enable efficient deployment of these genes in breeding; an international collaborative network for the nomenclature of the genes is needed. The availability of

genetically uniform single spore-derived *P. brassicae* isolates and our knowledge of Brassica genomes can greatly facilitate this. Ineffectiveness of some of the major resistance genes has been reported after growing a resistant cultivar for a period; in this regard, a pyramiding of multiple major genes and race non-specific QTL may increase resistance durability. However, the effective deployment of these genes also needs a knowledge of the ecology and life history of the host and pathogen and their interactions. Although significant progress has been made in understanding the complex hormonal and metabolic changes resulting in gall formation in roots, further research is needed to understand this reprogramming at cellular and molecular levels. Furthermore, the knowledge of the mechanisms underlying plant's response to *P. brassicae* via transcriptional and post-transcriptional (miRNA and lncRNA) means is still narrow; research is needed to fill the gaps in understanding these regulatory processes. Genome editing technologies such as *CRISPR*/*Cas9* system can be used to facilitate the validation and use of the candidate genes in breeding for clubroot resistance.

1.9. Research objective

The long-term goal of this research project is to understand the genetic control of clubroot resistance in Brassica, map the genes/QTL conferring resistance to this disease, and develop molecular markers linked to the resistance genes for use in marker-assisted selection (MAS) for the development of clubroot resistant cultivars. The primary objectives of this PhD thesis research are:

1. Study the genetic control of clubroot resistance in rutabaga (*Brassica napus* var. *napobrassica*) and molecular mapping of resistance.
2. Map the QTL affecting agronomic and seed quality traits and clubroot resistance, and study their associations in a rutabaga × spring canola population.
3. Study the genetic control of clubroot resistance in *Brassica rapa* ssp. *pekinensis* cv. Bilko and development of genetic markers for use in marker-assisted breeding.

4. Study the effect of A-genome-specific clubroot resistance in the resynthesized *Brassica juncea*.

I hypothesized that

1. Rutabaga cv. Brookfield carries major genes or QTL for resistance to *P. brassicae* pathotypes 2F, 3H, 5I, 6M and 8N.
2. Rutabaga possesses alleles that can improve the agronomic and seed quality traits of canola.
3. Clubroot resistance of rutabaga affects the agronomic and seed quality traits.
4. Chinese cabbage (*Brassica rapa* var. *pekinensis*) cv. Bilko carries a major clubroot resistance gene, and this resistance can be introduced in *B. napus* spring canola.
5. Clubroot resistance can be introduced into *B. juncea* from clubroot resistant *B. rapa* ssp. *rapifera* (turnip) through resynthesis of this species.

Results from my thesis research have been presented in the Chapters 2 to 5, and a general discussion on this thesis research has been built in Chapter 6.

1.10. Tables

Table 1.1: Clubroot resistance gene/loci mapped in *Brassica rapa*

Source (Germplasm)	Gene	LG	Reported as	Physical position (bp)[a]	Resistant to *P. brassicae*	Reference
B. rapa ssp. *pekinensis* cv. KU-101	*CR6a*	A01	QTL	-	Pathotype 6	Lee et al. (2002)
B. rapa ssp. *rapifera* cv. Siloga	*Crr2*	A01	Modifier gene	5,589,418 – 6,527,791	Pathotype 4	Suwabe et al. (2003, 2006)
B. rapa ssp. *rapifera* cv. Debra	*CRc*	A02	QTL, possibly as major gene	24,031,900 - 24,032,693	Race 2	Sakamoto et al. (2008)
B. rapa ssp. *rapifera* cv. Pluto	*Rcr8*	A02	QTL	22,502,031 – 26,348,249	Pathotype 5X	Yu et al. (2017)
B. rapa ssp. *rapifera* cv. Debra	*CRk*	A03	QTL, possibly as major gene	15,328,592 - 15,329,521	Race 2	Sakamoto et al. (2008)
B. rapa ssp. *chinensis* line 85-74	*CRd*	A03	Major gene	15,986,124 - 16,046,801	Race 4	Pang et al. (2018)
B. rapa ssp. *rapifera* 'Milan White'	*Crr3*	A03	Major gene	16,048,432 - 16,289,350	Pathotype 2	Hirai et al. (2004); Saito et al. (2006)
B. rapa ssp. *rapifera* cv. Pluto	*Rcr4*	A03	QTL, possibly as major gene	23,823,130 - 26,806,601	Pathotype 2, 3, 5, 6 & 8	Yu et al. (2017)
B. rapa ssp. *rapifera* cv. Purple Top White Globe	*Rcr5*	A03	Major gene	24,462,632 - 24,587,821	Pathotype 3	Huang et al. (2019)
B. rapa ssp. *chinensis* cv. Flower Nabana	*Rcr1*	A03	Major gene	24,574,673 - 25,715,499	Pathotype 3	Chu et al. (2014)
B. rapa ssp. *pekinensis* cvs. CR Saerona, Akiriso, CR Shinki DH	*CRb*	A03	Major gene	25,372,383 - 25,551,199	Race 4, Pathotype 2, 3, 4 & 8	Piao et al. (2004); Cho et al. (2008); Kato et al. (2012, 2013)
B. rapa ssp. *chinensis* cv. Jazz	*Rcr2*	A03	Major gene	25,287,088 - 25,568,685	Pathotype 3	Huang et al. (2017)
B. rapa ssp. *chinensis*	*CRa*	A03	Major gene	25,523,177 – 25,752,008	Race 2	Ueno et al. (2012)
B. rapa ssp. *chinensis*	*CRq*	A03	QTL, possibly as a major gene	25.7 Mb - 26.0 Mb[b]	Pathotype 2	Yuan et al. (2015)
B. rapa ssp. *pekinensis* line KU-101	*CR6b*	A03	QTL	-	Pathotype 6	Lee et al. (2002)
B. rapa ssp. *pekinensis* line 20-2cc1	*CrrA5*	A05	Gene	-	Field population	Nguyen et al. (2018)
B. rapa ssp. *rapifera* cv. Siloga	*Crr4*	A06	QTL	-	-	Suwabe et al. (2006)
B. rapa ssp. *chinensis* cv. Maikno	*qBrCR38-1*	A07	QTL	20,107,291 – 20,209,491	Race 7	Zhu et al. (2019b)
B. rapa ssp. *chinensis* cv. Akimeki	*CRs*	A08	Major gene	11,344,437 – 12,169,364	Pathotype 4	Laila et al. (2019)
B. rapa ssp. *rapifera* cv. Waaslander	*Rcr3*	A08	Gene	11,385,371 - 11,627,069	Pathotype 3	Karim et al. (2020)
B. rapa ssp. *rapifera* cv. Siloga	*Crr1a*	A08	Major gene	12,266,348 – 12,283,424	Pathotype 2	Suwabe et al. (2003, 2006); Hatakeyama et al. (2013)
B. rapa ssp. *rapifera* cvs. Pluto & Waaslander	*Rcr9*	A08	QTL	12,311,942 – 12,646,299	Pathotype 5X	Yu et al. (2017); Karim et al. (2020)
B. rapa ssp. *rapifera* cv. Siloga	*Crr1b*	A08	Gene	-	Pathotype 2	Hatakeyama et al. (2013)
B. rapa ssp. *chinensis* cv. Maikno (Pakchoi)	*qBrCR38-2*	A08	QTL	20,254,038 – 21,748,038	Race 7	Zhu et al. (2019b)

[a] Physical position has been determined by aligning flanking markers and co-segregating makers with *Brassica rapa* cv. Chiifu-401 whole-genome sequence assembly v.3.0 on http://brassicadb.org/brad/blastPage.php
[b] Physical position as reported in the article by Yuan et al. (2015)

Table 1.2: Clubroot resistance QTL/loci mapped in *Brassica oleracea*

Source (germplasm)	Gene or QTL	LG	LOD	R^2 [a]	Physical position (bp)	Resistant to *P. brassicae*	Reference
B. oleracea var. *capitata* cv. Böhmerwaldkohl	4 QTLs	-			-		Yoshikawa (1993)
B. oleracea var. *capitata* cv. Bindsachsener	pb-3	C03	27.5	54	-	EDC 16/3/30 (Buczacki et al. 1975)[b]	Voorrips et al. (1997)
	pb-4	C01	3.5	6	-		
B. oleracea var. *acephala* cv. K269	QTL1	C01	2.96	-	-	Pathotype 1 & 3 (Williams 1966) [b]	Moriguchi et al. (1999)
	QTL3	C03	16.34	-	-		
	QTL9	C09	3.52	-	-		
B. oleracea var. *acephala*	*Pb-Bo1*	C01	6.4 - 64.3	20.7 - 80.7	-	Pathotype 1, 2, 4 & 7 (Some et al. 1996) [b]	Rocherieux et al. (2004)
	Pb-Bo2	C02	5.6 - 13.0	2.5 - 21.0	-		
	Pb-Bo3	C03	2.6	4.7	-		
	Pb-Bo4	C04	3.9	2.8	-		
	Pb-Bo5a	C05	3.0 - 6.2	3.3 - 4.3	-		
	Pb-Bo5b	C05	3.8 - 4.3	3.0 - 6.1	-		
	Pb-Bo8	C08	4.1	2.1	-		
	Pb-Bo9a	C09	3	5.5	-		
	Pb-Bo9b	C09	5.2	3.4	-		
B. oleracea var. *capitata* cv. Anju	*PbBo(Anju)1*	C02	13.7	47	-	Race 4 (Williams 1966) [b]	Nagaoka et al. (2010)
	PbBo(Anju)2	C02	4.9	4	-		
	PbBo(Anju)3	C03	4.1	9	-		
	PbBo(Anju)4	C07	3.1	3	-		
	PbBo(GC)1	C05	5.1	9	-		
B. oleracea var. *capitata*	CRQTL_YC	C03	8.7	47.1	-	Race 2	Lee et al. (2015)
	CRQTL-GN_1	C02	3.4 - 4.9	22.0 - 29.7	-	Race 9 (Williams 1966) [b]	
	CRQTL-GN_2	C03	3.7 - 4.7	23.5 - 29.1	-		
B. oleracea var. *capitata* cv. Tekila	Rcr7	C07	-	-	42,863,773 – 42,889,307	Pathotype 3 & 5X L-G2 (Strelkov et al. 2018) [b]	Dakouri et al. (2018)
B. oleracea inbred line GZ87	DIC.I-1	C08	6.91	17.7	-	Race 4 (Williams 1966) [b]	Peng et al. (2018)
	DIC.II-1	C08	7.28	14.8	-		
B. oleracea diversity accessions (n = 135)	*PbC4.1*	C04	-	9.7	-	Pathotype 3A (Strelkov et al. 2018) [b]	Farid et al. (2020)
	PbC6	C06	-	8.3	-		
	PbC7.1	C07	-	9.2	-		
	PbC7.2	C07	-	7.8	-		
	PbC8	C08	-	17.9	-		
	PbC9.1	C09	-	8.4	-		
	PbC3	C03	-	8.9	-	Pathotype 5X L-G2 (Strelkov et al. 2018) [b]	
	PbC4.2	C04	-	8.4	-		
	PbC7.3	C07	-	12	-		
	PbC9.2	C09	-	13.5	-		

[a] Percent phenotype variation explained by the QTL
QTL with R^2 value > 15% is considered as major effect QTL, <15% is considered as minor effect QTL
[b] *Plasmodiophora brassicae* classification system

Table 1.3: Clubroot resistance gene/loci mapped in *Brassica napus*

Source (Germplasm)	Gene or QTL	LG	Physical position (bp)[a]	Identified as	Resistant to *P. brassicae*	Reference
B. napus var. *oleifera* cv. Darmor-bzh	*Pb-Bn1*	DY4	-	Major gene	Pb137-522	Manzanares-Dauleux et al. (2000)
	2 QTL	DY4 and DY15	-	QTL	K92-16	
245 *B. napus* diversity panel	2 QTL	A02 and A03	-	QTL	ECD 17/31/31	Hejna et al. (2019)
Resynthesized *B. napus*	19 QTL	A02, A03, A08, A09, C05, C06 and C09	-	QTL	Isolates 'Korporal', 'K', '01.07', '1', '01:60', 'e4x04' and 'a'	Werner et al. (2008)
B. napus var. *oleifera* cv. Mendel	*CRa* or *CRb*	A03	25,522,010 - 25,835,881	Major gene	Pathotype 3	Fredua-Agyeman and Rahman (2016)
B. napus var. *oleifera* line 12-3	*CRa*	A03	25,523,177 - 25,752,008	Major gene	Pathotype 3	Zhang et al. (2016a)
B. napus var. *napobrassica* cv. Brookfield	Single gene	A08	12,116,024 - 13,046,214	Major gene	Pathotype 2, 3, 5, 6 & 8	Hasan and Rahman (2016)
B. napus var. *pabularia* cv. NZ Clubroot Resistant Rape	Single gene	-	-	Major gene	Race B	Lammerink (1967)
B. napus var. *napobrassica* cv. Wilhelmsburger	Single gene	-	-	Major gene	Race C	
B. napus var. *pabularia* cv. NZ Clubroot Resistant Rape	Single gene	-	-	Major gene	Race N4	Johnston (1970)
B. napus var. *napobrassica* cv. Wilhelmsburger	Single gene	-	-	Major gene	Race N4	
B. napus var. *napobrassica* cv. Wilhelmsburger	Single gene	-	-	Major gene	Race 3	Ayers and Lelacheur (1972)
	Two genes	-	-	Major gene	Race 2	
B. napus var. *napobrassica* cv. York	Single gene	-	-	Major gene	Race 2	
	Single gene	-	-	Major gene	Race 3	

[a] Physical position has been determined by aligning flanking markers and co-segregating makers with *Brassica rapa* cv. Chiifu-401 whole-genome sequence assembly v.3.0 on http://brassicadb.org/brad/blastPage.php

Table 1.4: List of transcriptomic studies conducted in Brassica for identification of genes conferring resistance to *Plasmodiophora brassicae* causing clubroot disease

Brassica sp.	Plant materials		Days/hours (dai/hai) after inoculation	No. of DEGs involved in resistance	Number of genes validated by qRT-PCR	Reference
	Resistant	Susceptible				
B. oleracea	B2013	90196	0, 7 and 14 dai	4,516	8	Zhang et al. (2016b)
B. rapa	CR BJN3-2	BJN3-2	0, 12, 72 and 96 hai	151	40	Chen et al. (2016)
B. rapa	R635-10	S177-47	30 dai	89	28	Jia et al. (2017)
B. rapa	85-74	–	0, 4 and 8 dai	94	35	Fu et al. (2019)
B. napus	ZHE-226	Zhongshuang 11	0, 3, 6, 9 and dai	175	–	Mei et al. (2019)
B. oleracea	XG	JF	7 and 28 dai	141	13	Ning et al. (2019)
B. oleracea	CR21	CS54	3 dai	165	10	Wang et al. (2019)
B. napus	28,669	YJ-8	12, 24, 60 and 96 hai	–	29	Li et al. (2020)

1.11. Figures

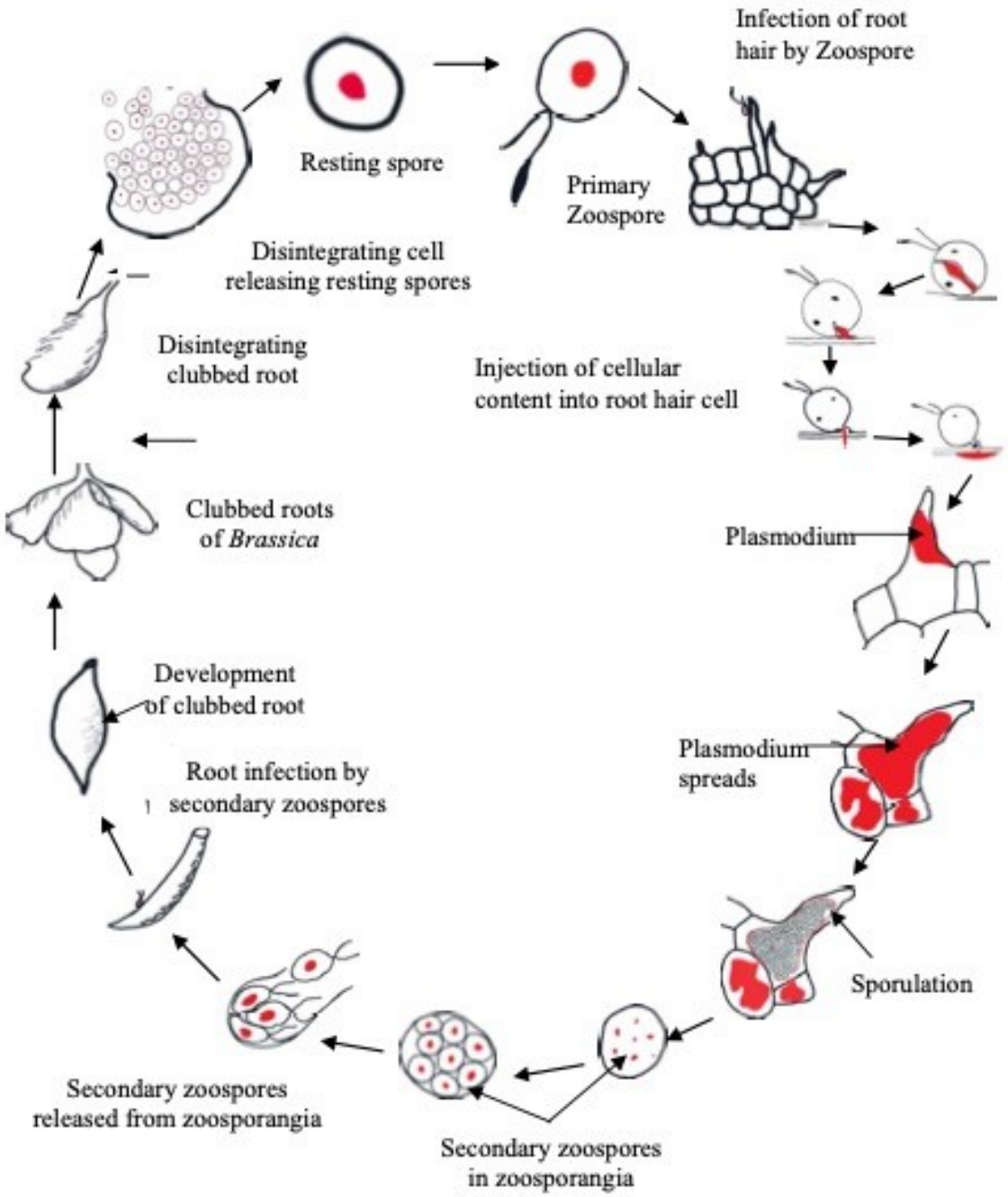

Figure 1.1: Disease cycle of clubroot of crucifers caused by *Plasmodiophora brassicae* (Hasan 2010)

Figure 1.2: Disease severity class for clubroot diseases in Brassica based on gall development (Score 0 = No visible gall, healthy roots; Score 1 = One or few small galls on lateral roots; Score 2 = Moderate galling on lateral roots; Score 3 = Sever galling on the lateral root or in the main root)

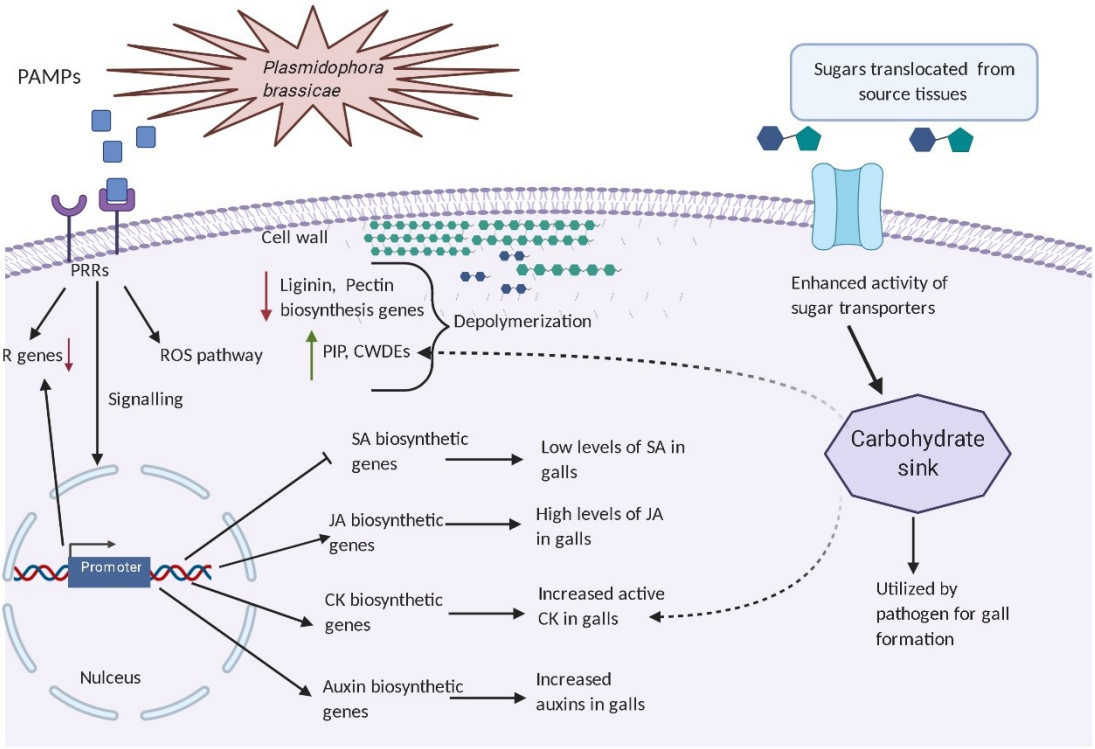

Figure 1.3: Schematic representation of putative metabolic and transcriptional changes in susceptible host plants after infection with *Plasmodiophora brassicae*. The pattern recognition receptors (PRRs) present in the plasma membrane of the host recognizes the pathogen-associated molecular patterns (PAMPs) released by the pathogen and triggers activation of reactive oxygen species (ROS) pathway signalling through various effector molecules such as Ca^{2+}, and finally, transcription of various genes involved in hormone signalling, sugar transport, sugar metabolism and cell wall degradation.

2. Chapter 2: Genetic control of clubroot resistance in rutabaga (*Brassica napus* var. *napobrassica*) and molecular mapping of resistance[2]

2.1. Introduction

Clubroot disease of Brassica, caused by *Plasmodiophora brassicae* Woronin, is an emerging threat to canola production in Canada. In Canada, Alberta was the first province to report this disease in canola fields in 2003 (Tewari et al. 2005), followed by Saskatchewan in 2008 (Dokken-Bouchard et al. 2010). Many pathotypes, such as pathotypes 2, 3, 5, 6 and 8, were reported in Canadian canola fields (Strelkov et al. 2007, 2016; Xue et al. 2008), of which pathotype 3 has been the most prevalent and virulent one in Alberta (Strelkov et al. 2006). However, a few new *P. brassicae* populations, such as L-G1, L-G2, and L-G3, have recently evolved in the canola fields in this province, are highly virulent on the available clubroot resistant Canadian canola cultivars (Strelkov et al. 2016a). According to Tewari et al. (2005), yield loss of canola in Alberta due to this disease can be about 30%. Pageau et al. (2006) reported a 6 % reduction in oil content in seeds harvested from the infected plants. The longevity of the resting spores of this pathogen in soil (Wallenhammar 1996) is the major constraint for efficient control of this disease by cultural and chemical practices (Voorrips 1995). Therefore, growing resistant cultivars can secure the production of this crop, and it is also a vital component of the integrated management of this disease.

A field population of *P. brassicae* is often composed of several pathotypes (Manzanares-Dauleux et al. 2001; Strelkov et al. 2006). The genetic makeup of the population can change over time, as well (Wallenhammar et al. 2011). In the host plant, resistance to a specific

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[2] A version of this chapter has been published as

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**(10): 805–815. doi:10.1139/gen-2016-0034.

pathotype is often controlled by a dominant gene (Ayers and Lelacheur, 1972; Toxopeus and Janssen, 1975; Voorrips and Visser, 1993; Piao et al., 2004). Therefore, a cultivar carrying a pathotype-specific resistance is often not durable. Oxley (2007) reported a breakdown of clubroot resistance after a few years of cultivation in an infested field. Therefore, the durability of resistance is necessary for growing a cultivar for a more extended period, and pyramiding of different resistance genes can achieve this (Mundt 1990). The accumulated genes in a cultivar may provide resistance to multiple races of a pathogen or can impart multilayered resistance to a single race (Chisholm et al. 2006). Matsumoto et al. (2012) pyramided three clubroot resistance genes (*CRa*, *CRk*, and *CRc*) in four Chinese cabbage lines through marker-assisted breeding and this makes the genotypes highly resistant to six isolates of *P. brassicae*. Similarly, Tomita et al. (2013) also showed that a single major QTL or accumulation of multiple minor QTL does not impart strong resistance to this disease in *B. oleracea*. However, a major QTL along with two to three minor QTL can confer moderate resistance, while the accumulation of all five QTL including the major one results in the strongest resistance to multiple isolates.

The success of gene pyramiding in a cultivar depends on several factors, such as the number of genes to be transferred and the ease of genotyping and phenotyping the plants (Joshi and Nayak 2010). Validation of the number of resistance genes pyramided in a single plant or a cultivar is challenging as a plant carrying one or multiple resistance genes can show a similar resistance phenotype unless the different resistance genes confer resistance to different pathotypes. Also, testing a plant for resistance to multiple pathotypes is not feasible in a recurrent backcross-breeding program. In this regard, the development of gene-specific markers and use in marker-assisted selection would facilitate the gene-pyramiding task greatly.

Among the cultivated Brassicas, resistance to *P. brassicae* in *B. rapa* often shows a race-specific reaction controlled by major genes, while resistance in *B. oleracea* is often controlled

by multiple QTL (reviewed by Piao et al. 2009; Nagaoka et al. 2010). In the case of *B. napus*, some of the swede or rutabaga (*B. napus* var. *napobrassica*) genotypes were reported to be possessing resistance to different *P. brassicae* pathotypes (Ayers and Lelacheur 1972; Buczacki et al. 1975; Crute et al. 1983; Gustafsson and Falt 1986) including the pathotypes prevalent in Canada (Hasan et al. 2012). Ayers and Lelacheur (1972) reported resistance to *P. brassicae* races 2 and 3 in rutabaga cv. York is controlled by a single dominant gene, whereas the cv. Wilhelmsburger carries one gene for resistance to race 3 and two genes for resistance to race 2. Hasan et al. (2012) reported that the rutabaga cultivars Brookfield and Polycross carry resistance to pathotypes 2, 3, 5, 6 and 8, which are threats to the Canadian canola crop. The objectives of this research were to study the genetic basis of resistance in the cultivar Brookfield and map the resistance genes through the construction of a linkage map and identify molecular markers for use in marker-assisted selection.

2.2. Materials and methods

2.2.1. Plant material

The parent materials included a clubroot-resistant rutabaga inbred line Rutabaga-BF and a susceptible spring *B. napus* canola cultivar A07-29NI (UA AlfaGold) (Rahman 2017). Rutabaga-BF was developed from the cultivar Brookfield through self-pollination of a single clubroot-resistant plant (Spaner 2002). Seeds of Brookfield were received from Dr. Dean Spaner, Professor, Department of Agricultural, Food and Nutritional Science, University of Alberta, and A07-29NI, a canola cultivar developed in the Canola Program University of Alberta. The line Rutabaga-BF showed complete resistance to Canadian *P. brassicae* pathotypes 2, 3, 5, 6 and 8.

2.2.2. Crossing and population development

The parent Rutabaga-BF was vernalized for eight weeks at 4°C temperature with an 8/16 hr (day/night) photoperiod and crossed to the susceptible parent A07-29NI. The F_1 plants

were self-pollinated to produce F_2 seed after eight weeks of vernalization. The F_1 plants were crossed to A07-29NI to produce Backcross (BC_1) seeds. The F_2 and BC_1 seeds were harvested from the individual F_1 plants and maintained as separate families.

2.2.3. Doubled haploid (DH) lines production

Doubled haploid lines were produced from five F_1 plants by applying the microspore culture technique (Coventry et al. 1988). For this, flower buds of 3.5 - 4.5 mm in length, where the size of the petals was about two-thirds of the length of the anther, were used for microspore isolation. The buds were collected in a Falcon tube and placed on an ice bed to prevent microspore degeneration.

Before microspore isolation, the buds were sterilized by treating with 6% calcium hypochlorite [$Ca(OCl)_2$] solution for 15 min and were rinsed three times with cold, sterile Milli-Q Millipore water. About 15-20 sterilized flower buds were homogenized in 25 ml liquid B5 medium (Gamborg et al. 1968) containing 13% sucrose using a pair of sterile mortar and pestle. The homogenate was poured through two layers of nested sterile filters [64-micron top (NTX64) and 41-micron bottom (NTX41)] into a sterile Falcon tube. The Falcon tube was centrifuged for 5 min at 1000 rpm (rotation per minute), and the dark green supernatant was decanted, leaving the pallet of yellow spores at the bottom of the tube. Microspores were washed three times with the B5 liquid medium. After washing, the clean microspore pellet was re-suspended in 30 ml NLN 13 medium (Lichter 1982) containing 50 mg/L colchicine and poured into sterile Petri plates. The Petri plates were sealed with parafilm and incubated in the dark in an incubator at 30°C for 24 hrs. After 24 hrs, the suspension was transferred to a Falcon tube and centrifuged at 1000 rpm for 5 min. The supernatant was decanted, and 25 ml of NLN 13 medium (without colchicine) was added to the microspores and cultured in Petri plates for 14 days at 30ºC.

After 14 days, the plates were transferred on a shaker rotating at 59 rpm to provide enough air to the developing embryos. The torpedo-shaped embryos were transferred to a solid B5 medium (liquid B5 with 20% sucrose and 2% agar) at a density of 20 embryos per Petri plate. The plates were kept at 4ºC for 14 days and then transferred to room temperature under light (16/8 h day/night) to germinate the embryos and develop plantlets. Seedlings with well-developed roots and true leaves were transferred to soil-free growth media (Sungro Horticulture) and covered with clear cups for about a week to maintain high humidity.

At flowering, the plants producing visible pollen were considered doubled haploids, while the sterile plants with smaller size flower/petal and lacking pollen grains were considered as haploids. The haploid plants were treated with a 0.35% aqueous solution of colchicine for chromosome doubling. For this, roots were washed and immersed in colchicine solution for one hour. After this, roots were washed thoroughly under running tap water, and the shoots were clipped off, leaving only a few auxiliary buds (also called lateral buds). The treated plants were transplanted to the soil-free growth medium in pots. Fertile plants with visible pollen grains were self-pollinated by bag isolation and harvested as doubled haploids.

2.2.4. Pathogen isolate

Single-spore derived isolates of *P. brassicae*, classified as pathotype 2, 3, 5, 6, or 8 based on Williams's (1966) differentials, were used. *Plasmodiophora brassicae* pathotypes 2, 3, 5, 6, and 8 have been recently classified as pathotype F, H, I, M, and N, respectively, by using Canadian Clubroot Differential (CCD) (Strelkov et al. 2018). Dr. Stephen Strelkov, Professor, Department of Agricultural, Food and Nutritional Sciences, the University of Alberta, kindly provided these isolates in the form of galls. Resting spore suspensions (inoculum) from the preserved galls were prepared following the protocol described by Strelkov et al. (2007), and the concentration of the suspension was adjusted to 1×10^7 - 1×10^8 resting spores/ml.

2.2.5. Resistance test

Four F_2 and their corresponding BC_1 families were evaluated for resistance to *P. brassicae* pathotype 3, and the DH population was assessed for resistance to *P. brassicae* pathotypes 2, 3, 5, 6, and 8. Seven day old seedlings germinated on moistened Whatman filter paper No. 1 were inoculated by dipping the roots in resting spore suspension (Nieuwhof and Wiering 1961). The inoculated seedlings were planted to 3 × 3 × 5 cm (L × W × D) cells filled with Sunshine Professional Natural and Organic Mixes # 4 (Sungro Horticulture), placed in a tray (2 × 4 m), and were grown in a greenhouse at 21 ± 2 °C temperature with a 16-hr photoperiod. After transplanting, 2 mL inoculums were pipetted to each cell to ensure successful inoculation. During the first seven days, the cells were kept saturated with water to ensure sufficient moisture for the development of the pathogen. To ensure acidic condition of the soil, HCl solution (10% v/v) @ 20 mL/tray (2 × 4 m) was added each day. From the beginning of the second week, watering was done once a day. Seedlings were evaluated for clubroot resistance at 42 to 45 days after inoculation, and the severity of gall development was scored on a 0 to 3 scale: where 0 = no galling, 1 = one or few tiny galls on lateral roots, 2 = moderate galling on lateral roots, and 3 = severe galling on the lateral or main root. The inoculum preparation, inoculation technique, and scoring are described elsewhere (Rahman et al. 2011; Hasan et al. 2012). Based on disease score, plants with scores 0 or 1 were classified as resistant, while the plants with scores 2 or 3 were classified as susceptible.

2.2.6. DNA extraction

Leaf samples of the parents and the DH population (3-5 weeks old plants) grown in the greenhouse were collected in aluminum foil and kept at -80°C until use. DNA was extracted using the Wizard Genomic DNA purification kit (Promega Corporation 2010), following the manufacturer's instructions. The DNA pellet was air-dried for 60 min and rehydrated in 200 µl DNA rehydration solution (TE buffer) overnight at 4ºC. The quality and quantity of the DNA

were estimated by spectrometry using a 260/280 nm absorbance ratio, and the concentration of DNA was estimated at 260 nm with an ND-2000 Nanodrop spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE, USA).

2.2.7. Molecular markers

Simple sequence repeats (SSR) primers were used to map the clubroot gene(s). Most of these SSR primer sequences were received from Agriculture and Agri-Food Canada (AAFC), Saskatoon, through a material transfer agreement (currently available http://aafc-aac.usask.ca/BrassicaMAST/). The SSR primers reported by Lowe et al. (2002, 2004), Suwabe et al. (2002, 2003), Piquemal et al. (2005), Saito et al. (2006), Cheng et al. (2009) and Kato et al. (2012) were used, too. Besides, SSR markers designed based on *B. rapa* cv. Chiifu-401 whole-genome sequence assembly version 1.5 (Cheng et al. 2011) was used in this study. To design SSR primers from the *B. rapa* genome sequence, the open-access software program, QDD version 3, was used to detect microsatellites (SSRs) in the whole genome, and primers were designed from the targeted regions (Meglécz et al. 2010).

2.2.8. Polymerase chain reaction (PCR)

The polymerase chain reaction was performed in a total volume of 14 µl reaction mixture. The mixture contained 45 ng (15 ng/µL × 3 µL) DNA, 2.5 µL 1× PCR buffer, 1.0 µL 25 mM $MgCl_2$, 0.35 µL 0.4 mmol/L dNTPs (Promega Corporation 2010), 0.5 µmol/L of each of forward and reverse SSR primers, 0.25 pmol/L of labelling dye (FAM, VIC, NED, and PET; Applied Biosystem, Foster City, CA, USA), 0.125 U of GoTaq DNA Polymerase enzyme, and 6.995 µL ddH_20. Amplification reactions were carried out using a GeneAmp PCR system 9700 (Applied Biosystems) starting with 95ºC for 2 min followed by 35 cycles of denaturation at 95ºC for 30 sec, annealing at 56ºC for 30 sec, and extension at 72ºC for 45 sec, with a final extension at 72ºC for 15 min. The PCR products were labelled with the M13 tailing technique as described by Schuelke (2000). For this, each forward SSR primer was attached to a universal

M13 primer sequence 5-CACGACGTTGTAAAACGAC-3 fluorescently labelled with infra-red (IRD; MWG-Biotech AG, Ebensburg, Germany) FAM, VIC, NED, and PET (Applied Biosystems, Foster City, CA) dyes. Electrophoretic separation and detection of the amplification products was performed using a capillary ABI sequencer No. 3730 (Applied Biosystems, Foster City, CA), and scored and analyzed using GeneMapper software version 3.7.

2.2.9. Map construction and identification of the locus controlling clubroot resistance

All primer pairs that showed polymorphism between the two parents were used for genotyping the DH population. Based on segregation in F_2, BC_1, and DH populations, it was evident that a major gene is involved in the control of resistance to *P. brassicae* pathotypes in Rutabaga-BF. Therefore, in the first step, single marker analysis was done with the DH population using the polymorphic SSR markers to identify the chromosome(s) carrying the resistance gene(s). This analysis indicated that SSR markers from chromosome A08 to be associated with resistance. Therefore, a genetic linkage map of this chromosome was constructed to identify the genomic region associated with resistance and to identify the markers from this region. The linkage map was constructed using the program JoinMap 4.0 (Van Ooijen 2006). A LOD threshold of 3.0 and a recombination frequency of 0.40 were used to group the marker loci. The recombination frequencies were transformed into centimorgan (cM) using the Kosambi mapping function (Kosambi 1943).

Composite interval mapping analysis was performed using the linkage map to identify the genomic region carrying the clubroot resistance gene and identify the linked markers for use in marker-assisted selection. For this, genotypic and phenotypic data were subjected to Inclusive Composite Interval Mapping (ICIM) analysis using the software program QTL IciMapping version 4.0 (http://www.isbreeding.net). The linkage map was scanned for association with phenotypic variation for resistance to *P. brassicae* pathotype 2, 3, 5, 6, and 8

at every 0.1 cM intervals. Empirical threshold levels, which confirmed the existence of the locus at a significance level of 0.05, were obtained by analyzing 1000 permutations of the data set following the method described by Churchill and Doerge (1994). The location of the resistance locus was declared at the position where the LOD score exceeded the threshold significance level of 3.0.

2.2.10. Candidate gene prediction

In order to identify the physical location of the clubroot resistance gene in the Brassica genome, SSR marker sequences flanking the resistance locus were mapped on the *B. rapa* cultivar Chiifu-401 genome sequence assembly version 1.5 (Cheng et al. 2011; www.brassicadb.org) by using electronic-PCR (e-PCR) (Schuler 1997; Rotmistrovsky et al. 2004). e-PCR is a computational procedure that searches DNA sequences that closely match with the PCR primers in the context of correct order, orientation, and spacing, and thereby identifying the sequence(s) within the genome whose size is similar to the PCR product generated by the use of these primer pairs. When the primer sequences matched with a fragment of DNA sequence from the target chromosome and the length of the deduced fragment to be amplified by the primers is similar to the actual size of the fragment amplified by these primers, the sequences were considered to be the marker sequence. The sequence of the A08 chromosome segment of *B. rapa* cultivar Chiifu-401 (genome sequence assembly version 1.5), flanked by the SSR markers associated with resistance, was scanned using Augustus, a server-based software program that predicts the ab initio gene in the eukaryotic genomic sequence (Stanke et al. 2004, http://www.augustus.gobics.de), for detection of the gene(s) in this genomic region to be associated with resistance. The protein sequences predicted based on the gene sequences were scanned for similarity with *Arabidopsis thaliana* protein(s) of known function (https://www.arabidopsis.org) using the software program WU-BLAST2 (Gish 2003, https://www.arabidopsis.org/wublast/index2.jsp).

2.2.11. Other statistical analysis

A χ^2 test for segregation for resistance in F_2, BC_1 and DH population was done using SAS software version 9.3 (SAS Institute Inc. 2011).

2.3. Results

2.3.1. Inheritance of resistance to pathotype 3 in F_2, backcross (BC_1) and doubled haploid (DH) populations

A total of 806 F_2 plants and 445 BC_1 plants were evaluated for resistance to *P. brassicae* pathotype 3. In both cases, a precise bi-modal distribution for resistance and susceptibility was found (Figure 2.1a and 2.1b). The F_2 and BC_1 populations were derived from four F_1 plants; therefore, a homogeneity χ^2 test was performed. The results indicated that all families followed a similar segregation pattern (Table 2.1) and thus, justify the pooling of data of the four families. Pooled data from the four families fit well to 3:1 segregation in F_2 and 1:1 segregation in BC_1 populations (Table 2.1). Data from the individual families also followed simple Mendelian segregation in most cases. Analysis of data considering disease score 0 as resistant and scores 1, 2 and 3 as susceptible also showed similar segregation patterns (data not shown). Thus, the F_2 and BC_1 data suggest that a major gene is involved in the control of resistance to *P. brassicae* pathotype 3.

A total of 94 DH lines were produced from five F_1 plants. The homogeneity χ^2 test showed that segregation for resistance to *P. brassicae* pathotype 3 among the DH families derived from five F_1 plants was similar (Table 2). The distribution of the DH lines for resistance phenotype was bimodal (Figure 2.1c). A 1:1 segregation for resistance was found when the five DH families were subjected to χ^2 tests individually (Table 2.2); however, pooled data failed to fit into 1:1 segregation. In all DH families, the number of resistant DH lines was higher than the number of susceptible lines, and this cumulatively resulted in a significantly higher number of resistant lines in the whole DH population. However, the F_2 and BC_1 families did not show such a deviation for segregation. Taken together, segregation in the F_2, BC_1 and, DH

population, it is apparent that a major gene is involved in the control of resistance to *P. brassicae* pathotype 3 in Rutabaga-BF.

The DH lines were also evaluated for resistance to pathotypes 2, 5, 6 and 8 (Table 2.3). In all cases, the distribution of the DH population for resistance to these pathotypes was bimodal (Figure 2.2). In general, most of the DH lines showing resistance to pathotype 3 also showed resistance to the other pathotypes. Of the 68 DH lines considered resistant to pathotype 3 (score 0 and 1), 61 lines showed resistance to the other pathotypes (2, 5, 6 and 8) as well. This result is also evident from the strong correlation between resistance to pathotype 3 and resistance to other pathotypes (Table 2.4).

2.3.2. Molecular marker analysis and mapping the resistance locus

A total of 1,154 SSR primer pairs from 19 linkage groups of *B. napus* were evaluated for polymorphism between the resistant and susceptible parents. Of the tested markers, 260 (23%) failed PCR amplification, while 519 (45%) found to be polymorphic between the parents that can be detected by ABI sequencer. Of the 519 markers, 342 (66%) markers produced fragments with more than 4 bp differences. Based on a clear difference between the size of fragments and reproducibility of the genotyping data, 217 markers were selected and used to study linkage association with resistance in the DH population. The reason for using the DH population for molecular mapping was that the same DH line (genotype) could be phenotyped in replicated trials as well as can be tested against multiple pathotypes, which is not possible with the other two populations (F_2 or BC_1).

Of the 217 SSR markers, 207 produced only two fragments – either of the A or the C genome, while 10 markers produced more than two fragments – probably amplifying both the A and C genomes. Single marker analysis identified markers sS1702, sS2329, sN4145, sR0841, sN0809, A08_5024, A08_5021, sR6068, A08_5229b, BrUA2628, sNRF19, A08_5255, BrUA2654, sN0990bNP, BrUA2650, BrUA2616, A08_4603, BrUA2632, A08_5222b,

A08_4987, BRMS097, BrUA2659b, sN12295, BrUA2612, BrUA2641, BrUA2659a, A08_7000, A08_6688, BrUA2620, BrEST33, sN1958a, A08_5500, sN13095a434, BRMS070, sR02133, sR1868, CB10179, BnGMS389, sN2711 and sR688 to be associated with resistance to pathotypes 2, 3, 5, 6 and 8 in this DH population. The markers prefixed with "s", "CB", "BR" and "Bn" were previously reported on the chromosome A08 of *Brassica* spp., while the markers prefixed with "Br" and "A08" were designed in this study based on A08 chromosome sequence of *B. rapa* cultivar Chiifu-401 genome sequence v.1.5. No marker from chromosomes other than A08 showed association with resistance to any of the pathotypes. Based on this, a genetic linkage map of this chromosome was constructed using 48 markers. This linkage map included seven markers published by different researchers, 18 markers developed by AAFC, and 23 markers developed based on *B. rapa* cultivar Chiffu-401 genome sequence, and the map covered 107.6 cM with a mean distance of 2.24 cM between the markers (Figure 2.3). The loci controlling clubroot resistance to the pathotypes 2, 3, 5, 6 and 8 were located in between 19.20 cM and 20.39 cM position of this linkage map and were detected with LOD scores of 26.58, 20.69, 22.65, 23.78 and 23.47 for the five pathotypes, respectively (Figure 2.3). This region is flanked by the SSR markers sS1702 and A08_5024 and explained 100% of the phenotypic variation.

2.3.3. Candidate gene prediction

BLAST search of the region flanked by the markers sS1702 [genetic position 19.203 cM; physical position between 10,692,332 nt (start position) and 10,692,602 nt (end position)] and A08_5024 [genetic position 20.393 cM; physical position between 11,617,700 nt (start position) and 11,617,968 nt (end position)] detected a segment of approximately 925,366 nt in length on the chromosome A08. Scanning of this 925,366 nt long sequence in the *A. thaliana* database using the software program, Augustus predicted 181 genes encoding 201 proteins (scanning was conducted on December 6, 2015) (complete list of the predicted 181 genes and

201 proteins are not shown here). These 201 protein sequences were annotated using *A. thaliana* proteins with known function, and one of the sequences (Table S2.1) showed similarity with 137 proteins, of which 97 are TIR-NBS-LRR type proteins, and 26 are Toll-Interleukin-Resistance (TIR) domain family proteins (Table S2.2). The TIR-NBS-LRR class protein participates in the processes such as defence response, apoptosis, signal transduction (www.ncbi.nlm.nih.gov). The expression of the gene, encoding TIR-NBS-LRR class protein, is controlled by DNA methylation in its promoter region (Li et al. 2007b). Domain Enhanced Lookup Time Accelerated BLAST (DELTA-BLAST) scanning of this predicted protein sequence with NCBI protein database showed sequence similarity with TIR (Toll/Interleukin-1 Receptor) domain of a clubroot disease resistance protein in *B. rapa* (Locus: BAM77406, accession AB605024.1; E-value 2 $x10^{-56}$).

2.4. Discussion

The present study indicated that resistance to *P. brassicae* pathotype 3 in Rutabaga-BF is controlled by a major gene located on chromosome A08, where the resistant phenotype exerts dominance over susceptibility. The genomic region carrying this gene also confers resistance to four other *P. brassicae* pathotypes studied in this experiment. Nine clubroot resistance loci have been identified and mapped on five chromosomes of the Brassica A-genome (Kuginuki et al. 1997; Hirai et al. 2004; Piao et al. 2004; Suwabe et al. 2006; Sakamoto et al. 2008; Chu et al. 2014). Five of these loci, namely *CRa*, *CRb*, *Crr3, CRk,* and *Rcr1*, are mapped on chromosome A03 (Matsumoto et al. 1998; Hirai et al. 2004; Piao et al. 2004; Sakamoto et al. 2008; Chu et al. 2014), while the loci *Crr2*, *CRc* and *Crr4* are mapped on chromosome A01 (Suwabe et al. 2006), A02 (Sakamoto et al. 2008), and A06 (Suwabe et al., 2006), respectively. Suwabe et al. (2006) reported the locus *Crr1* and mapped this on chromosome A08 of *B. rapa*. This locus was found to be flanked by the SSR markers BRMS-088 and BRMS-173 and accounted for 26.8% of the total phenotypic variation for resistance to *P. brassicae* isolate

Wakayama-01 (Suwabe et al. 2006). According to Suwabe et al. (2012), the genetic distance between these two flanked SSR markers was 3.9 cM; however, neither of these markers showed polymorphism between the resistant and susceptible parents (Rutabaga-BF and A07-29NI) used in this study. Further study by Suwabe et al. (2012) and Hatakeyama et al. (2013) revealed that the genomic region carrying *Crr1* comprises two gene loci; the locus with major effect was named *Crr1a,* and the minor locus as *Crr1b*. They also reported that the gene *Crr1a* comprises four open reading frames (ORFs) and shows similarity with a TIR-NBS-LRR type *R*-gene.

The NBS-LRR is the largest class of known plant disease resistance genes (*R*-genes), and TIR-NBS-LRR is one of the subclasses of this gene family (Akira and Hemmi 2003). Yu et al. (2014) reported 157 and 206 NBS-LRR protein-encoding genes in *B. oleracea* and *B. rapa*, respectively, and 167 in *A. thaliana*. NBS-LRR imparts plant immunity through hypersensitive response by monitoring the status of plant proteins targeted by pathogen virulence effector (Dangl and Jones 2001; Nandety et al. 2013). This type of resistance, imparted by NBS-LRR, is effective against obligate biotrophic or hemibiotrophic pathogens but not against necrotrophs (as reviewed by Glazebrook 2005). Genes encoding TIR-NBS-LRR class proteins have been described to confer clubroot resistance in *B. rapa* (Ueno et al. 2012; Hatakeyama et al. 2013). Swiderski et al. (2009) reported that the TIR domain of the TIR-NBS-LRR gene plays a signalling role in the induction of the defence response, and the LRR domain plays a role in recognition specificity (Collier and Moffett 2009). Hatakeyama et al. (2013) observed a complete susceptibility to clubroot disease in *B. rapa* due to the lack of more than half of the TIR domain. Swiderski et al. (2009) also observed a complete loss of function of the TIR-NBS-LRR gene due to a mutation at the TIR domain.

A BLASTn search of the cDNA of the *Crr1a* reported by Hatakeyama et al. (2013) has been done, and this identified two genomic regions of 11,105,764 to 11,635,861 nt and

15,371,893 to 15,723,317 nt of the A08 chromosome of *B. rapa* cv. Chiifu-401 sequence assembly version 1.5 (Cheng et al. 2011; www.brassicadb.org). Based on this, a total of 78 SSR markers were designed from the genomic region of 10,353,228 to 12,646,253 nt and 13 markers from the region of 13,604,267 to 16,005,042 nt and screened for polymorphism between the resistant and susceptible parents. However, only 12 markers from the region of 10,353,228 to 12,646,253 nt and four markers from the region of 13,604,267 to 16,005,042 nt were polymorphic between the parents and mapped on the linkage group A08. Composite Interval Mapping analysis indicated that the SSR markers sS1702 and A08_5024 flank the resistance locus in Rutabaga-BF. These two markers were found to be located in the genomic region of 10,692,602 to 11,617,968 nt of chromosome A08 of *B. rapa* cv. Chiifu-401 assembly version 1.5 (Cheng et al. 2011; www.brassicadb.org). Based on these pieces of evidence, resistant parent Rutabaga-BF can be anticipated to carry *Crr1a* and confer resistance to *P. brassicae* pathotypes 2, 3, 5, 6 and 8, or a cluster of genes conferring resistance to different pathotypes is present in this genomic region. Based on the fine mapping of a 1.6 cM genetic region of A08, located between the markers BRMS-088 and BRMS-173, and comparing this region with *A. thaliana* chromosome 4, Suwabe et al. (2012) found evidence that this region carries two clubroot resistance genes. Data from the evaluation of the DH population for resistance to multiple pathotypes also provide evidence that a cluster of clubroot resistance genes might be present in chromosome A08. A cluster of other resistance genes, such as resistance to blackleg disease caused by *Leptosphaeria maculans*, can also be found in the Brassica genome as reported by Delourme et al. (2004) for four *Rlm* genes (*Rlm3, Rlm4, Rlm7* and *Rlm9*) on chromosome A10. The cluster of disease resistance genes has been reported in other plant species, such as lettuce (Meyers et al. 1998) and potato (Kuang et al. 2005).

2.5. Conclusion

In conclusion, mapping the clubroot resistance locus and the SSR markers identified in this study provide valuable knowledge and tools for breeding clubroot-resistant *B. napus* cultivars. Earlier efforts on the breeding of clubroot-resistant Brassica crop cultivars, such as Chinese cabbage, was based on the use of resistance controlled by a single Mendelian gene (Yoshikawa 1993; Matsumoto et al. 1998; Suwabe et al. 2003; Hirai et al. 2004; Piao et al. 2004; Sakamoto et al. 2008). However, a breakdown of single-gene resistance has been reported by different researchers (Hirai et al. 2004; Hirai 2006); therefore, a pyramiding of multiple resistance genes into a cultivar would ensure the durability of resistance under field conditions. In this regard, the genomic region and the markers identified in this study provide valuable information, as this region is associated with resistance to multiple pathotypes. Furthermore, the knowledge gained from this study can also be used for the pyramiding of resistance genes from different genomic regions through marker-assisted selection. The genomic region identified in this study can also be used to fine map this region well as map-based cloning of the resistance gene(s).

2.6. Tables

Table 2.1: Segregation for resistance to *Plasmodiophora brassicae* pathotype 3 in F_2 and backcross (BC_1) populations of Rutabaga-BF × A07-29NI cross of *Brassica napus*.

Generation	Family	Total plants	No. resistant (% plants)	No. susceptible (% plants)	Ratio	Homogeneity		Segregation (R:S)	
						χ^2	*p*	χ^2	*p*
F_2	Family 1	223	169 (75.8)	54 (24.2)	3:1	-	-	0.073	0.79
	Family 2	165	121 (73.3)	44 (26.7)	3:1	-	-	0.244	0.62
	Family 3	206	155 (75.2)	51 (24.8)	3:1	-	-	0.006	0.94
	Family 4	212	174 (82.1)	38 (17.9)	3:1	-	-	5.66	0.02
	Total	806	619 (76.8)	187 (23.2)	3:1	4.592	0.2042	1.39	0.24
BC_1	Family 1	105	64 (61.0)	41 (39.0)	1:1	-	-	5.04	0.025
	Family 2	113	59 (52.2)	54 (47.8)	1:1	-	-	0.22	0.64
	Family 3	113	49 (43.4)	64 (56.6)	1:1	-	-	1.99	0.16
	Family 4	114	56 (49.1)	58 (50.9)	1:1	-	-	0.04	0.85
	Total	445	228 (51.2)	217 (48.8)	1:1	7.013	0.0715	0.27	0.60

Table 2.2: Segregation for resistance to *Plasmodiophora brassicae* pathotype 3 in doubled haploid (DH) population of Rutabaga-BF × A07-29NI cross of *Brassica napus*.

Cross	Total DH	No. of resistant lines (%)	No. of susceptible lines (%)	Ratio	Homogeneity		Segregation (R:S)	
					χ^2	*p*	χ^2	*p*
Family 1	26	18 (69.2)	8 (30.8)	1:1	-	-	3.85	0.05
Family 2	8	5 (62.5)	3 (37.8)	1:1	-	-	0.50	0.48
Family 3	34	19 (55.9)	15 (44.1)	1:1	-	-	0.47	0.49
Family 4	13	9 (69.2)	4 (30.8)	1:1	-	-	1.92	0.16
Family 5	13	8 (61.5)	5 (38.5)	1:1	-	-	0.69	0.40
Total	94	59 (62.8)	35 (37.2)	1:1	1.30	0.86	6.13	<0.05

Table 2.3: Resistance to *Plasmodiophora brassicae* pathotypes in a doubled haploid (DH) population derived from Rutabaga-BF × A07-29NI cross of *Brassica napus*

Pathotype	No. of resistant DH line	No. of susceptible DH lines
2	64	43
3	68	39
5	67	40
6	65	42
8	64	43
All pathotypes	61	35

Table 2.4: Coefficient of correlation for resistance to different *Plasmodiophora brassicae* pathotypes in the doubled haploid (DH) population derived from a Rutabaga-BF × A07-29NI cross of *Brassica napus*

	Pathotype 3	Pathotype 5	Pathotype 6	Pathotype 8
Pathotype 2	0.947	0.957	0.942	0,922
Pathotype 3	-	0.899	0.946	0.887
Pathotype 5	-	-	0.896	0.878
Pathotype 6	-	-	-	0.903

2.7. Figures

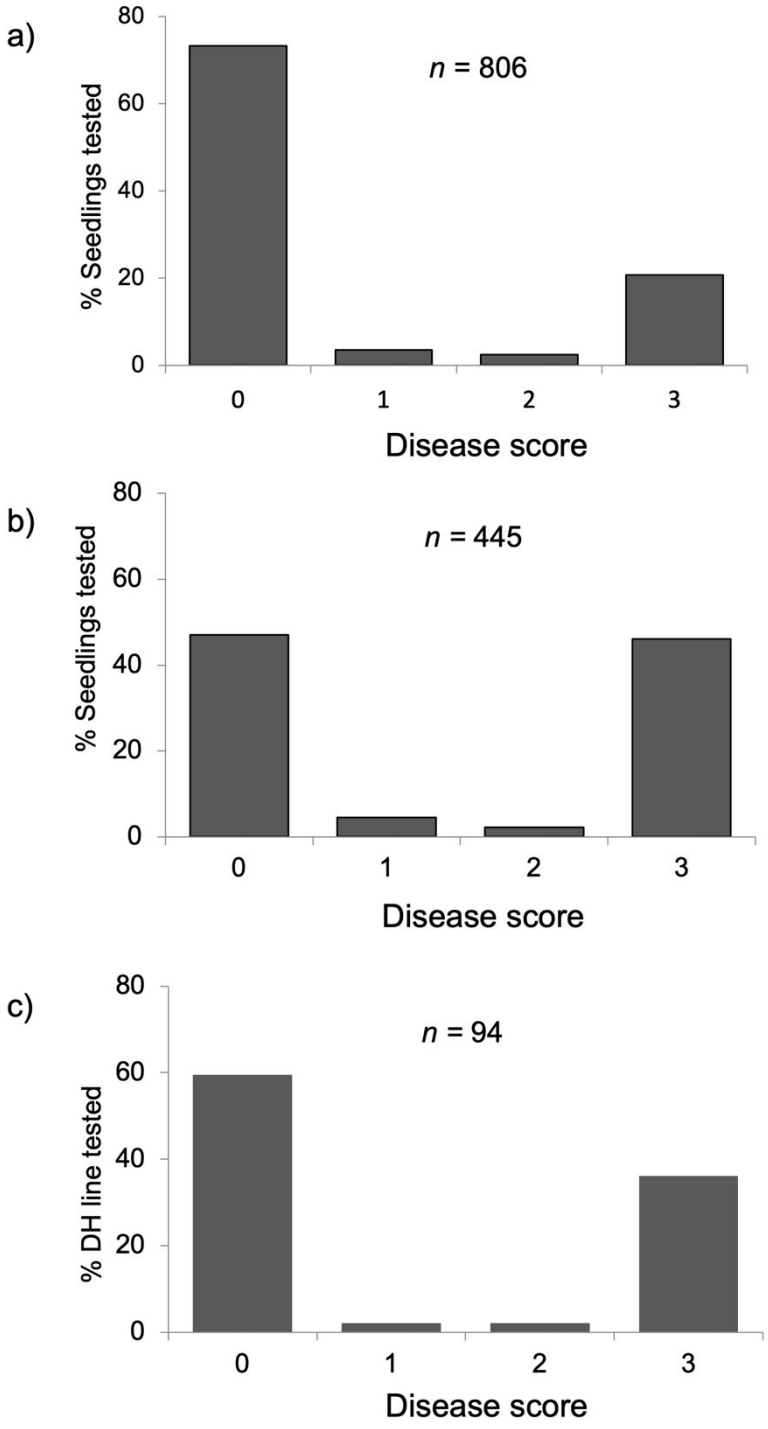

Figure 2.1: Frequency distribution of (a) F_2, (b) backcross (BC_1), and (c) doubled haploid (DH) populations of Rutabaga-BF × A07-29NI cross of *Brassica napus* for resistance to *Plasmodiophora brassicae* pathotype 3. '*n'* indicates the total number of seedlings/DH lines tested.

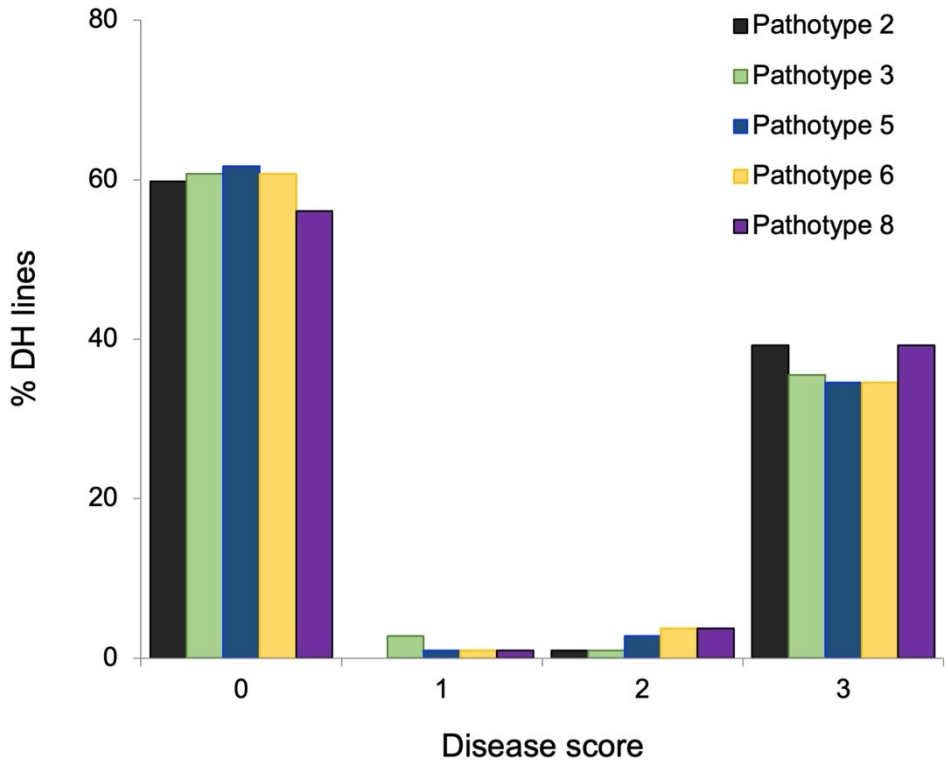

Figure 2.2: Frequency distribution of the doubled haploid (DH) population derived from Rutabaga-BF × A07-29NI cross of *Brassica napus* for resistance to five *Plasmodiophora brassicae* pathotypes

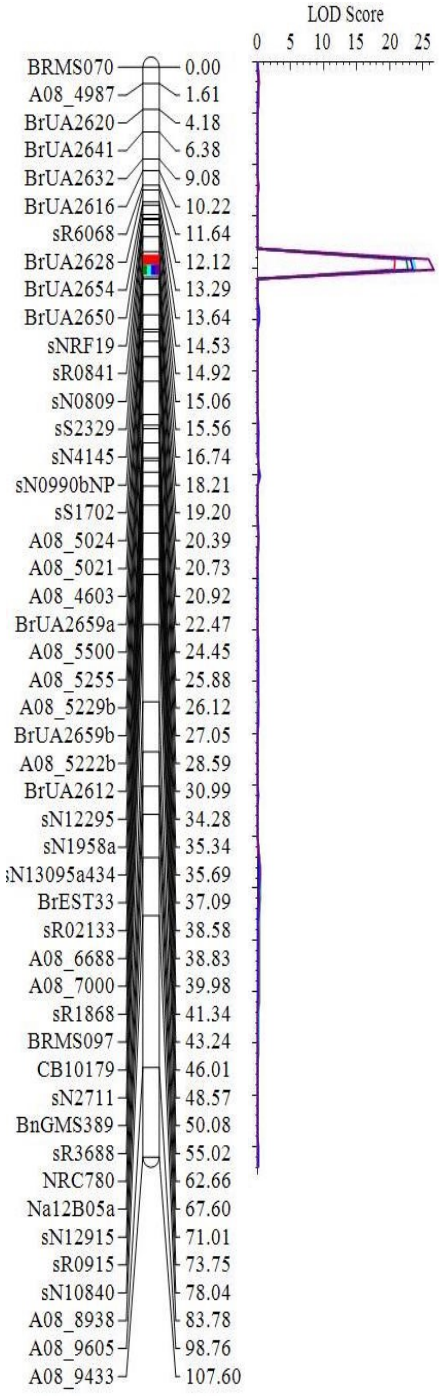

Figure 2.3: Linkage map of A08 of *Brassica napus* carrying resistance to clubroot disease. Marker names and their genetic distances (cM), calculated using the Kosambi mapping function, are indicated at the left and right sides of the linkage map, respectively. Right: The QTL likelihood profile for the clubroot resistance trait. LOD score and map distance are indicated on the x and y axes, respectively. Red, blue, turquoise, green, and violet LOD peaks indicate *P. brassicae* pathotypes 2, 3, 5, 6 and 8.

2.8. Supplemental materials

Table S2.1: Nucleotide sequence of a gene identified by Augustus gene prediction software within the *Brassica rapa* cv. Chifu-401 version 1.5 genome sequence flanked by SSR markers sS1702 and A08_5024 and the amino acid sequences encoded by this predicted gene which show sequence similarity with *A. thaliana* TIR-NBS-LRR protein sequences encoded by *At4g11170* gene

Nucleotide sequence:

ATGAAGAGGAACGCGGACGAGCTCTGGAATCCAGTGAGAAAGAATATCATAG
TGGAGAACCAAAGCCAAATTCAAGTGCCATCATCACATCCGTCTTCTTTGTCTC
TTTTATCAGTTCCATCTTCTTCATCTCACAACTGGACACACGATGTCTTTCCAAG
CTTTCGCGGGGAAGATGTCCGCATAAAATTTCTCAGCCACCTTAAGAAGGAGT
TTAAAAGAAAAGGAACCACACCATTCATTGACAATGAGATCAGGAGAGGAGA
ATCCATCGGTCCCGAACTCATAAGGGCCATTAGAGAATCTAAGATCGCAATCA
TCTTGATCTCGAGGAGCTATGCATCTTCAAAGTGGTGTGTTGACGAGTTGGTGG
AGATTATGAAGTGCAAAGAAGAGTTAGGCCAAACCGTAATCCCCGTTTTCTAT
AAAATAGATCCTTCTGATGTAAAGAAGCTGACTGGATATGTTGGCAAGGTTTTT
GAAAAAACTTGTGTTGGTAAAAGTAAGGAGGATACTGAAAAATGGAGACATG
CTTTGAAGAAGGTGGCCACAATTGCTGGTTACGATTAA

Amino acid sequence:

MKRNADELWNPVRKNIIVENQSQIQVPSSHPSSLSLLSVPSSSSHNWTHDVFPSFRG
EDVRIKFLSHLKKEFKRKGTTPFIDNEIRRGESIGPELIRAIRESKIAIILISRSYASSKW
CVDELVEIMKCKEELGQTVIPVFYKIDPSDVKKLTGYVGKVFEKTCVGKSKEDTE
KWRHALKKVATIAGYD

Table S2.2: List of *Arabidopsis thaliana* proteins with a known function that shows sequence similarity with the predicted protein sequence

Protein	Function	Score	E-value
AT1G69550	Disease resistance protein (TIR-NBS-LRR class) family	235	1.00E-62
AT5G11250	Disease resistance protein (TIR-NBS-LRR class) family	230	3.00E-61
AT3G04220	Disease resistance protein (TIR-NBS-LRR class) family	229	1.00E-60
AT1G65850	Disease resistance protein (TIR-NBS-LRR class) family	228	3.00E-60
AT5G44510	TAO1 | target of AVRB operation1	214	2.00E-56
AT5G18370	Disease resistance protein (TIR-NBS-LRR class) family	211	2.00E-55
AT3G25510	Disease resistance protein (TIR-NBS-LRR class) family	201	3.00E-52
AT3G44400	Disease resistance protein (TIR-NBS-LRR class) family	201	3.00E-52
AT5G38344	Toll-Interleukin-Resistance (TIR) domain family protein	201	3.00E-52
AT3G44480	RPP1, cog1 | Disease resistance protein (TIR-NBS-LRR class) family	199	8.00E-52
AT3G44630	Disease resistance protein (TIR-NBS-LRR class) family	197	3.00E-51
AT2G14080	Disease resistance protein (TIR-NBS-LRR class) family	194	4.00E-50
AT3G44670	Disease resistance protein (TIR-NBS-LRR class) family	189	6.00E-49
AT5G18350	Disease resistance protein (TIR-NBS-LRR class) family	189	700E-49
AT5G38340	Disease resistance protein (TIR-NBS-LRR class) family	189	1.00E-48
AT3G04210	Disease resistance protein (TIR-NBS-LRR class) family	186	1.00E-47
AT4G11170	Disease resistance protein (TIR-NBS-LRR class) family	182	1.00E-46
AT5G22690	Disease resistance protein (TIR-NBS-LRR class) family	181	3.00E-46
AT4G16890	SNC1, BAL | disease resistance protein (TIR-NBS-LRR class) putative	180	4.00E-46
AT5G46470	RPS6 | disease resistance protein (TIR-NBS-LRR) family	179	8.00E-46
AT5G41750	Disease resistance protein (TIR-NBS-LRR class) family	178	2.00E-45
AT5G41540	Disease resistance protein (TIR-NBS-LRR class) family	177	4.00E-45
AT5G46450	Disease resistance protein (TIR-NBS-LRR class) family	177	4.00E-45
AT5G51630	Disease resistance protein (TIR-NBS-LRR class) family	175	2.00E-44
AT5G18360	Disease resistance protein (TIR-NBS-LRR class) family	172	2.00E-43
AT5G40910	Disease resistance protein (TIR-NBS-LRR class) family	171	3.00E-43
AT5G49140	Disease resistance protein (TIR-NBS-LRR class) family	171	3.00E-43
AT1G64070	RLM1 | Disease resistance protein (TIR-NBS-LRR class) family	171	2.00E-43
AT1G56510	WRR4, ADR2 | Disease resistance protein (TIR-NBS-LRR class) family	171	4.00E-43
AT1G56520	Disease resistance protein (TIR-NBS-LRR class) family	169	8.00E-43
AT5G41550	Disease resistance protein (TIR-NBS-LRR class) family	169	8.00E-43
AT5G58120	Disease resistance protein (TIR-NBS-LRR class) family	167	5.00E-42
AT1G56540	Disease resistance protein (TIR-NBS-LRR class) family	166	8.00E-42
AT4G08450	Disease resistance protein (TIR-NBS-LRR class) family	166	1.00E-41
AT1G57630	Toll-Interleukin-Resistance (TIR) domain family protein	164	3.00E-41
AT5G38850	Disease resistance protein (TIR-NBS-LRR class) family	163	6.00E-41
AT1G63730	Disease resistance protein (TIR-NBS-LRR class) family	162	1.00E-40
AT4G16990	RLM3 | disease resistance protein (TIR-NBS class), putative	162	9.00E-41

Protein	Function	Score	E-value
AT1G63880	Disease resistance protein (TIR-NBS-LRR class) family	161	3.00E-40
AT2G16870	Disease resistance protein (TIR-NBS-LRR class) family	161	3.00E-40
AT4G16950	RPP5 | Disease resistance protein (TIR-NBS class), putative	161	2.00E-40
AT5G17970	Disease resistance protein (TIR-NBS-LRR class) family	160	4.00E-40
AT1G66090	Disease resistance protein (TIR-NBS-LRR class) family	160	6.00E-40
AT5G46510	Disease resistance protein (TIR-NBS-LRR class) family	158	2.00E-39
AT5G46260	Disease resistance protein (TIR-NBS-LRR class) family	157	3.00E-39
AT5G46490	Disease resistance protein (TIR-NBS-LRR class) family	155	1.00E-38
AT1G31540	Disease resistance protein (TIR-NBS-LRR class) family	154	2.00E-38
AT5G46270	Disease resistance protein (TIR-NBS-LRR class) family	154	4.00E-38
AT1G63870	Disease resistance protein (TIR-NBS-LRR class) family	153	7.00E-38
AT4G19500	Nucleoside-triphosphates; trans membrane receptors; nucleotide binding; ATP binding	153	5.00E-38
AT4G36140	Disease resistance protein (TIR-NBS-LRR class) family	152	1.00E-37
AT4G16920	Disease resistance protein (TIR-NBS-LRR class) family	151	3.00E-37
AT4G16900	Disease resistance protein (TIR-NBS-LRR class) family	150	4.00E-37
AT5G46520	Disease resistance protein (TIR-NBS-LRR class) family	150	5.00E-37
AT4G04110	Disease resistance protein (TIR-NBS-LRR class) family	149	1.00E-36
AT5G41740	Disease resistance protein (TIR-NBS-LRR class) family	149	1.00E-36
AT1G63860	Disease resistance protein (TIR-NBS-LRR class) family	148	2.00E-36
AT1G63750	Disease resistance protein (TIR-NBS-LRR class) family	148	3.00E-36
AT5G17680	Disease resistance protein (TIR-NBS-LRR class) family	144	3.00E-35
AT4G16960	Disease resistance protein (TIR-NBS-LRR class) family	143	6.00E-35
AT4G16860	RPP4 | Disease resistance protein (TIR-NBS-LRR class) family	141	3.00E-34
AT1G63740	Disease resistance protein (TIR-NBS-LRR class) family	139	1.00E-33
AT5G36930	Disease resistance protein (TIR-NBS-LRR class) family	138	2.00E-33
AT4G14370	Disease resistance protein (TIR-NBS-LRR class) family	134	5.00E-32
AT1G72890	Disease resistance protein (TIR-NBS-LRR class) family	124	3.00E-29
AT4G19520	Disease resistance protein (TIR-NBS-LRR class) family	121	3.00E-28
AT1G72940	Toll-Interleukin-Resistance (TIR) domain-containing protein	118	2.00E-27
AT4G12010	Disease resistance protein (TIR-NBS-LRR class) family	116	7.00E-27
AT1G72950	Disease resistance protein (TIR-NBS-LRR class) family	116	9.00E-27
AT1G72920	Toll-Interleukin-Resistance (TIR) domain-family protein	116	1.00E-26
AT1G72860	Disease resistance protein (TIR-NBS-LRR class) family	115	2.00E-26
AT1G72900	Toll-Interleukin-Resistance (TIR) domain-family protein	115	2.00E-26
AT1G72930	TIR | toll/interleukin-1 receptor-like protein	112	1.00E-25
AT1G72910	Toll-Interleukin-Resistance (TIR) domain-family protein	112	1.00E-25
AT1G27170	Trans-membrane receptors; ATP binding protein	112	1.00E-25

Protein	Function	Score	E-value
AT4G16940	Disease resistance protein (TIR-NBS-LRR class) family	109	1.00E-24
AT1G17615	Disease resistance protein (TIR-NBS-LRR class) family	108	2.00E-24
AT4G36150	Disease resistance protein (TIR-NBS-LRR class) family	108	2.00E-24
AT2G20142	Toll-Interleukin-Resistance (TIR) domain-family protein	108	1.00E-24
AT5G48780	Disease resistance protein (TIR-NBS-LRR class) family	106	8.00E-24
AT1G57670	Toll-Interleukin-Resistance (TIR) domain-family protein	106	1.00E-23
AT5G45200	Disease resistance protein (TIR-NBS-LRR class) family	105	3.00E-23
AT1G57850	Toll-Interleukin-Resistance (TIR) domain-family protein	103	9.00E-23
AT1G72840	Disease resistance protein (TIR-NBS-LRR class) family	102	1.00E-22
AT1G27180	Disease resistance protein (TIR-NBS-LRR class) family	101	4.00E-22
AT5G45070	AtPP2-A8, PP2-A8 | phloem protein 2-A8	100	5.00E-22
AT1G57830	Toll-Interleukin-Resistance (TIR) domain-family protein	100	5.00E-22
AT4G19530	Disease resistance protein (TIR-NBS-LRR class) family	99	1.00E-21
AT5G45060	Disease resistance protein (TIR-NBS-LRR class) family	99	2.00E-21
AT5G45250	RPS4 | Disease resistance protein (TIR-NBS-LRR class) family	99	2.00E-21
AT4G19510	Disease resistance protein (TIR-NBS-LRR class) family	98	4.00E-21
AT2G32140	Trans membrane receptors	98	4.00E-21
AT5G17880	CSA1 | Disease resistance protein (TIR-NBS-LRR class) family	97	9.00E-21
AT1G09665	Toll-Interleukin-Resistance (TIR) domain-family protein	97	7.00E-21
AT5G48770	Disease resistance protein (TIR-NBS-LRR class) family	96	1.00E-20
AT1G47370	Toll-Interleukin-Resistance (TIR) domain-family protein	95	2.00E-20
AT2G03300	Toll-Interleukin-Resistance (TIR) domain-family protein	95	3.00E-20
AT4G19920	Toll-Interleukin-Resistance (TIR) domain-family protein	95	3.00E-20
AT5G40100	Disease resistance protein (TIR-NBS-LRR class) family	94	7.00E-20
AT1G17600	Disease resistance protein (TIR-NBS-LRR class) family	93	1.00E-19
AT4G09430	Disease resistance protein (TIR-NBS-LRR class) family	92	2.00E-19
AT3G51570	Disease resistance protein (TIR-NBS-LRR class) family	92	3.00E-19
AT1G60320	Toll-Interleukin-Resistance (TIR) domain-family protein	92	3.00E-19
AT2G17050	Disease resistance protein (TIR-NBS-LRR class) family	91	7.00E-19
AT2G03030	Toll-Interleukin-Resistance (TIR) domain-family protein	90	9.00E-19
AT4G23515	Toll-Interleukin-Resistance (TIR) domain-family protein	89	2.00E-18
AT5G44900	Toll-Interleukin-Resistance (TIR) domain-family protein	89	2.00E-18
AT5G44870	LAZ5 | Disease resistance protein (TIR-NBS-LRR class) family	88	3.00E-18
AT1G65390	ATPP2-A5, PP2-A5 | phloem protein 2 A5	86	1.00E-17
AT5G45230	Disease resistance protein (TIR-NBS-LRR class) family	86	2.00E-17
AT2G17060	Disease resistance protein (TIR-NBS-LRR class) family	84	5.00E-17
AT5G45220	Disease resistance protein (TIR-NBS-LRR class) family	84	6.00E-17
AT5G45000	Disease resistance protein (TIR-NBS-LRR class) family	83	1.00E-16

Protein	Function	Score	E-value
AT4G19925	Toll-Interleukin-Resistance (TIR) domain-family protein	83	1.00E-16
AT4G11340	Disease resistance protein (TIR-NBS-LRR class) family	82	2.00E-16
AT1G72870	Disease resistance protein (TIR-NBS-LRR class) family	80	6.00E-16
AT5G44920	Toll-Interleukin-Resistance (TIR) domain-family protein	80	6.00E-16
AT1G72850	Disease resistance protein (TIR-NBS-LRR class) family	75	2.00E-14
AT4G09420	Disease resistance protein (TIR-NBS-LRR class) family	75	3.00E-14
AT1G51270	Structural molecules; trans-membrane receptors; structural molecules	75	3.00E-14
AT5G45080	AtPP2-A6, PP2-A6 | phloem protein 2-A6	73	1.00E-13
AT4G23510	Disease resistance protein (TIR-NBS-LRR class) family	71	5.00E-13
AT4G19910	Toll-Interleukin-Resistance (TIR) domain-family protein	71	4.00E-13
AT5G45210	Disease resistance protein (TIR-NBS-LRR class) family	65	3.00E-11
AT1G52900	Toll-Interleukin-Resistance (TIR) domain-family protein	65	2.00E-11
AT3G51560	Disease resistance protein (TIR-NBS-LRR class) family	64	7.00E-11
AT4G16930	Toll-Interleukin-Resistance (TIR) domain-family protein	61	5.00E-10
AT5G45240	Disease resistance protein (TIR-NBS-LRR class) family	55	2.00E-08
AT5G44910	Toll-Interleukin-Resistance (TIR) domain-family protein	55	2.00E-08
AT5G45050	TTR1, ATWRKY16, WRKY16 | Disease resistance protein (TIR-NBS-LRR class) family	52	3.00E-07
AT1G17610	Disease resistance protein (TIR-NBS-LRR class) family	51	4.00E-07
AT2G17055	Toll-Interleukin-Resistance (TIR) domain-family protein	51	5.00E-07
AT1G61105	Toll-Interleukin-Resistance (TIR) domain-family protein	48	3.00E-06
AT5G40090	Disease resistance protein (TIR-NBS-LRR class) family	41	5.00E-04
AT4G12020	WRKY19, ATWRKY19, MAPKKK11, MEKK4 | Protein kinase family protein	41	4.00E-04
AT5G45090	AtPP2-A7, PP2-A7 | phloem protein 2-A7	38	0.004
AT5G56220	P-loop containing nucleoside triphosphate hydrolases	38	0.004
AT2G38840	Guanylate-binding family protein	31	0.61
AT3G08950	HCC1 | electron transport SCO1/SenC family protein	30	0.85
AT5G45260	RRS1, ATWRKY52, SLH1 | Disease resistance protein	28	3.7
AT5G17890	CHS3, DAR4 | DA1-related protein 4	27	6.6
AT3G48320	CYP71A21 | cytochrome P450, family 71, subfamily A, polypeptide 21	27	9.9

3. Chapter 3: QTL mapping of agronomic and seed quality traits and clubroot resistance, and their associations in a rutabaga × spring canola population[3]

3.1. Introduction

Rutabaga (*Brassica napus* var. *napobrassica*), a vitamin C- and mineral-rich root vegetable (USDA 2019), carries the same genome of *B. napus* canola (2*n* = 38, AC genome). This variety of *B. napus* possesses a winter growth habit, requiring vernalization for flowering, but genetically distinct from winter and spring canola (Diers and Osborn 1994; Bus et al. 2011). Rutabaga seed is low in oil content, and the oil contains erucic acid; the seed meal also contains a high level of glucosinolates.

Rutabaga, being genetically distinct from canola (Bus et al. 2011), may contribute favourable alleles to improve the genetic base of spring canola for seed yield, agronomic and seed quality traits (Shiranifar et al. 2020, 2021). However, this variety of *B. napus* can also introduce undesirable traits, such as vernalization requirement, the lateness of flowering and maturity, and high erucic acid and glucosinolate contents, in spring canola. Despite these challenges, Shiranifar et al. (2020) succeeded in developing several advanced generation spring canola lines with seed yield and oil content higher than the canola parents from rutabaga × spring canola crosses. Therefore, knowledge of the rutabaga gene pool, especially its alleles contributing to agronomic and seed quality traits, is important for the rational utilization of this gene pool in canola breeding. To our knowledge, no study has so far been conducted to map quantitative trait loci (QTL) for different traits in *B. napus* by using rutabaga.

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[3] A version of this chapter has been published in Euphytica as
Hasan, J., Shaikh, R., Megha, S., Herrmann, D.T., Kebede, B. and Rahman, H. 2021b. Mapping of flowering time, seed quality and clubroot resistance in rutabaga x spring canola populations and their association. Euphytica, **217**(8): 160. doi: https://doi.org/10.1007/s10681-021-02889-7

Several rutabaga cultivars were reported to carry resistance to clubroot disease caused by *P. brassicae* (Lammerink 1967; Johnston 1970; Ayers and Lelacheur 1972; Hasan et al. 2012; Yu 2019). Lammerink (1967) reported a dominant gene conferring resistance to *P. brassicae* race B and a second non-allelic dominant gene conferring resistance to race C in cv. Wilhelmsburger, while Johnston (1970) reported that a single dominant gene in this cultivar could confer resistance to race N4. Ayers and Lelacheur (1972) reported that this rutabaga cultivar carries one gene for resistance to race 3 and two genes for resistance to race 2. In the case of rutabaga, cv. York, a single dominant gene, controls resistance to both races 2 and 3. Hasan and Rahman (2016) identified a genomic region of chromosome A08 of rutabaga cv. Brookfield conferring resistance to pathotypes 2, 3, 5, 6 and 8; this resistance has been extensively used in Canada to develop clubroot resistant hybrid canola cvs., such as PV580 GC and PV585 GC (Nutrien Ag Solution, personal communication). Fredua-Agyeman et al. (2020) reported three genomic regions in rutabaga accessions from Nordic countries, conferring resistance to 16 *P. brassicae* pathotypes. Thus, it is apparent that rutabaga carries multiple clubroot resistance (CR) loci, and a single genomic region can confer resistance to multiple pathotypes. However, our knowledge of whether a single genomic region carries multiple resistance genes or a single gene exerting a pleiotropic effect on resistance to multiple pathotypes is limited. The occurrence of CR in rutabaga and its genetic distinctness from canola made this variety of *B. napus* attractive for use in canola breeding (Rahman et al. 2014b; Shiranifar et al. 2020, 2021).

Incorporation of disease resistance genes into crop cultivars generally secures crop production; however, this benefit, in some instances, can come at the cost of some economically important traits. For example, the mildew resistance gene *mlo* of barley has been reported to decrease 1000-grain weight and thus reduces grain yield by about 4% (Kjær et al. 1990). Similarly, *Fusarium* head blight (FHB) resistance QTL of spring wheat, located on

chromosome 2DL, decreases grain weight, and the QTL located on chromosome 6BS delays heading time (Suzuki et al. 2012). The eyespot resistance gene *Pch1* of *Aegilops ventricosa* (Doussinault et al. 1983), when introgressed into wheat (Groos et al. 2003), cost about 8% grain yield (Summers and Brown 2013). Similarly, the powdery mildew resistance gene *Pm16* of *Aegilops speltoides* (Chen et al. 2005) reduces grain yield in wheat by 15% (Summers and Brown 2013). In the case of *B. napus*, Zhao et al. (2006b), Wei et al. (2014) and Wu et al. (2019) reported a negative correlation between Sclerotinia stem rot resistance and flowering time. Rahman and Franke (2019) found a genomic region of chromosome A03 of winter canola cv. 'Mendel' conferring resistance to clubroot disease is associated with susceptibility to fusarium wilt disease in spring canola background. The clubroot resistance in the experimental materials used in this study has been introgressed from rutabaga (Hasan and Rahman 2016); therefore, an insight into the effect of the resistance genes on other traits due to linkage drag or pleiotropic effect is vital. To our knowledge, no study has so far been conducted to investigate the effect of the clubroot resistance genes of rutabaga on other plant traits. Thus, the objectives of this research were to identify the genomic regions associated with clubroot resistance, vernalization requirement, days-to-flowering, seed oil, protein, glucosinolate and erucic acid contents in rutabaga, and investigate the effect of the clubroot resistance on the flowering and seed quality traits.

3.2. Materials and methods

3.2.1. Plant material

A population of 102 doubled haploid (DH) lines, developed from F_1 plants of a rutabaga breeding line Rutabaga-BF crossed to a Canadian spring canola cultivar UA AlfaGold (Rahman 2017), were used in this study. Rutabaga-BF was developed from the rutabaga cv. Brookfield (Spaner 2002) through self-pollination of clubroot-resistant single plants. The details of the DH line development have been described elsewhere (Hasan and Rahman 2016).

3.2.2. Field trial and phenotyping

The DH population and their parents were evaluated in four field trials over three years at Edmonton Research Station (ERS) of the University of Alberta, Canada (latitude 53.49697, longitude -113.536750; 645 m above sea level). The trials were grown as two types of experiments: Direct seeding in the field (Exp. 1) or seeding in the greenhouse and transplanting the plants into the field after vernalization (Exp 2). In the case of Exp. 1, seeding was done on May 25th in 2012 and on May 28th in 2013; these two trials are designated as 2012NV and 2013NV, respectively. In this case, seeding was done in eight-foot-long single-row plots in three replications. In the case of the Exp. 2, the seedlings were grown in 2-inch × 2-inch cells in a greenhouse at 21±2 ºC temperature and 16/8 h (day/night) photoperiod for about four weeks and were vernalized for eight weeks at 4 °C with an 8/16 h (day/night) photoperiod. After vernalization, the seedlings, acclimatized at 10 °C for one week and 15 °C for another week, were transplanted in the field plots. The vernalized plants were transplanted in the field on May 24th in 2012 and on June 13th in 2016; these two trials are designated as 2012V and 2016V, respectively. In Exp 2, the number of plants grown in a plot was eight, and the number of replications was three in 2012 and two in 2016. Randomization of the trials was done following a Row-Column design with Microsoft Excel.

Days to flowering (DTF) data were recorded as the number of days from seeding or transplanting to the date when 50% of the plants in a plot had at least one open flower. Flowering data were recorded three times a week. Seed oil, protein and glucosinolate contents were estimated from open-pollinated bulk seeds harvested from Expt 2 using the near-infrared spectroscopy (NIRS) method (Osborne 2000) on a FOSS NirSystems 6500 (FOSS, Denmark), and data was adjusted at an 8.5% seed moisture basis. Erucic acid (EA) content in seed oil was estimated following the methodology to measure methyl ester of fatty acids of the oil on an Agilent 7890a gas chromatograph (Agilent, USA).

3.2.3. Resistance test to *Plasmodiophora brassicae* pathotypes

The DH lines and their parents were phenotyped for resistance to single spore isolates of *P. brassicae* pathotypes 2 or 2F, 3 or 3H, 5 or 5I, 6 or 6M and 8 or 8N, as classified on the differentials of Williams' (1966) or the Canadian Clubroot Differentials Set (Strelkov et al. 2018), and data reported elsewhere by Hasan and Rahman (2016). In brief, the seedlings were evaluated for resistance to these pathotypes at 42–45 days after inoculation. The severity of gall development was scored on a 0 to 3 scale, where 0 = no galling, 1 = one or few tiny galls on the lateral roots, 2 = moderate galling on the lateral roots, and 3 = severe galling on the lateral or main root. Plants with a disease score of 0 were classified resistant (R), while the plants with disease scores of 1, 2 or 3 were classified susceptible. By using the resistance scores, Disease Severity Index (DSI) was calculated for each DH line and parent by using the following formula (modified from Horiuchi and Hori 1980):

$$\boldsymbol{DSI}\ (\%) = \frac{\sum(\boldsymbol{n} \times \mathbf{0} + \boldsymbol{n} \times \mathbf{1} + \boldsymbol{n} \times \mathbf{2} + \boldsymbol{n} \times \mathbf{3})}{\boldsymbol{N} \times \mathbf{3}} \times \mathbf{100}$$

Where $\boldsymbol{n}$ is the number of plants in a class and 0, 1, 2, and 3 are the disease symptom severity classes, and $\boldsymbol{N}$ is the total number of plants.

A total of 47 and 38 advanced generations (BC_1F_5, BC_1F_7 and BC_1F_9) lines derived from Rutabaga-BF × Hi-Q and Rutabaga-BF × A07-26NR crosses, respectively, were evaluated for resistance to pathotype 3 to validate the QTL detected using the DH population. Hi-Q (Stringam et al. 2000) and A07-26NR (1918) are spring canola cultivars developed at the University of Alberta.

3.2.4. Genotyping, linkage map construction and QTL analysis

3.2.4.1. *Whole-genome resequencing (WGRS)*

QTL-seq approach following whole-genome resequencing (WGRS) was used to identify the genomic regions associated with clubroot resistance in the DH population. QTL-seq combines bulked-segregant analysis (BSA) (Michelmore et al. 1991) and WGRS allow

rapid identification of the genomic regions affecting the trait (Takagi et al. 2013). A total of three samples, that is, a resistant parent (RP) (Rutabaga-BF), one resistant bulk (R-pool) and one susceptible bulk (S-pool), were sequenced on Illumina HiSeq 2500 (Illumina Inc., San Diego, CA, USA). The R-pool was prepared by combining an equal amount of DNA from 10 resistant (disease score 0 for pathotype 3), and the S-pool was prepared by combining DNA from 10 susceptible (disease score 3 for pathotype 3) DH lines. The DNA libraries were prepared using the Paired-End DNA Sample Prep kit (Illumina). The libraries were used for cluster generation and sequenced on an Illumina HiSeq® platform (Illumina Inc., San Diego, CA, USA). All samples were sequenced twice to increase the depth and breadth of coverage. Sequencing and data analysis, including SNP calling and alignment to the reference genome, was done by Novogene Inc. (8801 Folsom Blvd, Sacramento, CA, USA).

A consensus whole-genome sequence for the resistant parent (RP) Rutabaga-BF was generated using *B. rapa* cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011; www.brassicadb.cn) as reference. The original image data was transformed to sequence data or raw reads by base calling with CASAVA v1.8.2 and stored as FASTAQ files. Adapter sequences and sequence read with >50% bases having Phred quality score <5 (Ewing and Green 1998) and reads with >10% of unknown bases were trimmed-off. Trimmed reads of the resistant parent (RP) were aligned to the reference genome assembly using BWA (Burrows-Wheeler Aligner) v.0.7.12 (Li and Durbin 2009). The aligned sequences were processed to extract the exact position of the mapped reads and then filtered using the mpileup function of Samtools v.1.3.1 (Li et al. 2009). The variants (single nucleotide polymorphism, SNP and Indels) between RP reads and the reference genome assembly was called using Genome Analysis Toolkit (GATK) (McKenna et al. 2010). A consensus sequence assembly of the resistant parent (hereafter referred to as RP assembly, RPA) was developed by substituting *B. rapa* cv. Chiifu-401 v1.5 nucleotides with variants from RP. The sequence read with read-

depth >7 and base quality ≥100 from R- and S-pool were aligned onto the reference-guided RPA and used to calculate their SNP-index. The SNP index of the R- and S-pool was calculated using the following formula suggested by Abe et al. (2012):

$$\text{SNP index} = \frac{\text{Number of alternate (alternate to RPA) nucleotide reads in bulk}}{\text{Total number of nucleotide reads in bulk}}$$

Delta (Δ) SNP-index was calculated by subtracting the R-pool SNP-index from the S-pool SNP-index. Only those SNP positions with ΔSNP-index = −1 were considered the causal SNPs for the trait of interest. ΔSNP-index = −1 indicates that the resistant bulk allele is the same as that of the resistant parent, while the alternate base is present in the susceptible bulk (Pandey et al. 2017), indicating the association of the SNPs with the trait.

Based on WGRS data, a total of 106 SNP-allele-specific PCR (AS-PCR) primers were designed using BatchPrimer3 (You et al. 2008) from the chromosome region associated with resistance to *P. brassicae* pathotype 3. The DH population was genotyped using these AS-PCR markers following the PCR conditions reported elsewhere by Hasan and Rahman (2016). Electrophoretic separation of the PCR amplicons was conducted on 2.0% agarose gel, stained with SYBR Green 1, and then imaged with a Typhoon FLA 7000 laser scanner (GE_Healthcare 2010).

3.2.4.2. ***Genotyping by 60K SNP array and KASP marker development***

The 47 + 38 = 85 inbred lines (BC_1F_5, BC_1F_7 and BC_1F_9) of Rutabaga-BF × Hi-Q and Rutabaga-BF × A07-26NR crosses were genotyped by *Brassica* 60K Illumina Infinium SNP genotyping array (San Diego, CA, USA). Genotyping, SNP data analysis including alignment to the *B. napus* reference genome (Chalhoub et al. 2014; www.genoscope.cns.fr/brassicanapus) was done by the Delta Genomics, Edmonton, AB (http://www.deltagenomics.com/). After removing the monomorphic markers, low coverage site markers, markers with minor allele frequency (MAF) ≥0.05, and the markers showing missing data in >5% of the accessions, a

total of 1,437 A-genome SNP markers polymorphic between the resistant (Rutabaga-BF) and susceptible parents (Hi-Q and A07-26NR) were identified and used for single-marker analysis.

The *Brassica* 60K SNP genotyping data was also used to design kompetitive allele-specific PCR (KASP) primers for genotyping the DH mapping population. Seventy-two KASP markers were designed based on 72 SNPs from the A08 QTL region (Hasan and Rahman 2016). The KASP analysis, including designing the primers and genotyping the DH population, was carried out by LGC Genomics LLC (100 Cummings Centre, Beverly, MA, USA; current name LGC Biosearch Technologies; https://www.lgcgroup.com/).

3.2.4.3. ***Linkage map construction and QTL analysis***

The DH population was genotyped using simple sequence repeat (SSR), AS-PCR, KASP and SNP markers. The details of the genotyping by SSR markers have been reported elsewhere by Hasan and Rahman (2016). The population was genotyped by 24 polymorphic AS-PCR markers designed based on the WGRS approach. The DH lines and their parents were also genotyped using a sub-set of 1,378 SNP from the *Brassica* 60K Illumina Infinium SNP genotyping array (San Diego, CA, USA) at Cargill Seed Innovation Centre (Fort Collins, CO). These SNPs were optimized as single-locus markers for *B. napus* (Clarke et al. 2016) and were selected for their even distribution on the A and C genome chromosomes based on a set of Brassica germplasm (unpublished data) at Cargill Seed Innovation Centre (Fort Collins, CO). The SNP markers were also selected based on GenTrain genotype scores above 0.75, as Illumina suggested, followed by selection for those lacking inter-homeologous polymorphism (Trick et al. 2009). Clarke et al. (2016) proposed the nomenclature for the optimized single-locus SNP markers following their alignment to the *B. napus* DH12075 whole-genome assembly v3.1; this nomenclature was used in this paper.

A genetic linkage map of the DH population was constructed using the SSR, AS-PCR, KASP and SNP genotype data in the software program JoinMap v.4 (Van Ooijen 2006). A

framework map for the 19 linkage groups was constructed using polymorphic markers showing a 1:1 segregation for the parental alleles. The polymorphic markers that deviated from 1:1 segregation was later added to this framework map. A threshold recombination frequency of 0.25 and logarithm of the odds (LOD) value of 5.0 was used for grouping the loci to construct the framework map; however, a higher threshold for recombination frequency (0.35) and LOD value of 3.0 was used to integrate the distorted markers in the framework map. The genetic distance between the pairs of markers was calculated by using the Kosambi mapping function (Kosambi 1943), and the linkage groups were named following the recommendation by the Multinational *Brassica* Genome Project (MBGP) (http://www.brassica.info/resource/maps/lg-assignments.php). The chromosome maps were illustrated with MapChart v. 2.32 (Voorrips 2002) using linkage distribution calculated by JoinMap v.4 (Van Ooijen 2006).

QTL analysis was conducted using the software program QTL IciMapping version 4.2 (Meng et al. 2015). The genetic map was scanned with Inclusive Composite Interval Mapping of ADDitive QTL (ICIM-ADD) model (Li et al. 2007a) to estimate the likelihood of a QTL and its additive effect at every 0.1 cM interval following a stepwise regression probability of 0.001 (Manichaikul et al. 2009). To declare a QTL based on individual trial data, the empirical threshold of LOD (p-value < 0.05) was obtained from 1,000 permutations (Churchill and Doerge 1994). Data were re-analyzed following the Composite Interval Mapping (CIM) method in the R/qtl package (Broman et al. 2003; Arends et al. 2010) of the software program R version 3.6.1 (R Core Team 2013) to confirm the accuracy of the QTL detected in each environment following the ICIM-ADD method. The likelihood of a QTL and its effects at every 0.1 cM intervals was estimated in a window of 10 cM. The QTL detected in different environments was considered the same if their C.I. (Confidence Interval) overlapped with 2-LOD (equal to 95% C.I.). On the other hand, if the C.I. of one QTL from one environment did

not overlap the C.I. of a QTL in a different environment, the two QTL were considered independent.

The QTL was named using the 'designation' system described by McCouch et al. (1997) with minor modifications. For example, the name *qDTF-2012_A2* was used for a QTL mapped on chromosome A02 associated with days-to-flowering (DTF) and detected in the 2012 field trial. Similarly, *qVERN-2016_A2* was used for a QTL mapped on chromosome A02 associated with vernalization response and detected in 2016. However, the name *qVERN_A2* was used for a QTL mapped on chromosome A02 associated with vernalization response and detected in multiple trials. An additional suffix (*a, b*, *c,* and *d*) was used if more than one QTL for a trait was identified on the same chromosome. The additive effect of the QTL and the proportion of the total phenotypic variance (R^2) explained by the QTL were captured from the QTL peak area.

3.2.4.4. ***Single marker analysis***

Single marker analysis was carried out using 1,437 SNP data from the *Brassica* 60K array and DSI for resistance to pathotype 3 of the pedigree lines. The analysis was performed using the software program QTL IciMapping version 4.2 (Meng et al. 2015). The likelihood of association between the marker and the trait was performed following a stepwise regression probability of 0.001 (Manichaikul et al. 2009). To declare a marker-trait association, the empirical threshold of LOD (p-value < 0.05) was obtained from 1,000 permutations (Churchill and Doerge 1994).

3.2.5. Physical mapping of the genetic markers

The physical position of the marker was determined by aligning the forward and reverse primer or the marker sequences to the *B. napus* cv. Darmor-*bzh* whole-genome assembly v4.1 (Chalhoub et al. 2014; www.genoscope.cns.fr/brassicanapus) or to the *B. rapa* cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011; www.brassicadb.cn) by using BLASTn

search (Altschul et al. 1997) tool in *Brassica* Database server (BRAD, http://brassicadb.cn). When the forward and reverse primer sequences aligned with the DNA sequence of a chromosome and the deduced length of the fragment between the forward and reverse primer sequences or the markers match with the known or predicted amplicon size, the position of the marker was considered to be the physical position. In the case of the SNP markers detected based on the *B. napus* cv. Darmor-*bzh* whole-genome assembly v4.1 (Chalhoub et al. 2014), the most likely position of these markers in the *B. rapa* cv. Chiifu-401 genome assembly v1.5 (Cheng et al. 2011) was determined from BLASTn hit with the greatest E-value.

The genome sequences of *B. napus* and *B. rapa*, flanked by the QTL markers, were scanned for putative candidate genes using Generic Genome Browser v2.56 in *Brassica* Database (BRAD, http://brassicadb.cn). The putative candidate genes identified in the Brassica genome were aligned with the Arabidopsis genome (The Arabidopsis Information Resource TAIR, http://www.arabidopsis.org) to identify the homoeologous genes with known function.

3.2.6. Other analysis

Spearman's correlation coefficient and LSmean values were calculated using the software program R version 3.6.1 (R Core Team 2013). Broad-sense heritability (H^2) was calculated as

$$H^2 = \frac{\sigma_g^2}{(\sigma_g^2 + \frac{\sigma_{ge}^2}{n} + \frac{\sigma_e^2}{nr})}$$

where, σ_g^2 = genetic variance, σ_e^2 = error variance, σ_{ge}^2 = interaction variance between genotypes and environments, n = number of environments, and r = number of replications in each environment. The estimates of σ_g^2, σ_e^2 and σ_{ge}^2 were obtained from a two-way ANOVA, carried out by the software program R version 3.6.1 (R Core Team 2013). For variance analysis, the genotypes, environments and interactions between the genotypes and environments were considered random effects.

3.3. Results

3.3.1. Phenotypic variation for flowering time

In Expt 1, where seeds were planted directly in field plots, the parent Rutabaga-BF failed to flower, confirming its need for vernalization to transit from vegetative to reproductive phase. Of the 97 DH lines evaluated in this experiment, 41 did not flower, indicating their need for vernalization for flowering (Figure S3.1a). The DH population followed a 1:1 segregation (χ^2 = 2.3196, *df* = 1, *P*-value = 0.128) for spring and winter growth habit, suggesting a major locus in rutabaga for vernalization requirement.

In Expt 2, where the plants were transplanted in the field after vernalization, all DH lines and their parents flowered; Rutabaga-BF flowered the latest (Figure S3.1d, S3.1e). The frequency distribution of the DH lines evaluated in this experiment (Figure S3.1d and S3.1e) and the spring-growth habit DH lines evaluated in Expt 1 (Figure S3.1b, S3.1c) showed a continuous variation for days-to-flowering indicating quantitative genetic control of this trait. Broad-sense heritability for days-to-flowering was 97% in the vernalized experiment and 81% in the non-vernalized experiment.

3.3.2. Phenotypic variation for seed oil, protein, glucosinolate and erucic acid

Rutabaga-BF and UA AlfaGold differed significantly for seed oil, glucosinolates and erucic acid contents. The oil content of UA AlfaGold was 5.7% higher, while glucosinolate and erucic acid contents were 43.6 μmol/g seed and 24.0% (two-season average), respectively, which is lower than Rutabaga-BF (Table S3.3). The DH population exhibited a continuous variation for these seed quality traits (Figure S3.2), where transgressive segregation was found for oil (Figure S3.2a, S3.2b) and protein (Figure S3.2c, S3.2d) contents. In the case of erucic acid content, the DH population displayed a bimodal distribution (Figure S3.2g, S3.2h), indicating that a major gene is involved in controlling this trait in Rutabaga-BF. Broad-sense

heritability for oil, protein, glucosinolate and erucic acid was 74%, 71%, 86% and 98%, respectively.

3.3.3. Correlation between days-to-flowering and seed quality traits

Only oil content showed a weak but significant positive correlation (r = 0.21, P = 0.01) with days-to-flowering (Figure S3.3a). Seed oil content showed a strong negative correlation with seed protein (r = -0.82) (Figure S3.3j) and a weak negative correlation with seed glucosinolate content (r = -0.39) (Figure S3.3e).

3.3.4. Effect of clubroot resistance on days-to-flowering and seed quality traits

The disease reaction data of the DH lines to *P. brassicae* pathotypes 2, 3, 5, 6 and 8, reported by Hasan and Rahman (2016), were analyzed with growth habits, days-to-flowering, oil, protein, erucic acid and glucosinolate data. Resistance to these pathotypes did not show a statistically significant correlation with the growth habit, flowering, and seed quality traits (Table S3.1). To further confirm this, the DH population was partitioned into resistant and susceptible types for each pathotype; and these two groups were compared for flowering and seed quality traits. Two-sample *t*-tests showed no significant difference between the resistant and susceptible groups for growth habit (winter or spring), days-to-flowering, seed oil, protein, glucosinolate, and erucic acid contents (Table S3.2).

3.3.5. Identification of the genomic region for clubroot resistance based on WGRS data

Calculation of ΔSNP-index identified 2,159 SNPs from 10 A-genome chromosomes. Among these, chromosome A08 carried 93.8% (2,025/2,159) of the SNPs indicating that the gene for resistance to pathotype 3 is present on this chromosome (Figure 1a). Of the total number of SNPs of A08, the great majority is located at about 10.0 to 15.0 Mb region (Figure 1b), suggesting that this genomic region carries resistance to this pathotype. Based on the highly confident SNPs from A08, 118 SNP-based AS-PCR primers (118 × 3 = 354 primers) were designed.

3.3.6. Linkage map construction

Of the 118 SNP AS-PCR and 72 KASP markers, 24 (20.3%) AS-PCR and 42 KASP (58.3%) markers were polymorphic between the two parents Rutabaga-BF and UA AlfaGold. The DH population was genotyped using the polymorphic 24 AS-PCR and 42 KASP markers. In the case of the SNP markers from the *Brassica* 60 K Illumina (San Diego, CA, USA) Infinium SNP genotyping array (genotyped at Cargill Seed Innovation Centre), 1,378 SNP probes were tested on the two parents, of which 40.28% (555/1,378) were polymorphic. Thus, the genotypic data of the 227 SSR (Hasan and Rahman 2016), 24 AS-PCR, 42 KASP and 555 SNP markers were used to construct the linkage map. Among these, a total of 585 markers, which included 108 SSR, 13 AS-PCR, 29 KASP and 435 SNP markers, could be assigned to 19 linkage groups (Figure 2). This linkage map had a total length of 1,202.1 cM, where 648.3 cM for the A-genome and 553.8 cM for the C-genome chromosomes. The smaller size map, as compared to many of the published maps, apparently resulted from limited genome coverage for some of the chromosomes, such as A5, C6, C7 and C8. The linkage map's average marker density was 2.05 cM between the markers, where chromosome A08 had the highest density (0.83 cM). The number of marker loci in the linkage groups varied from 10 (A02) to 108 (A08), and the length varied from 24.9 cM (A05) to 104.1 cM (C03). As compared to the genetic map of A08 reported by Hasan and Rahman (2016), the A08 of this map contained 73 additional markers (5 SSR, 13 SNP AS-PCR, 29 KASP and 26 SNP), and this reduced the length of this chromosome from 107.60 cM to 90.1 cM.

3.3.7. QTL for clubroot disease resistance

The disease reaction data of the DH lines to *P. brassicae* pathotypes, estimated qualitatively (disease score ‘0’ as resistant and scores 1, 2, and 3 as susceptible) and quantitatively (DSI ranging from 0 to 100), were used to map the QTL associated with resistance. QTL analysis using the quantitative (DSI) data identified one QTL, *qCR_A8*, on

chromosome A08 and one QTL, *qCR*_A3, on chromosome A03 (Table 3.1). *qCR_A8*, located in the interval of 20.4 to 31.8 cM, explained 84.4%, 74.6%, 80.1%, 89.1% and 89.1% of the total phenotypic variance for resistance to pathotype 2, 3, 5, 6 and 8, respectively. BLAST alignment of the flanking marker sequences, UACSSR3667 and UACAS4263(T), positioned the *qCR_A8* in an interval of 9,303,840 to 12,229,696 bp of the A08 of *B. rapa* cv. Chiifu-401 genome-assembly v1.5 (Cheng et al. 2011). The QTL *qCR_A3*, located in the interval of 15.5 to16.1 cM of chromosome A03, explained about 12.4% of the total phenotypic variance for resistance to *P. brassicae* pathotype 3 (Table 3.1). QTL analysis using the qualitative data (resistant or susceptible) also broadly confirmed the two QTL mentioned above on A03 and A08. In all cases, the resistance alleles were derived from the parent Rutabaga-BF.

Single marker analysis of the pedigree lines, derived from Rutabaga-BF × Hi-Q and Rutabaga-BF × A07-26NR crosses, detected 17 SNP markers with LOD>10 associated with resistance to pathotype 3 (Figure 3.3, Table S3.4). These markers are positioned in the interval of 9,644,955 to 12,930,447 bp of A08, where the greatest number of markers is located at about 10 Mb position. This further confirms the location of the *qCR_A8* QTL conferring resistance to pathotype 3.

3.3.8. QTL for vernalization requirement and days-to-flowering

QTL analysis identified a major QTL, *qVERN_A2,* associated with vernalization requirement for flowering in the non-vernalized field trials (Expt. 1) (Table 3.3; Figure 3.2). The *qVERN*_A2, located in the interval of 16.4 to 19.8 cM on chromosome A02, explained about 15% of the total phenotypic variance (Table 3.2).

In the case of the vernalized field trials (Expt. 2), two QTL, *qDTF_A2* and *qDTF_C6*, affecting days-to-flowering, were identified on chromosomes A02 and C06, respectively (Table 3.2; Figure 3.2). The *qDTF_A2* is positioned in the genomic region of A02, where the *qVERN_A2* is located. *qDTF_A2* explained 16.3% and 21.5% of the total phenotypic variance

in 2012 and 2016 trials, respectively. The other QTL, *qDTF_C6*, also explained a similar phenotypic variance (13.1% and 19.5%) in these two growing seasons. QTL analysis of the spring growth habit DH lines (n = 58) from Expt. 1 (2012NV and 2013NV) also identified the 17.9 cM genomic region of A02, where the QTL *qDTF_A2* or *qVERN_A2* is located, affecting days-to-flowering.

The QTL *qVERN_A2/qDTF_A2* is located in a 5.3 cM region flanked by the SNP markers Bn-N2-p10848961 and Bn-N2-p8281271. BLAST alignment of the flanking marker sequences positioned *qVERN_A2/qDTF_A2* in the interval of 8,293,255 bp to 10,096,464 bp of A02 of the *B. napus* cv. Darmor-*bzh* genome v4.1 (Chalhoub et al. 2014). The QTL *qDTF_C6* is located in a 2.5 cM region flanked by the SNP markers Bn-N16-p7584100 and Bn-N16-p15822788; these two markers could be positioned at 7,257,006 bp and 15,708,434 bp of C06 of the *B. napus* cv. Darmor-*bzh* genome v4.1 (Chalhoub et al. 2014). For these two QTL, Rutabaga-BF alleles contributed 1.6- and 1.5-days variation in flowering, respectively (Table 3.2).

Environmental influence on the *qVERN_A2* was small, as evident from a minor difference between the total phenotypic variance (PVE) and the phenotypic variance explained by the genetic (PVE-A) effect at the QTL position. However, the environment exerted a greater influence on the *qDTF_A2* for days-to-flowering in the DH lines with spring growth habit; this is evident from about 26% difference between the total phenotypic variance (PVE) and phenotypic variance explained by the genetic (PVE-A) effect at the QTL position (Table 3.2).

3.3.9. QTL for oil, protein, glucosinolates and erucic acid

QTL analysis mapped *qOIL_C3*, a QTL for seed oil content, at about 73 cM of chromosome C03, explaining 13.3% and 14.7% of the total phenotypic variance in 2012 and 2016, respectively. BLAST alignment of the flanking primer sequences positioned the *qOIL_C3* in the interval of 47,390,156 bp to 50,573,817 bp of the *B. napus* cv. Darmor-*bzh*

genome assembly v4.1 (Chalhoub et al. 2014). The G×E analysis indicated a substantial environmental influence on this QTL, evident from the difference between the PVE and PVE-A (14.1 vs. 7.3) (Table 3.3). A major QTL for erucic acid, *qERUCIC_C3*, was identified at about 16 cM away from this seed oil QTL (*qOIL_C3*). The *qERUCIC_C3* explained 31.1% and 40.6% of the total phenotypic variance in 2012 and 2016 trials, respectively. BLAST alignment of the flanking primer sequences positioned the *qERUCIC_C3* in the interval of 49,267,208 bp to 55,062,494 bp of the *B. napus* cv. Darmor-*bzh* genome assembly v4.1 (Chalhoub et al. 2014). The high oil and erucic acid alleles are derived from rutabaga.

QTL analysis identified *qPROTEIN_C3, a* minor QTL for seed protein content, on chromosome C03 and *qGSLs_A9*, a major QTL for seed glucosinolate content on chromosome A09. The *qPROTEIN_C3*, located in the genomic region where *qOIL_C3* is located, explained 9% of the total phenotypic variance for seed protein content (Table 3.3). The *qGSLs_A9* explained 32.2% and 28.7% of the trait variance in 2012 and 2016 trials, respectively, and exerted an additive effect of 6.5 µmol glucosinolate per g seed (Table 3.3). BLAST alignment of the flanking marker sequences positioned the *qGSLs_A9* in the interval of 3,791,683 bp to 4,094,922 bp of the *B. napus* genome (Chalhoub et al. 2014). The high-glucosinolate allele of this QTL derived from rutabaga.

3.4. Discussion

Canola oil contains <2% erucic acid, and a gram of oil-free seed meal contains <30 µmol glucosinolates. It is the 2nd major oil crop (FAO 2020) grown in many parts of the world either as a winter, semi-winter or spring crop. The winter canola cultivars require vernalization, while the spring type does not require this to initiate flowering. Early flowering and delayed maturity often results in higher seed yield in canola (Habekotté 1997). Flowering is the transition of a plant from vegetative to the reproductive phase in its life cycle. This transition determines how much the plant invests into vegetative growth and, consequently, how much nutrients will be

available at the time of grain filling (Méndez-Vigo et al. 2011). The flowering time has implications on plasticity in water-use efficiency (Kenney et al. 2014), carbohydrate availability (Graf et al. 2010), plant vigour (Ni et al. 2009) and resistance to disease (Wei et al. 2014).

In this research, QTL affecting vernalization response, days-to-flowering, clubroot resistance, seed oil, protein, glucosinolate, and erucic acid content have been identified by using 102 doubled haploid (DH) lines derived from rutabaga × spring canola cross. The use of a DH population allowed to phenotype for various traits in multiple experiments. A larger mapping population is generally preferred for detecting both large and small effect QTL (Li et al. 2006). However, large effect QTL can also be detected using a smaller mapping population, although this can give an underestimation of the number of QTL and an overestimation of the percent phenotypic variance explained by the marker or QTL (Bradshaw et al. 1995, 1998; Vales et al. 2005).

By phenotyping the DH population with or without vernalization, this study identified an 8-10 Mb region of chromosome A02 associated with vernalization response and days-to-flowering. Several researchers have studied the genetics of vernalization response and days-to-flowering in Brassica (e.g. Ferreira et al. 1995; Delourme et al. 2006; Zhao et al. 2010; Raman et al. 2013; Javed et al. 2016; Arifuzzaman et al. 2017; Rahman et al. 2017, 2018; Yu et al. 2019; Ghanbari et al. 2020; Tudor et al. 2020) where most of the studies focused on these two traits separately. Individual research groups have reported as few as one QTL (Ferreira et al. 1995; Zhao et al. 2010; Tudor et al. 2020) to as high as six QTL (Ghanbari et al. 2020) for vernalization and as few as two QTL (Arifuzzaman et al. 2017) to as high as 20 QTL (Raman et al. 2013) for days-to-flowering in a single mapping population. These studies reported QTL for vernalization response on chromosomes A02, A03, A06, A07, C02, C03 and C05, and QTL affecting days-to-flowering on most of the A and C genome chromosomes, including A01 and

C06. As I have investigated these two traits in a single study, QTL mapping of these two traits has also been carried out in *B. rapa* by Zhao et al. (2010) and in winter oilseed *B. napus* by Tudor et al. (2020); these two studies reported a genomic region from the top of the chromosome A02 affecting both vernalization-responsive flowering and flowering time variation. Thus, the results from this study by using a different variant of *B. napus* (var. *napobrassica*) further substantiate the results reported by Zhao et al. (2010) and Tudor et al. (2020). According to Tudor et al. (2020), allelic variation in *FLOWERING LOCUS C* (*BnaFLC.A02*) and *FLOWERING LOCUS T* (*BnaFT.A02*) located in the 10 Mb region of A02 affects vernalization requirement and flowering time variation, respectively. The QTL *qVERN_A2/qDTF_A2* detected in this study encompassed a large region (about 7 cM) of the chromosome A02; a BLAST search of this genomic region identified several flowering time genes, including a *FLOWERING LOCUS C* (*FLC*) gene *BnaA02g00370D* (ID# 106383096), a *VERNALIZATION INSENSITIVE 3* like protein-coding *VIN3* gene LOC106388998, and a flowering time control protein FY encoding gene *BnaA02a01670D* (ID# 106384227). Vernalization activates the *VIN3* gene (Sung and Amasino 2004), and this gene represses the expression of *FLC* and promotes flowering. The flowering time control protein FY also decreases the *FLC* mRNA and regulates flowering time through the autonomous pathway (Henderson et al. 2005). This study further demonstrates that *qDTF_A2* could exert more than double the magnitude of effect on the non-vernalized spring growth habit plants compared to the vernalized plants for days-to-flowering. The occurrence of a single major QTL affecting both vernalization requirement and days to flowering and a second major QTL for days to flowering in rutabaga cv. Brookfield has an implication in spring canola breeding. In this case, the breeding population derived from rutabaga × canola will segregate for one locus for vernalization; thus, a relatively smaller population will be required to select a plant with spring growth habit.

QTL for seed oil content have been reported almost in all 19 chromosomes of *B. napus* (Ecke et al. 1995; Burns et al. 2003; Delourme et al. 2006; Qiu et al. 2006; Zhao et al. 2008, 2012; Jiang et al. 2014; Liu et al. 2016b; Chao et al. 2017; Tang et al. 2019), where an individual research group reported as few as three QTL (Ecke et al. 1995) to as many as 67 QTL (Chao et al. 2017) in a single mapping population. Most of the QTL in *B. napus* has been detected by using either winter (Ecke et al. 1995; Burns et al. 2003; Delourme et al. 2006) or spring (Javed et al. 2016) growth habit populations or by using populations derived from winter × Chinese semi-winter crosses (Qiu et al. 2006; Jiang et al. 2014). It is well known that rutabaga is genetically distinct from oilseed *B. napus* (Bus et al. 2011); therefore, this variant of *B. napus* may carry favorable alleles for various agronomic and seed quality traits. To my knowledge, very few studies have so far been conducted to extend our knowledge in this gene pool (Shiranifar et al. 2020, 2021). The resulted reported from this study disclosed a QTL, *qOIL_C3*, on chromosome C03, where the allele for increased oil content derived from Rutabaga-BF. Oil content in this rutabaga line is about 6% less than that of the spring canola cv. UA AlfaGold; however, the *qOIL_C3* allele of Rutabaga-BF contributed about a 0.7% increase in seed oil content (Table 3.3). QTL analysis also identified an erucic acid QTL, *qERUCIC_C3*, at about 16 cM or 5 Mb away from the oil QTL, *qOIL_C3*, on chromosome C03. The occurrence of the high erucic acid and high oil QTL alleles on the same chromosome could be advantageous for using this genomic region to breed high-erucic acid high-oil rapeseed, but this can impede the breeding of canola for high oil content. However, the occurrence of these two QTL at about 16 cM apart suggests that recombination between these two loci can occur, and the high-oil C03 QTL allele of rutabaga can be used in breeding for high-oil canola. In fact, Shiranifar et al. (2020) reported a few canola (zero erucic acid, low glucosinolate) lines from Rutabaga-BF × spring canola crosses with 1–2% higher seed oil content than their spring canola parents.

Two major QTL, located on A08 and C03, regulate erucic acid biosynthesis in *B. napus* (Ecke et al. 1995; Qiu et al. 2006; Cao et al. 2010; Tang et al. 2019). The results reported from this study demonstrated that the line Rutabaga-BF carries one of the two major erucic acid QTL. This knowledge has implications on *B. napus* canola breeding. In this case, the breeding population derived from Rutabaga-BF × canola will segregate for one of the two erucic acid loci; thus, a relatively smaller size population will be required to select a plant with zero erucic acid in the oil.

Rutabaga-BF contains about 55 µmol glucosinolates per g seed, which is about 5x greater than the content of the spring canola parent UA AlfaGold. QTL for seed glucosinolate content have been reported from at least nine A- and C-genome chromosomes (e.g. A01, A02, A03, A04, A08, A09, C02, C03 and C09) (Schatzki et al. 2014; Rahman et al. 2014a; Liu et al. 2016a; He et al. 2018; Kittipol et al. 2019; Rahman and Kebede 2021). This study identified a major QTL located at about 3.8-4.1 Mb position of A09. A major QTL at one end of chromosome A09 (Rahman et al. 2014a; Liu et al. 2016a; He et al. 2018) or about 5 Mb position of this chromosome (Li et al., 2014; Kittipol et al., 2019) has also been reported in *Brassica*. It is estimated that the non-oilseed *B. napus*, which also includes rutabaga, diverged from oilseed type *B. napus* about 277 years ago (Lu et al. 2019). Based on the approximate genomic location of the A09 seed glucosinolate QTL in oilseed *B. napus* and rutabaga, it can be anticipated that no major structural change in this chromosome region occurred since the divergence of rutabaga (non-oilseed type) from oilseed *B. napus*.

Several rutabaga cultivars, including Brookfield, have been reported to carry resistance to clubroot disease (Hasan et al. 2012). By constructing a genetic linkage map of chromosome A08, Hasan and Rahman (2016) reported a genomic region of this chromosome of the rutabaga cv. Brookfield carries resistance to *P. brassicae* pathotypes 2, 3, 5, 6 and 8. This study further confirmed the A08 QTL by using a genetic map of 19 *B. napus* chromosomes and also detected

an additional QTL on A03 contributing resistance to pathotype 3. Pathotype 3 has been one of the most prevalent and virulent pathotypes in Canada. A recent study showed that the rutabaga cv. Brookfield also carries resistance to the newly evolved pathotypes, such as 3A and 5X (Shaikh et al. 2020). Whether the A03 QTL plays a role in resistance to the newly evolved pathotypes would need further investigation.

Previous reports have identified at least five race-specific CR loci, such as *Crr1*, *CRs*, *Rcr3*, *Rcr9*^{wa} and *qBrCR38-2*, on chromosome A08 (Suwabe et al. 2003; Yu et al. 2017; Laila et al. 2019; Zhu et al. 2019b; Karim et al. 2020). Among these, *Crr1* (Suwabe et al. 2003), *CRs* (Laila et al. 2019), *Rcr3* and *Rcr9*^{wa} (Karim et al. 2020) are located at about 9.6 - 11.8 Mb and *qBrCR38-2* (Zhu et al. 2019b) at about 18.7 - 20.2 Mb position of A08 of *B. rapa* cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011) (for review, see Hasan et al. 2021). These CR loci of chromosome A08 have been reported independently by different researchers by using different pathotypes. Therefore, the possibility of naming the same locus with different names cannot be ruled out, as has been reported in the case of *CRa* and *CRb* of the chromosome A03 (Hatakeyama et al. 2017). Based on the physical position of the CR loci reported on A08, the *qCR_A8* that has been detected in this study would correspond to the *Crr1*, *CRs*, *Rcr3* or *Rcr9*^{wa}.

Chromosome A03 carries two genomic regions (about 14.4 - 15.4 Mb and 23.5 - 25.0 Mb), harboring multiple clubroot resistance loci (for review, see Hasan et al. 2021). The 14.4 - 15.4 Mb region carry *CRd* (Pang et al. 2018), *CRk* (Sakamoto et al. 2008) and *Crr3* (Hirai et al. 2004; Saito et al. 2006), while the 23.8 - 25.0 Mb region carry *CRa/CRb* (Fredua-Agyeman and Rahman 2016; Hatakeyama et al. 2017), *CRq* (Yuan et al. 2015), *Rcr1* (Chu et al. 2014), *Rcr2* (Huang et al. 2017), *Rcr4* (Yu et al. 2017) and *Rcr5* (Huang et al. 2019). By using 124 rutabaga accessions from Nordic countries and following association mapping approach, Fredua-Agyeman et al. (2020) reported four SNPs positioned at the 23.8 -25.0 Mb region of

chromosome A03 associated with resistance to *P. brassicae* pathotype 3/3H. However, based on the flanking marker sequences of *qCR_A3,* which has been detected in this study, this locus can be positioned at 15.1 – 15.8 Mb region of A03 of *B. rapa* cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011). Therefore, it is likely that the locus *qCR_A3* corresponds to *CRd, CRk* or *Crr3*, reported previously by other researchers. Thus, QTL mapping of clubroot resistance and agronomic and seed quality traits as well as analysis of the DH population for all these traits also demonstrated that clubroot resistance of the rutabaga cv. Brookfield does not exert any effect on vernalization requirement and days to flowering, as well as on seed oil, protein, glucosinolate and erucic acid contents.

3.5. Conclusion

This is the first report on the QTL mapping of flowering and seed quality traits in rutabaga. This study demonstrated that rutabaga carries an allele which increases seed oil content, suggesting the utility of this gene pool for increasing seed oil content in spring oilseed *B. napus*. By evaluating the DH population with or without vernalization, this study confirmed the presence of a major QTL for vernalization requirement on chromosome A02 and demonstrated that this genomic region also contributes to the variation in flowering time. In the case of clubroot resistance, in addition to the QTL on A08, this study identified a QTL on A03 conferring resistance to *P. brassicae* pathotype 3. This study also demonstrated that the clubroot resistance of rutabaga does not affect days-to-flowering and seed quality traits. The occurrence of a single major QTL for erucic acid and a single QTL for vernalization requirement in the rutabaga cv. Brookfield has implications in spring canola breeding. In this case, the breeding population derived from rutabaga × canola will segregate for one erucic acid locus and one locus for vernalization; thus, a relatively smaller population will be required to select a plant with spring growth habit and zero erucic acid in the oil. The knowledge of the QTL and molecular markers identified in this study can potentially be used to breed *B.*

napus canola for improved agronomic and seed quality traits, including resistance to clubroot disease. It could be inferred that the rutabaga gene pool can also be used to improve the winter oilseed *B. napus*; however, this needs to be demonstrated experimentally.

3.6. Tables

Table 3.1: QTL for resistance to *Plasmodiophora brassicae* pathotypes 2, 3, 5, 6 and 8 identified by using a doubled haploid (DH) population derived from Rutabaga-BF × UA AlfaGold of *Brassica napus*. QTL analysis was carried out by using quantitative data for resistance to these pathotypes

QTL	*P. brassicae* pathotype	Left flanking marker	Right flanking marker	Chr.	Position (cM)	C.I. (cM)	LOD	PVE (%)	Additive effect	Marker with highest LOD score
qCR_A3	Path 3	Bn-N3-p16098951	Bn-N3-p15592124	A03	15.7	15.5 – 16.1	11.5	12.4	-19.6	Bn-N3-p15493124
qCR_A8	Path 2	UACSSR3667	UACAS4263(T)	A08	24.7	20.4 – 31.8	43.3	84.4	-41.4	sS1702
	Path 3	UACSSR3667	UACAS4263(T)	A08	24.7	20.4 – 31.8	27.9	74.6	-35.3	sS1702
	Path 5	UACSSR3667	UACAS4285(G)	A08	24.7	20.4 – 30.8	36.6	80.1	-36.5	sS1702
	Path 6	UACSSR3667	UACAS4263(T)	A08	24.7	20.4 – 31.8	50.1	89.1	-41.1	UACAS3643(A)
	Path 8	UACSSR3667	UACAS4263(T)	A08	24.7	20.4 – 31.8	48.0	89.1	-41.0	UACAS3643(A)

C.I.: Confidence Interval
PVE: Percent of the total phenotypic variance explained by both genetic and environmental effects at the current scanning position.
Additive effect (in negative value) is the reduction of disease severity index (DSI) due to substitution of the 'UA AlfaGold' allele by one 'Rutabaga-BF' allele

Table 3.2: QTL for vernalization (VERN) requirement and days-to-flowering (DTF) identified by using a doubled haploid (DH) population derived from Rutabaga-BF × UA AlfaGold of *Brassica napus*.

QTL	Analysis method[1]	Expt.[2]	Left marker	Right marker	Chr.	Position (cM)	C. I. (cM)[3]	LOD[4]	PVE[5]	LOD- A[6]	PVE -A[7]	Additive effect[8]
qVERN_A2	IT	2012NV	Bn-N2-p10848961	Bn-N2-p7866337	A02	19.7	13.1 – 20.3	4.5	14.5			-
	IT	2013NV	Bn-N2-p10848961	Bn-N2-p7866337	A02	19.7	13.1 – 20.3	3.9	15.1			-
	MET	2012NV + 2013NV	Bn-N2-p10848961	Bn-N2-p7866337	A02	19.7	13.1 – 20.3	7.8	12.9	7.8	12.9	-
qDTF_A2	IT	2012V	Bn-N2-p10848961	Bn-N2-p7866337	A02	16.5	13.1 – 20.3	4.0	16.3			1.8
	IT	2016V	Bn-N2-p10848961	Bn-N2-p7866337	A02	19.8	13.1 – 20.3	5.6	21.5			1.6
	MET	2012V + 2016V	Bn-N2-p10848961	Bn-N2-p7866337	A02	18.8	13.1 – 20.3	9.2	18.8	8.9	18.8	1.6
	IT	2012NV-S	Bn-N2-p10848961	Bn-N2-p8281271	A02	17.9	13.1 – 19.8	4.6	30.9			7.1
	IT	2013NV-S	Bn-N2-p10848961	Bn-N2-p8281271	A02	17.9	13.1 – 19.8	5.3	35.6			8.2
	MET	2012NVS + 2013 NVS	Bn-N2-p10848961	Bn-N2-p8281271	A02	19.8	16.5 – 19.8	8.3	46.6	3.7	20.5	4.0
qDTF_C6	IT	2012V	Bn-N16-p7584100	Bn-N16-p15822788	C06	10.9	8.9 – 11.4	3.2	13.1			1.6
	IT	2016V	Bn-N16-p7584100	Bn-N16-p15822788	C06	10.0	8.3 – 11.4	5.0	19.5			1.6
	MET	2012V + 2016V	Bn-N16-p7584100	Bn-N16-p15822788	C06	10.6	8.9 – 11.4	8.1	16.1	7.6	16.0	1.5

[1]IT: QTL mapping based on individual trial data; MET: QTL analysis based on multiple environment trial data
[2]NV: Non-vernalized trials; V: Vernalized trials; NVS: Spring flowering type from non-vernalized trials
[3]C.I.: Confidence Interval
[4]LOD: LOD score.
[5]PVE: Phenotypic variance explained by genetic and environmental effects at the current scanning position.
[6]LOD-A: LOD score for additive genetic effect from QTL analysis based on multiple environment trial data.
[7]PVE-A: Phenotypic variance by additive genetic effect at the current scanning position from QTL analysis based on multiple environment trial data.
[8]Positive value indicates that the rutabaga-allele increased the trait value.

Table 3.3: QTL for seed oil, protein, glucosinolate and erucic acid contents identified by using a doubled haploid (DH) population derived from Rutabaga-BF × UA AlfaGold of *Brassica napus*.

QTL	Analysis method[1]	Expt.[2]	Left marker	Right marker	Chr.	Position (cM)	C. I. (cM)[3]	LOD[4]	PVE[5]	LOD-A[6]	PVE-A[7]	Additive effect[8]
qOIL_C3	IT	2012V	Bn-N13-p54026938	Bn-N13-p56717165	C03	72.9	71.5 – 77.4	2.8	13.3			1.1
	IT	2016V	Bn-N13-p54026938	Bn-N13-p56717165	C03	72.1	69.7 – 73.0	3.5	14.7			1.3
	MET	2012V + 2016V	Bn-N13-p54026938	Bn-N13-p55065650	C03	73.8	73.4 – 78.0	3.9	14.1	2.5	7.3	0.7
qPROTEIN_C3	IT	2016V	Bn-N13-p54026938	Bn-N13-p56717165	C03	72.0	69.7 – 72.9	2.4	7.7			-0.6
	MET	2012V + 2016V	Bn-N13-p54026938	Bn-N13-p55065650	C03	73.8	73.4 – 77.9	5.0	9.4	4.8	9.4	-0.6
qGSLs_A9	IT	2012V	NRC1040	Bn-N9-p4426894	A09	1.7	1.0 – 2.6	4.9	32.2			8.0
	IT	2016V	Bn-N9-p4796113	Bn-N9-p4426894	A09	1.4	1.0 – 2.6	4.1	28.7			6.9
	MET	2012V + 2016V	NRC1040	Bn-N9-p4426894	A09	1.5	1.2 – 2.2	8.7	28.3	6.9	16.9	6.5
qERUCIC_C3	IT	2012V	Bn-N13-p53019263	A08_5222(b)	C03	88.0	69.7 – 90.0	26.8	31.3			7.9
	IT	2016V	A08_4857(b)	A08_5222(b)	C03	87.3	85.4 – 90.4	11.7	40.6			5.5
	MET	2012V + 2016V	A08_4857(b)	A08_5222(b)	C03	89.8	88.2 – 91.9	21.0	41.2	21.0	40.8	4.9

[1]IT: QTL mapping based on individual trial data
[2]V: Vernalized trials; MET: QTL analysis based on multiple environment trial data
[3]C.I.: Confidence Interval
[4]LOD: LOD score.
[5]PVE: Phenotypic variation explained by both genetic and environmental effects at the current scanning position.
[6]LOD-A: LOD score for additive genetic effect.
[7]PVE-A: Phenotypic variation by additive genetic effect at the current scanning position
[8]Positive value indicates that the rutabaga-allele increased the trait value while the negative value indicates that the UA AlfaGold allele increased the trait value

3.7. Figures

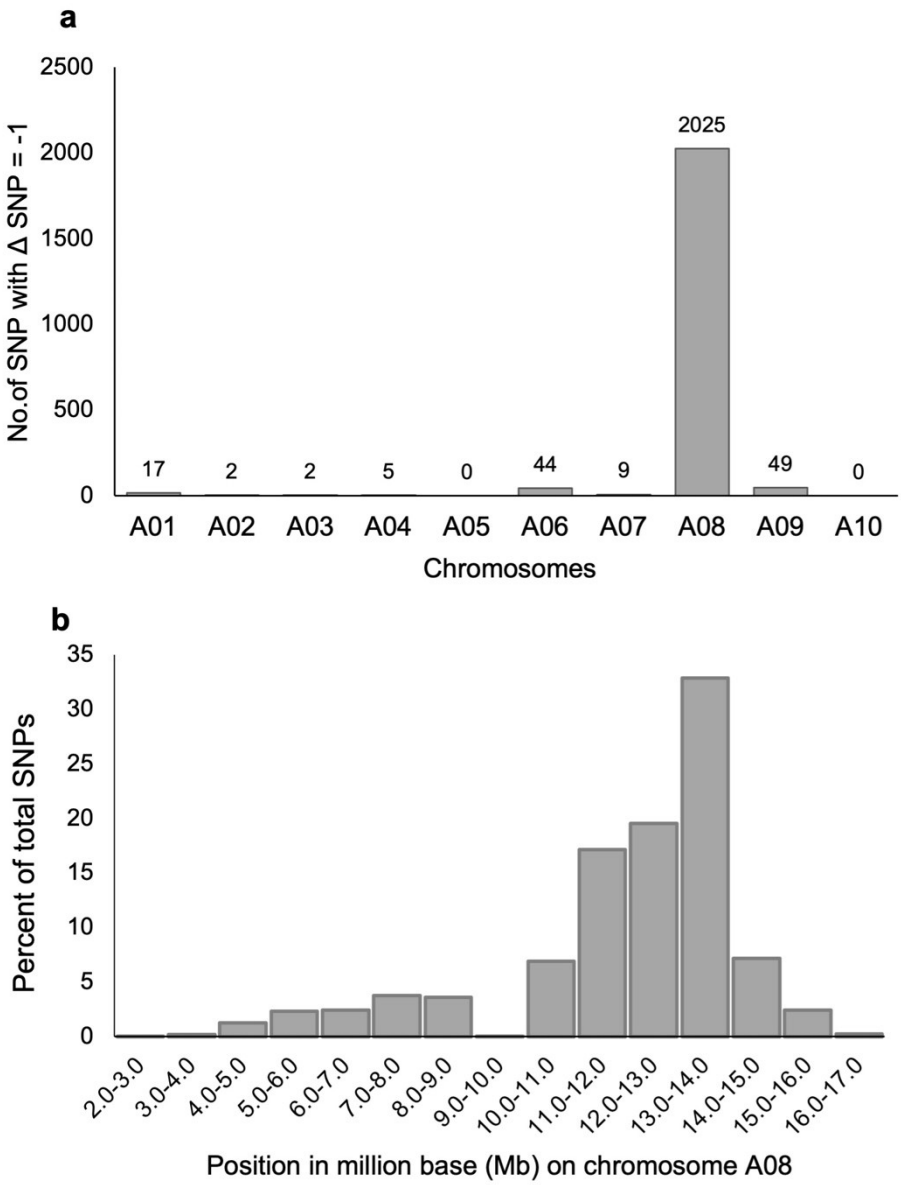
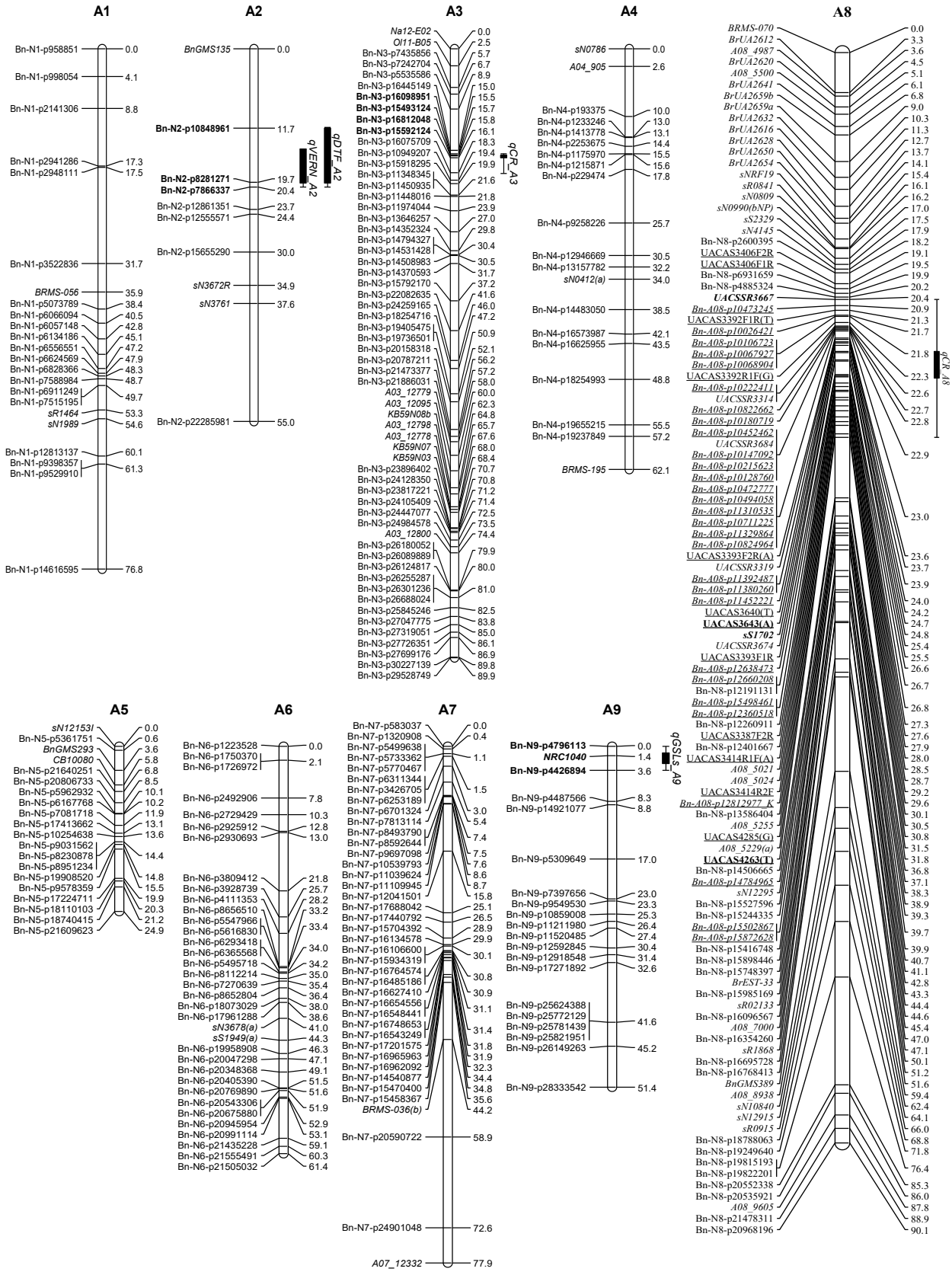


Figure 3.1: Whole-genome resequencing of the bulks of doubled haploid (DH) lines differing for resistance to *Plasmodiophora brassicae* pathotype 3 to identify the QTL associated with resistance; sequence alignment was done using the *B. rapa* cv. Chiifu-401 whole-genome assembly v1.5 (Cheng et al. 2011; www.brassicadb.cn). **a**) Number of SNPs with ΔSNP-index = -1 detected on different A genome chromosomes. **b**) Distribution of the SNPs on chromosome A08


A1
Bn-N1-p958851 0.0
Bn-N1-p998054 4.1
Bn-N1-p2141306 8.8
Bn-N1-p2941286 17.3
Bn-N1-p2948111 17.5
Bn-N1-p3522836 31.7
BRMS-056 35.9
Bn-N1-p5073789 38.4
Bn-N1-p6066094 40.5
Bn-N1-p6057148 42.8
Bn-N1-p6134186 45.1
Bn-N1-p6556551 47.2
Bn-N1-p6624569 47.9
Bn-N1-p6828366 48.3
Bn-N1-p7588984 48.7
Bn-N1-p6911249 49.7
Bn-N1-p7515195
sR1464 53.3
sN1989 54.6
Bn-N1-p12813137 60.1
Bn-N1-p9398357 61.3
Bn-N1-p9529910
Bn-N1-p14616595 76.8
A2
BnGMS135 0.0
Bn-N2-p10848961 11.7
qVERN_A2
qDTF_A2
Bn-N2-p8281271 19.7
Bn-N2-p7866337 20.4
Bn-N2-p12861351 23.7
Bn-N2-p12555571 24.4
Bn-N2-p15655290 30.0
sN3672R 34.9
sN3761 37.6
Bn-N2-p22285981 55.0
A3
Na12-E02 0.0
Ol11-B05 2.5
Bn-N3-p7435856 5.7
Bn-N3-p7242704 6.7
Bn-N3-p5535586 8.9
Bn-N3-p16445149 15.0
Bn-N3-p16098951 15.5
Bn-N3-p15493124 15.7
Bn-N3-p16812048 15.8
Bn-N3-p15592124 16.1
Bn-N3-p16075709 18.3
Bn-N3-p10949207 19.4
Bn-N3-p15918295 19.9
qCR_A3
Bn-N3-p11348345 21.6
Bn-N3-p11450935
Bn-N3-p11448016 21.8
Bn-N3-p11974044 23.9
Bn-N3-p13646257 27.0
Bn-N3-p14352324 29.8
Bn-N3-p14794327
Bn-N3-p14531428 30.4
Bn-N3-p14508983 30.5
Bn-N3-p14370593 31.7
Bn-N3-p15792170 37.2
Bn-N3-p22082635 41.6
Bn-N3-p24259165 46.0
Bn-N3-p18254716 47.2
Bn-N3-p19405475 50.9
Bn-N3-p19736501
Bn-N3-p20158318 52.1
Bn-N3-p20787211 56.2
Bn-N3-p21473377 57.2
Bn-N3-p21886031 58.0
A03_12779 60.0
A03_12095 62.3
KB59N08b 64.8
A03_12798 65.7
A03_12778 67.6
KB59N07 68.0
KB59N03 68.4
Bn-N3-p23896402 70.7
Bn-N3-p24128350 70.8
Bn-N3-p23817221 71.2
Bn-N3-p24105409 71.4
Bn-N3-p24447077 72.5
Bn-N3-p24984578 73.5
A03_12800 74.4
Bn-N3-p26180052
Bn-N3-p26089889 79.9
Bn-N3-p26124817 80.0
Bn-N3-p26255287
Bn-N3-p26301236 81.0
Bn-N3-p26688024
Bn-N3-p25845246 82.5
Bn-N3-p27047775 83.8
Bn-N3-p27319051 85.0
Bn-N3-p27726351 86.1
Bn-N3-p27699176 86.9
Bn-N3-p30227139 89.8
Bn-N3-p29528749 89.9
A4
sN0786 0.0
A04_905 2.6
Bn-N4-p193375 10.0
Bn-N4-p1233246 13.0
Bn-N4-p1413778 13.1
Bn-N4-p2253675 14.4
Bn-N4-p1175970 15.5
Bn-N4-p1215871 15.6
Bn-N4-p229474 17.8
Bn-N4-p9258226 25.7
Bn-N4-p12946669 30.5
Bn-N4-p13157782 32.2
sN0412(a) 34.0
Bn-N4-p14483050 38.5
Bn-N4-p16573987 42.1
Bn-N4-p16625955 43.5
Bn-N4-p18254993 48.8
Bn-N4-p19655215 55.5
Bn-N4-p19237849 57.2
BRMS-195 62.1
A8
BRMS-070 0.0
BrUA2612 3.3
A08_4987 3.6
BrUA2620 4.5
A08_5500 5.1
BrUA2641 6.1
BrUA2659b 6.8
BrUA2659a 9.0
BrUA2632 10.3
BrUA2616 11.3
BrUA2628 12.7
BrUA2650 13.7
BrUA2654 14.1
sNRF19 15.4
sR0841 16.1
sN0809 16.2
sN0990(bNP) 17.0
sS2329 17.5
sN4145 17.9
Bn-N8-p2600395 18.2
UACAS3406F2R 19.1
UACAS3406F1R 19.5
Bn-N8-p6931659 19.9
Bn-N8-p4885324 20.2
UACSSR3667 20.4
Bn-A08-p10473245 20.9
UACAS3392F1R(T) 21.3
Bn-A08-p10026421 21.7
Bn-A08-p10106723
Bn-A08-p10067927 21.8
Bn-A08-p10068904
qCR_A8
UACAS3392R1F(G) 22.3
Bn-A08-p10222411 22.6
UACSSR3314
Bn-A08-p10822662 22.7
Bn-A08-p10180719 22.8
Bn-A08-p10452462
UACSSR3684 22.9
Bn-A08-p10147092
Bn-A08-p10215623
Bn-A08-p10128760
Bn-A08-p10472777
Bn-A08-p10494058
Bn-A08-p11310535 23.0
Bn-A08-p10711225
Bn-A08-p11329864
Bn-A08-p10824964
UACAS3393F2R(A) 23.6
UACSSR3319 23.7
Bn-A08-p11392487 23.9
Bn-A08-p11380260 24.0
Bn-A08-p11452221 24.2
UACAS3640(T) 24.7
UACAS3643(A) 24.8
sS1702 25.4
UACSSR3674 25.5
UACAS3393F1R 26.6
Bn-A08-p12638473
Bn-A08-p12660208 26.7
Bn-N8-p12191131
Bn-A08-p15498461 26.8
Bn-A08-p12360518
Bn-N8-p12260911 27.3
UACAS3387F2R 27.6
Bn-N8-p12401667 27.9
UACAS3414R1F(A) 28.0
A08_5021 28.5
A08_5024 28.7
UACAS3414R2F 29.2
Bn-A08-p12812977_K 29.6
Bn-N8-p13586404 30.1
A08_5255 30.5
UACAS4285(G) 30.8
A08_5229(a) 31.5
UACAS4263(T) 31.8
Bn-N8-p14506665 36.8
Bn-A08-p14784965 37.1
sN12295 38.3
Bn-N8-p15527596 38.9
Bn-N8-p15244335 39.3
Bn-A08-p15502867
Bn-A08-p15872628 39.7
Bn-N8-p15416748 39.9
Bn-N8-p15898446 40.7
Bn-N8-p15748397 41.1
BrEST-33 42.8
Bn-N8-p15985169 43.3
sR02133 44.4
Bn-N8-p16096567 44.6
A08_7000 45.4
Bn-N8-p16354260 47.0
sR1868 47.1
Bn-N8-p16695728 50.1
Bn-N8-p16768413 51.2
BnGMS389 51.6
A08_8938 59.4
sN10840 62.4
sN12915 64.1
sR0915 66.0
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Bn-N8-p19249640 71.8
Bn-N8-p19815193
Bn-N8-p19822201 76.4
Bn-N8-p20552338 85.3
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Bn-N8-p21478311 88.9
Bn-N8-p20968196 90.1
A5
sN12153I 0.0
Bn-N5-p5361751 0.6
BnGMS293 3.6
CB10080 5.8
Bn-N5-p21640251 6.8
Bn-N5-p20806733 8.5
Bn-N5-p5962932 10.1
Bn-N5-p6167768 10.2
Bn-N5-p7081718 11.9
Bn-N5-p17413662 13.1
Bn-N5-p10254638 13.6
Bn-N5-p9031562
Bn-N5-p8230878 14.4
Bn-N5-p8951234
Bn-N5-p19908520 14.8
Bn-N5-p9578359 15.5
Bn-N5-p17224711 19.9
Bn-N5-p18110103 20.3
Bn-N5-p18740415 21.2
Bn-N5-p21609623 24.9
A6
Bn-N6-p1223528 0.0
Bn-N6-p1750370 2.1
Bn-N6-p1726972
Bn-N6-p2492906 7.8
Bn-N6-p2729429 10.3
Bn-N6-p2925912 12.8
Bn-N6-p2930693 13.0
Bn-N6-p3809412 21.8
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Bn-N6-p5616830 33.4
Bn-N6-p6293418
Bn-N6-p6365568 34.0
Bn-N6-p5495718 34.2
Bn-N6-p8112214 35.0
Bn-N6-p7270639 35.4
Bn-N6-p8652804 36.4
Bn-N6-p18073029 38.0
Bn-N6-p17961288 38.6
sN3678(a) 41.0
sS1949(a) 44.3
Bn-N6-p19958908 46.3
Bn-N6-p20047298 47.1
Bn-N6-p20348368 49.1
Bn-N6-p20405390 51.5
Bn-N6-p20769890 51.6
Bn-N6-p20543306 51.9
Bn-N6-p20675880
Bn-N6-p20945954 52.9
Bn-N6-p20991114 53.1
Bn-N6-p21435228 59.1
Bn-N6-p21555491 60.3
Bn-N6-p21505032 61.4
A7
Bn-N7-p583037 0.0
Bn-N7-p1320908 0.4
Bn-N7-p5499638
Bn-N7-p5733362 1.1
Bn-N7-p5770467
Bn-N7-p6311344 1.5
Bn-N7-p3426705
Bn-N7-p6253189 3.0
Bn-N7-p6701324 5.4
Bn-N7-p7813114
Bn-N7-p8493790 7.4
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Bn-N7-p9697098 7.5
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Bn-N7-p11109945 8.7
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Bn-N7-p17688042 25.1
Bn-N7-p17440792 26.5
Bn-N7-p15704392 28.9
Bn-N7-p16134578 29.9
Bn-N7-p16106600
Bn-N7-p15934319 30.1
Bn-N7-p16764574
Bn-N7-p16485186 30.8
Bn-N7-p16627410 30.9
Bn-N7-p16654556
Bn-N7-p16548441 31.1
Bn-N7-p16748653
Bn-N7-p16543249 31.4
Bn-N7-p17201575 31.8
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Bn-N7-p16962092 32.3
Bn-N7-p14540877 34.4
Bn-N7-p15470400 34.8
Bn-N7-p15458367 35.6
BRMS-036(b) 44.2
Bn-N7-p20590722 58.9
Bn-N7-p24901048 72.6
A07_12332 77.9
A9
Bn-N9-p4796113 0.0
NRC1040 1.4
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Bn-N9-p4426894 3.6
Bn-N9-p4487566 8.3
Bn-N9-p14921077 8.8
Bn-N9-p5309649 17.0
Bn-N9-p7397656 23.0
Bn-N9-p9549530 23.3
Bn-N9-p10859008 25.3
Bn-N9-p11211980 26.4
Bn-N9-p11520485 27.4
Bn-N9-p12592845 30.4
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Bn-N9-p17271892 32.6
Bn-N9-p25624388
Bn-N9-p25772129
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Bn-N9-p28333542 51.4

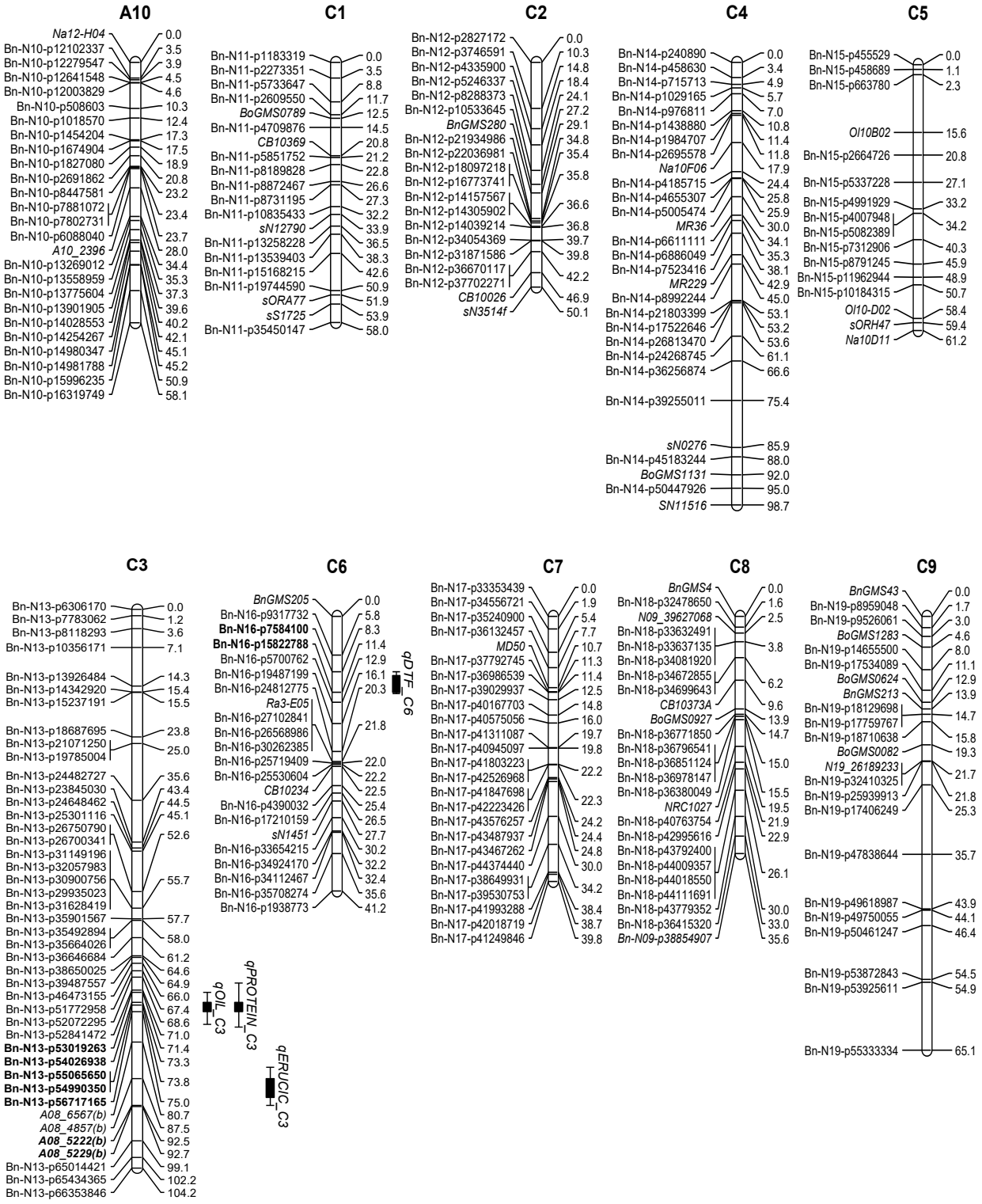

Figure 3.2: A genetic linkage map of *Brassica napus* and QTL locations for clubroot resistance, vernalization, days to flowering, and seed quality traits. The 19 linkage groups were represented by vertical bars, designated as A01-A10 for the A genome and C01-C09 for the C genome. The name of the marker loci is listed on the left side of the linkage groups, and the position (cM) of the markers given on the right side. SSR markers are italicized, allele-specific markers are underlined, KASP markers are italicized-underlined, and QTL flanking markers are bolded.

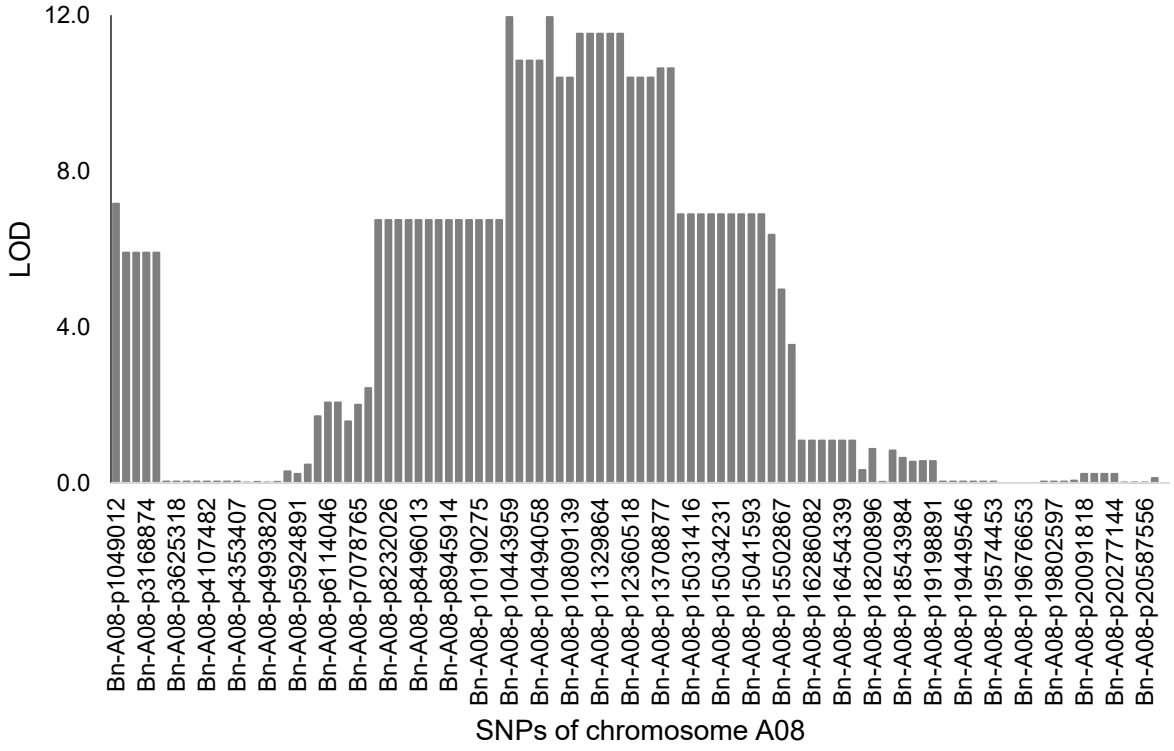

Figure 3.3: Single nucleotide polymorphism markers, identified through single marker analysis, showing association with resistance to *Plasmodiophora brassicae* pathotype 3 in a pedigree population derived from Rutabaga-BF × Susceptible canola (Hi-Q and A07-26NR) crosses. The name of all 17 SNPs with LOD>10 can be found in Supplemental Table S3.4

3.8. Supplemental materials

Table S3.1: Spearman's correlation of resistance to different *Plasmodiophora brassicae* pathotypes with agronomic and seed quality traits in a doubled haploid (DH) population of Rutabaga-BF × UA AlfaGold of *Brassica napus*

	Growth habit	Days to flowering	Oil	Protein	Erucic acid	Glucosinolates
Pathotype 2	-0.11 ^{NS}	-0.06 ^{NS}	-0.12^{NS}	0.02 ^{NS}	-0.10 ^{NS}	0.05 ^{NS}
Pathotype 3	-0.10 ^{NS}	-0.05 ^{NS}	-0.13 ^{NS}	0.04 ^{NS}	-0.06 ^{NS}	0.02 ^{NS}
Pathotype 5	-0.13 ^{NS}	-0.06 ^{NS}	-0.10 ^{NS}	-0.02 ^{NS}	-0.14 ^{NS}	0.03 ^{NS}
Pathotype 6	-0.12 ^{NS}	-0.06 ^{NS}	-0.13 ^{NS}	0.01 ^{NS}	-0.10 ^{NS}	0.02 ^{NS}
Pathotype 8	-0.14 ^{NS}	-0.10 ^{NS}	-0.15 ^{NS}	0.03 ^{NS}	-0.09 ^{NS}	0.06 ^{NS}

** and * indicate significant correlation at $P = 0.01$ and 0.05, respectively; NS = non-significant

Table S3.2: Two-sample *t*-test for significant difference between the clubroot resistant and susceptible doubled haploid (DH) lines of Rutabaga-BF × UA AlfaGold of *Brassica napus* for agronomic and seed quality traits

Variable	Resistant Mean ± SE	Susceptible Mean ± SE	*t*-value	*df*	*P*-value
Days to flowering	46.3 ± 0.6	45.5 ± 0.6	0.99	92	0.3279 ^{NS}
Flowering habit	S (29) + W (23)	S (27) + W (18)	0.65	95	0.5145 ^{NS}
Oil (%)	41.7 ± 0.4	40.7 ± 0.4	1.70	94	0.0920 ^{NS}
Protein (%)	27.8 ± 0.3	28.1 ± 0.3	0.76	94	0.4505 ^{NS}
Erucic acid (%)	10.1 ± 1.3	10.0 ± 1.5	0.04	94	0.9656 ^{NS}
Glucosinolate (µmol/g seed)	38.8 ± 1.5	39.8 ± 1.9	0.42	94	0.6764 ^{NS}

NS: not significantly different.
'S' and 'W' indicate DH lines with 'spring' and 'winter' growth habits in non-vernalized (NV) experiments, respectively. Therefore, the DH lines with spring growth habit were coded '1' and the lines with winter growth habit were coded '2', and the mean values were used for the *t*-test.

Table S3.3: Phenotypic variation for days to flowering, seed oil (% of seed), protein (% of seed), glucosinolates (µmol/g seed) and erucic acid (% of total fatty acids) contents and their variance components in a doubled haploid (DH) population derived from Rutabaga-BF × UA AlfaGold of *Brassica napus* tested in different field trials

Trait	Expt.[1]	Rutabaga-BF	UA AlfaGold	DH population		ANOVA[2]			Heritability (H^2)
				Mean ± SE	Range	σ_g^2	σ_e^2	σ_{ge}^2	
Days to flowering	2012V	54.0 ± 0.6	37.5 ± 0.5	46.1 ± 0.5	37.0 – 55.7	18.9	0.4		0.99
	2016V	56.0 ± 0.5	42.9 ± 0.4	45.7 ± 0.4	39.0 – 59.0	14.4	1.0		0.97
	Avg	55.5 ± 0.6	42.2 ± 0.5	46.0 ± 0.4	37.0 – 57.0	25.9	1.4	1.2	0.97
	2012NVS	-	51.3 ± 0.7	69.7 ± 1.4	54.7 – 96.7	88.1	4.1		0.98
	2013NVS	-	43.3 ± 1.7	65.9 ± 1.4	47.0 – 97.3	63.1	4.5		0.98
	Avg	-	47.3 ± 1.3	67.1 ± 1.2	47.0 – 90.0	62.1	4.5	28.0	0.81
Oil	2012V	39.8 ± 2.9	46.6 ± 0.5	40.4 ± 0.3	34.0 - 48.4	4.9	2.9		0.84
	2016V	39.4 ± 2.8	45.1 ± 0.7	43.3 ± 0.4	31.6 - 52.4	6.1	11.8		0.51
	Avg	39.5 ± 2.8	45.2 ± 0.7	41.2 ± 0.1	34.6 - 50.7	4.0	5.1	0.8	0.74
Protein	2012V	28.0 ± 0.4	29.2 ± 0.3	29.2 ± 0.2	23.5 - 33.9	2.0	2.1		0.74
	2016V	26.6 ± 0.4	26.9 ± 0.4	25.7 ± 0.2	19.8 - 32.1	2.0	3.8		0.52
	Avg	26.8 ± 0.4	27.3 ± 0.4	27.9 ± 0.0	19.8 - 32.2	1.6	2.5	0.3	0.71
Glucosinolate	2012V	65.3 ± 0.5	11.9 ± 0.3	41.5 ± 1.3	13.9 - 67.5	115.0	18.0		0.95
	2016V	50.4 ± 0.4	11.8 ± 0.3	34.4 ± 1.5	7.9 - 63.8	102.4	129.2		0.61
	Avg	55.4 ± 0.4	11.8 ± 0.3	39.3 ± 0.1	7.9 - 62.0	85.4	47.4	7.9	0.86
Erucic acid	2012V	24.0 ± 0.7	0.3 ± 0.1	10.3 ± 1.0	0.2 - 29.8	75.7	1.4		0.99
	2016V	24.1 ± 0.7	0.3 ± 0.1	9.4 ± 1.1	0.1 - 28.1	123.8	8.0		0.97
	Avg	24.0 ± 0.7	0.3 ± 0.1	10.1 ± 0.1	0.1 - 28.3	62.5	3.0	1.1	0.98

[1] NV= Non-vernalized trial; V = Vernalized trial; NVS = Spring flowering growth habit type from the non-vernalized trial.
[2]All variances were significant at $P < 0.01$
σ_g^2: Genetic variance
σ_e^2: Error variance
σ_{ge}^2: Trait variance due to Genotype × Environment interaction

Table S3.4: List of the 17 single nucleotide polymorphism markers, detected through single marker analysis, significantly associated (LOD>10) with resistance to *Plasmodiophora brassicae* pathotype 3 in a pedigree population (BC_1F_5, BC_1F_7 and BC_1F_9) of Rutabaga-BF × Susceptible canola (Hi-Q and A07-26NR) crosses

SNP ID	Chromosome	Position (base)	LOD	PVE (%)	Additive effect
Bn-A08-p10443959	A08	10,443,959	12.0	46.7	-25.1
Bn-A08-p10472777	A08	10,472,777	10.8	43.5	-24.1
Bn-A08-p10473245	A08	10,473,245	10.8	43.5	-24.1
Bn-A08-p10494058	A08	10,494,058	10.8	43.5	-24.1
Bn-A08-p10711225	A08	10,711,225	12.0	46.7	-25.1
Bn-A08-p10774582	A08	10,774,582	10.4	42.2	-24.0
Bn-A08-p10809139	A08	10,809,139	10.4	42.2	-24.0
Bn-A08-p11304602	A08	11,304,602	11.5	45.5	-25.1
Bn-A08-p11310535	A08	11,310,535	11.5	45.5	-25.1
Bn-A08-p11329864	A08	11,329,864	11.5	45.5	-25.1
Bn-A08-p11380260	A08	11,380,260	11.5	45.5	-25.1
Bn-A08-p11392487	A08	11,392,487	11.5	45.5	-25.1
Bn-A08-p12360518	A08	12,360,518	10.4	42.2	-24.0
Bn-A08-p12828472	A08	12,828,472	10.4	42.2	-24.0
Bn-A08-p12923799	A08	12,923,799	10.4	42.2	-24.0
Bn-A08-p13708877	A08	13,708,877	10.6	42.9	-24.2
Bn-A08-p13709853	A08	13,709,853	10.6	42.9	-24.2

PVE (%): Percent of the total phenotypic variance explained by the SNP allele
Additive effect (in negative value) is the reduction of disease severity index (DSI) due to substitution of one 'susceptible canola' allele by one 'Rutabaga-BF' allele

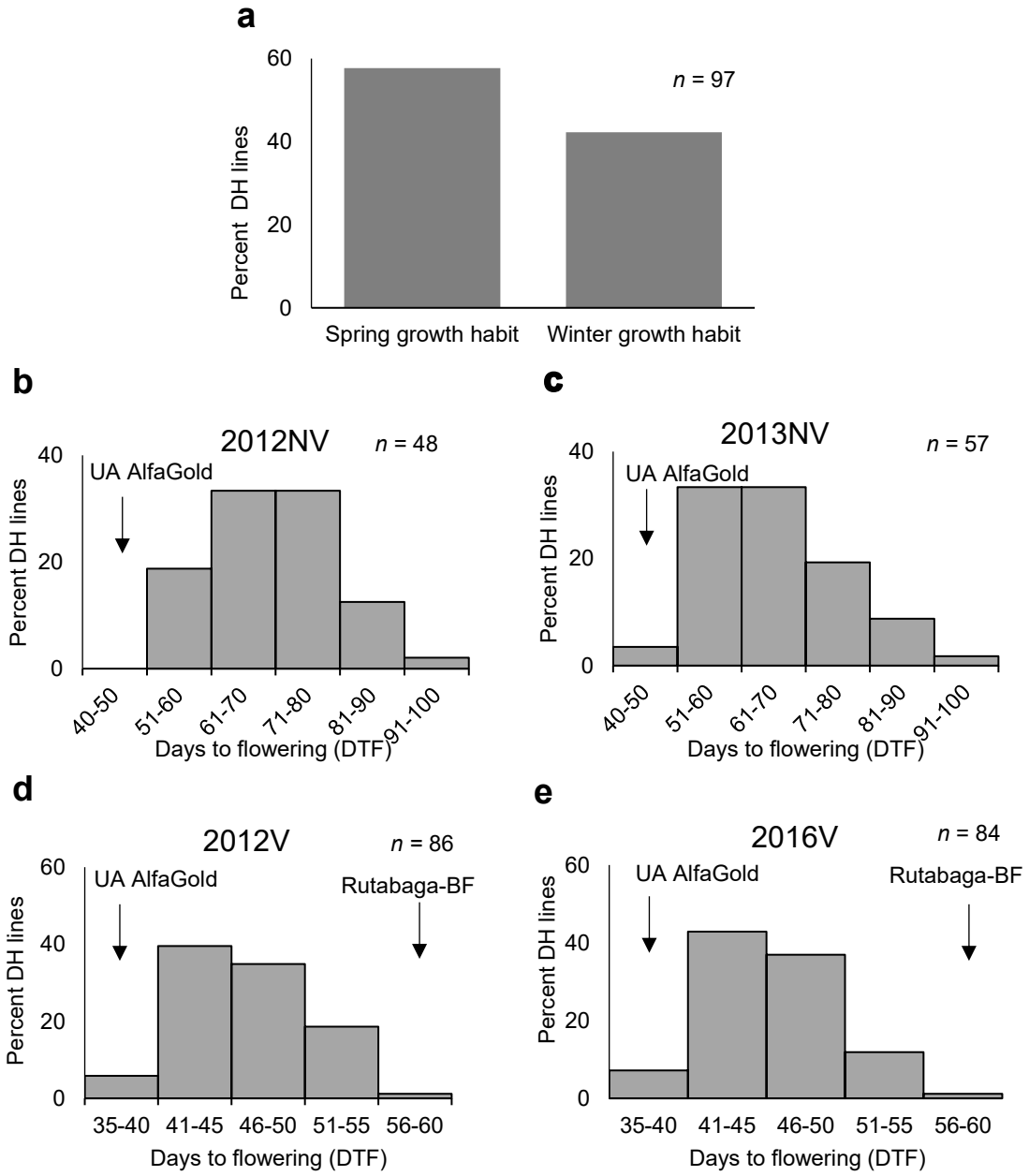

Figure S3.1: Frequency distribution of a doubled haploid (DH) population of Rutabaga-BF × UA AlfaGold of *Brassica napus*, tested in field trials, in 2012 and 2013 without vernalization for growth habit and days to flowering, and in 2012 and 2016 after vernalization for days to flowering. a) The proportion of the DH lines exhibiting winter or spring growth habit (pooled data of two years presented). b, c) The distribution of the spring growth habit DH lines for days to flowering in 2012 and 2013 trial, respectively. d, e) The distribution of the DH lines for days to flowering in 2012 and 2016 trials, respectively. 2012NV and 2013NV indicate the trials conducted in 2012 and 2013, respectively, without vernalization. 2012V and 2016V indicate the trials conducted in 2012 and 2016, respectively, after vernalization of the plants before transplanting to the field plots. Arrows indicate the mean values of the parents. The Rutabaga-BF failed to flower in the 2012 NV and 2013 NV field trials.

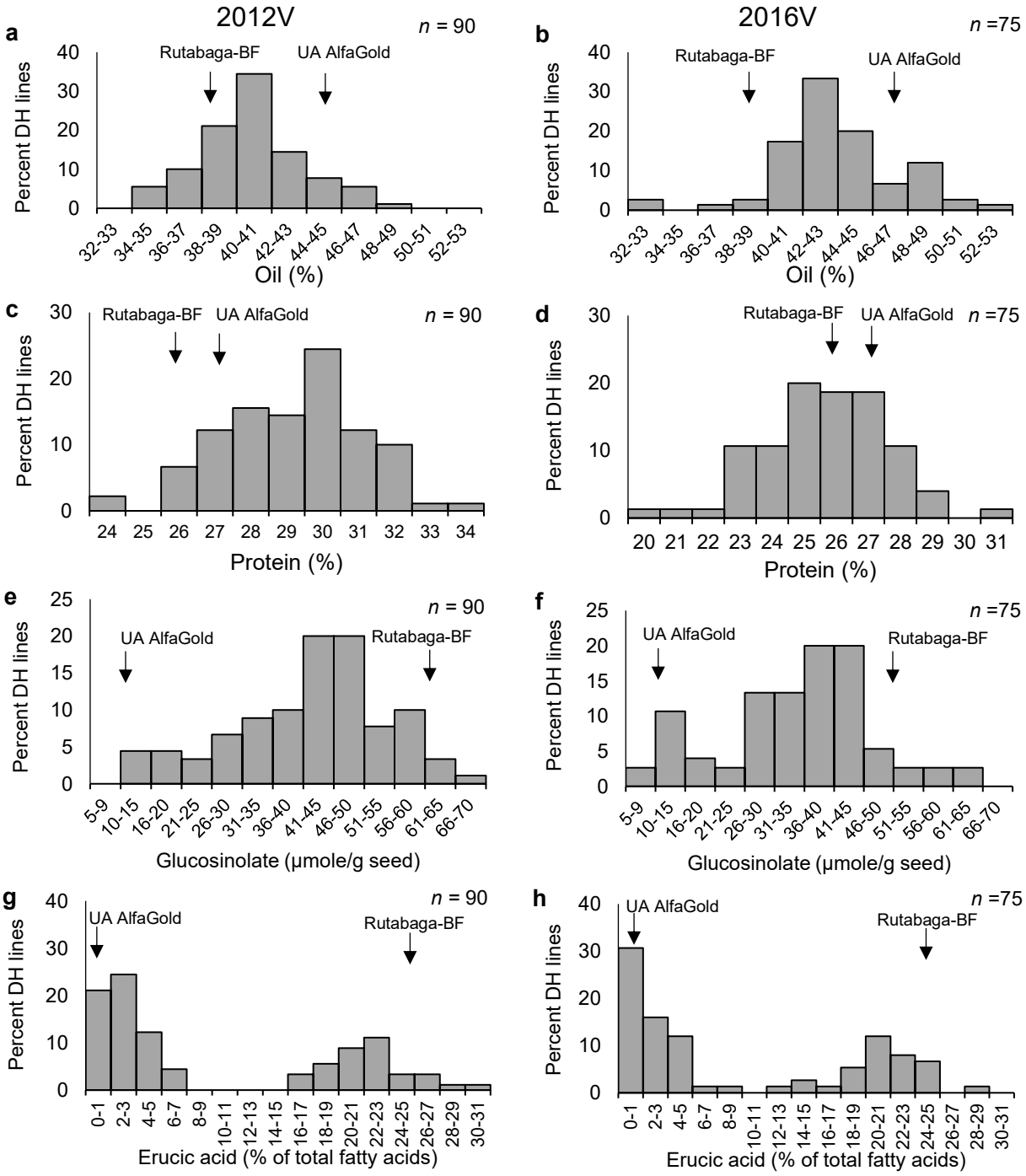

Figure S3.2: Frequency distribution of a doubled haploid (DH) population of Rutabaga-BF × UA AlfaGold of *Brassica napus* for seed oil (a, b), protein (c, d), glucosinolate (e, f) and erucic acid (g, h) contents. 2012V and 2016V indicate the trials conducted in 2012 and 2016, respectively, after vernalization of the plants before transplanting to the field plots. Arrow indicates the mean values of the parents.

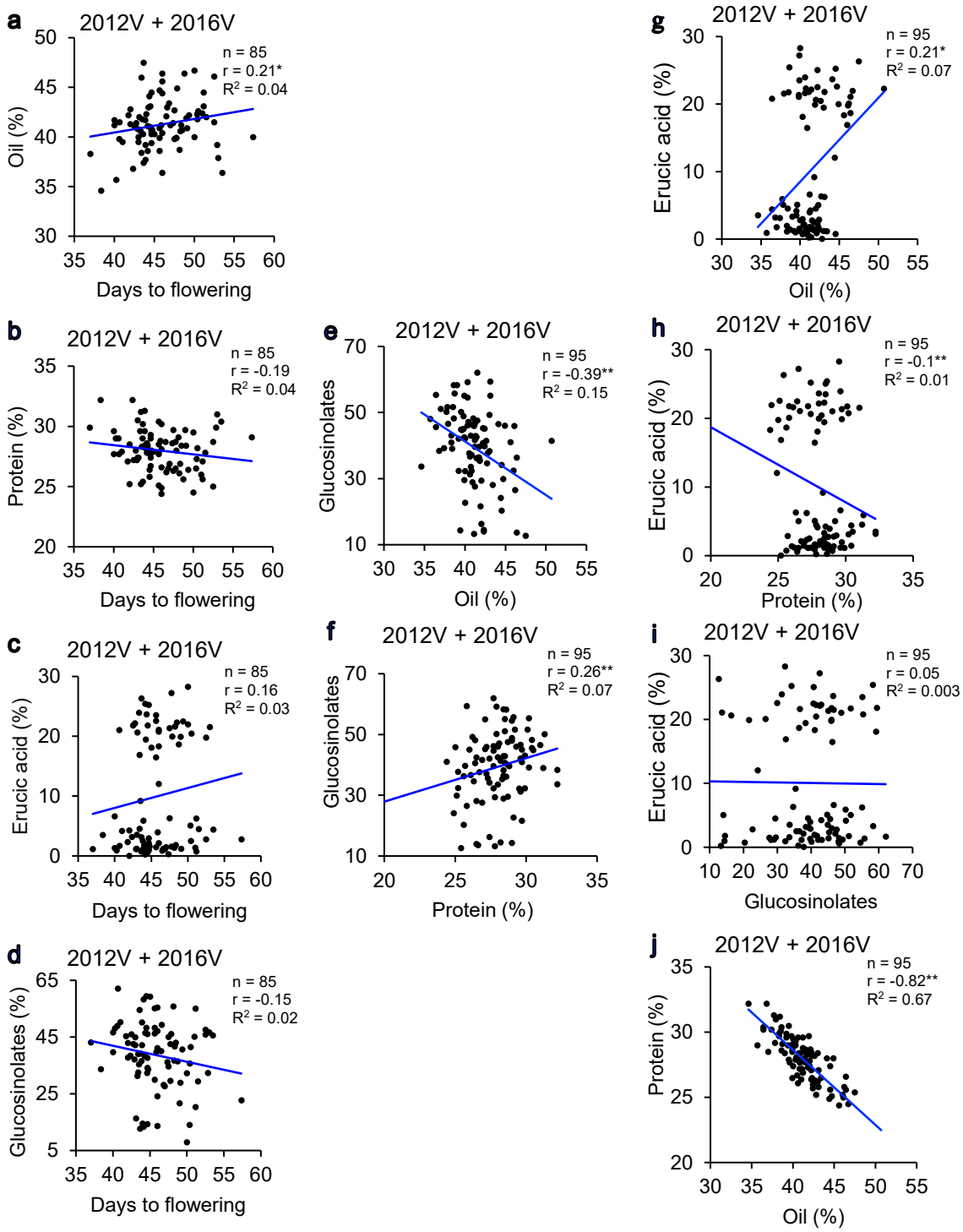

Figure S3.3: Scatter diagrams showing the relationship among the agronomic (days to flowering) and seed quality traits (oil, protein, glucosinolates and erucic acid) in a doubled haploid (DH) population derived from Rutabaga-BF × UA AlfaGold of *Brassica napus*. LSmean data of the two vernalized trials (Expt. 2) presented. n = No. of individual, r = correlation of coefficient, R^2 = Coefficient of determination, ** and * indicate significant at P = 0.01 and 0.05, respectively

4. Chapter 4: Mapping clubroot resistance of *Brassica rapa* introgressed into *Brassica napus* and development of molecular markers for the resistance[4]

4.1. Introduction

Clubroot disease, caused by *Plasmodiophora brassicae* (Woronin 1878), is a major threat to canola production in different countries, including North America (Tewari et al. 2005; Cao et al. 2009; Dokken-Bouchard et al. 2010; Chittem et al. 2014; Al-Daoud et al. 2018), and causes about 30% yield loss in canola in Alberta (Tewari et al. 2005). Aside from yield loss, this disease decreases seed oil content by about 2-6% (Engqvist 1994; Pageau et al. 2006) and increases the chlorophyll content in seed oil by 50% (Engqvist 1994). Growing cultivars carrying multiple resistance genes and adopting appropriate crop rotation are key components for successfully managing this disease (Donald and Porter 2003; Peng et al. 2015).

In Brassica, clubroot resistance (CR) has been reported in diploid species *B. rapa* (AA, 2*n* = 20), *B. oleracea* (CC, 2*n* = 18) and *B. nigra* (BB, 2*n* = 16), and in allopolyploid species *B. napus* (AACC, 2*n* = 38) (Johnston 1970; Ayers and Lelacheur 1972; Buczacki et al. 1975; Hasan et al. 2012; Peng et al. 2014a; Laperche et al. 2017; Fredua-Agyeman et al. 2019; Farid et al. 2020). Among these, the CR genes, mostly from the A-genome of *B. rapa,* have been used for breeding clubroot-resistant vegetables (Laila et al. 2019; Matsumoto et al. 2012; Piao et al. 2004) and oilseed (Frauen 1999; Hasan & Rahman 2016; Hirani et al. 2016; Rahman et al. 2011) Brassica crops. Genetic analysis and QTL mapping studies have identified at least 24 CR loci/QTL on seven chromosomes of *B. rapa, viz.* A01, A02, A03, A05, A06, A07 and

[4] A version of this chapter has been submitted to Crop Science as

Hasan, J., Shaikh, R., Basu, U. and Rahman, H. 2021. Mapping clubroot resistance of *Brassica rapa* introgressed into *Brassica napus* and development of molecular markers for the resistance. Crop Science

A08; these loci often confer race-specific resistance to *P. brassicae* pathotypes (Hasan et al. 2021). About half (12 out of 24 loci/QTL) of these CR loci, namely *CRa* or *CRb* (Matsumoto et al. 1998; Piao et al. 2004; Cho et al. 2008; Hayashida et al. 2008; Kato et al. 2012, 2013; Hatakeyama et al. 2017), *CRd* (Pang et al. 2018), *CRq* (Yuan et al. 2015), *CRs* (Laila et al. 2019), *CR6a, CR6b* (Lee et al. 2002), *Rcr1* (Chu et al. 2014), *Rcr2* (Huang et al. 2017), *CrrA5* (Nguyen et al. 2018), *qBrCR38-1* and *qBrCR38*-2 (Zhu et al. 2019b) have been mapped in Chinese cabbage (*B. rapa* var. *chinensis/pekinensis*). However, only a few of the CR loci have so far been introgressed into *B. napus* canola and have been used in breeding (Frauen 1999; Rahman et al. 2011; Hirani et al. 2016; Hasan and Rahman 2016). Breakdown of a single-gene resistance in canola due to the emergence of new virulent pathotypes has been reported in different countries, including Canada (Strelkov et al. 2016b, 2018; Cao et al. 2020). Therefore, introgression of additional CR genes in *B. napus* canola are needed to diversify the CR genes in this crop.

Pyramiding of multiple major resistance genes (Boskovic et al. 2001; Werner et al. 2005; Feechan et al. 2015) or major genes combined with QTL (Ellis et al. 2014; Fukuoka et al. 2015) can increase the durability of disease resistance in a cultivar (Mundt 1990; Li et al. 2016; Pilet-Nayel et al. 2017). For example, a pyramiding of three major CR loci, *CRa, CRk,* and *CRc*, in Chinese cabbage has been reported to confer resistance to six *P. brassicae* isolates (Matsumoto et al. 2012). The pyramiding of a major gene and minor genes has also been reported to increase resistance to multiple pathotypes in *B. napus* (Shah et al. 2019) and *B. oleracea* (Tomita et al. 2013). Gene pyramiding based on phenotypic evaluation is a challenging task and may not be feasible in many cases. A plant carrying a single resistance gene can exhibit a similar resistance phenotype to a plant carrying multiple resistance genes. In this regard, the development of gene-specific molecular marker(s) strongly co-segregating with the resistance gene (Ruane and Sonnino 2007) and their use in marker-assisted selection (MAS) can resolve this constraint.

Typically, DNA markers with a genetic distance of <1 cM from the gene of interest are considered tightly linked and suitable for use in MAS.

This paper reports the genetic basis of resistance to pathotype 3 in Chinese cabbage cv. Bilko, which has been introgressed into oilseed *B. napus*, identification of the genomic region associated with resistance in *B. napus*, and development of molecular markers for use in marker-assisted breeding of clubroot-resistant *B. napus* canola cultivars.

4.2. Materials and Methods

4.2.1. Plant material

A *B. napus* BC_1F_5 Recombinant Inbred Line (RIL) population comprising 247 individuals belonging to 47 families was used in this study. The RIL population was developed by crossing a clubroot susceptible *B. napus* spring canola line A04-73NA (2*n* = 38; AACC) and a clubroot resistant Chinese cabbage (*B. rapa* var. *pekinensis*) line Bilko-CR (2*n* = 20; AA). The Chinese cabbage line Bilko-CR was derived from the F_1 hybrid cv. Bilko through self-pollination for two generations (F_3 generation). The F_3 line of Bilko, *i.e.*, the Bilko-CR, was homozygous for resistance to *P. brassicae* pathotypes 3 or 3H, as classified on Williams' (1966) or Canadian Clubroot Differentials Set (Strelkov et al. 2018). In brief, the clubroot-resistant F_3 line was crossed as male to the *B. napus* line A04-73NA, and the F_1 plants were backcrossed with A04-73NA. The BC_1 plants were self-pollinated for five generations, and the BC_1F_5 RIL population was developed. During the development of this RIL population, plants producing viable pollen were self-pollinated to produce seeds for the next generation population; selection for CR was performed only in the BC_1F_2 generation. The BC_1F_5 population used in this study was fully fertile and the plants were morphologically similar to *B. napus*. Previously, Attri and Rahman (2018) reported that, upon self-pollination, progeny of the *B. napus* × *B. rapa* interspecific cross stabilizes into *B. napus* type where most of the population become *B. napus* type already in BC_1F_3 generation.

To better understand the CR trait in the RIL population, the inheritance of this trait was studied in Bilko. Ninety-two F_2 plants developed through self-pollination of three Bilko F_1 plants were evaluated for resistance to pathotype 3 (3H). Five F_2 plants showing resistance to pathotype 3 (3H) were self-pollinated to produce F_3 seeds, and a total of 141 F_3 plants of these five families were evaluated for resistance to pathotype 3 (3H).

4.2.2. Clubroot disease resistance test

The parents, RILs, and the segregating populations were phenotyped for resistance to single-spore isolate of *P. brassicae* pathotype 3 (3H). For this, seeds were sown in 3 cm × 3 cm × 5 cm (L × W × D) cells filled with Sunshine® Professional Growing Mix #4 (Sungrow Horticulture, 770 Silver Street, Agawam, MA, USA 01001) and seven days old seedlings were inoculated by pipetting 2 mL inoculum with spore concentration of 1×10^6 spores/mL. The inoculum preparation and inoculation method details have been reported elsewhere (Hasan et al. 2012). The seedlings were evaluated for resistance at 42-45 days after inoculation, and the severity of gall development was scored on a 0–3 scale, where 0 = no galling, 1 = one or a few tiny galls on the lateral roots, 2 = moderate galling on the lateral roots, and 3 = severe galling on the lateral or main root. Plants with a disease score of 0 were classified as resistant, and plants with disease scores of 1, 2, or 3 were classified as susceptible.

4.2.3. Whole-genome resequencing and SNP calling

Genomic DNA of the parents and the RILs of *B. napus* × *B. rapa* line Bilko-CR was extracted from leaf samples collected from 3-4 weeks old plants; the details of DNA extraction can be found in Hasan and Rahman (2016). For whole-genome resequencing (WGRS), four DNA samples, the resistant (RP) (*B. rapa* line Bilko-CR) and susceptible (SP) (*B. napus* line A04-73NA) parents, and a resistant (R-pool) and a susceptible (S-pool) bulk were prepared. The R-pool DNA was prepared by combining an equal amount of DNA from 50 plants belonging to 14 RIL families homozygous for resistance to pathotype 3 (3H). The S-pool DNA

was prepared by mixing DNA from 15 plants belonging to eight RIL families homozygous for susceptibility to pathotype 3 (3H). The four DNA sample libraries were prepared following the TruSeq DNA Sample Prep kit (Illumina) protocol for paired-end (2x 150 bp reads) sequencing using Illumina HiSeq 2500 platform (Illumina Inc San Diego, CA, USA). All four samples were sequenced 30× to increase the depth and breadth of coverage. Library preparation and sequencing were carried out by Genewiz (Genewiz Inc. South Plainfield, NJ, USA, www.genewiz.com).

Adapter sequences and low-quality sequence reads were trimmed off using Trimmomatic v0.36 (Bolger et al. 2014), and the sequence reads <35 bp in size were discarded. The RP reads were aligned to two *B. napus* reference genomes, the spring *B. napus* DH12075 whole-genome assembly v3.1 (Agriculture and Agri-Food Canada, unpublished), and the winter *B. napus* cv. Darmor-*bzh* whole-genome assembly v4.1 (Chalhoub et al. 2014), using BWA (Burrows-Wheeler Aligner) v.0.7.12 (Li and Durbin 2009). The aligned sequences were processed to extract the exact position of the mapped reads and then filtered using the mpileup function of Samtools v.1.3.1 (Li et al. 2009). The variants between the RP reads, and the A-genome of the reference genome assemblies were called from the mpileup output using VarScan v.2.3.9 (Koboldt et al. 2012). By using this information, two sequence assemblies of the RP were developed, and these two assemblies are hereafter referred to as RPA_DH12075 and RPA_Darmor. The R- and S-pool sequence reads were aligned onto the two RPAs, and SNP-index was calculated.

4.2.4. SNP-index and ΔSNP-index calculation

For each nucleotide position, SNP-index for both R-pool and S-pool was calculated by comparing their sequence reads with those of the two RPA's using the following formula (Abe et al. 2012):

$$\text{SNP index} = \frac{\text{Number of alternate (alternate to RPA) nucleotide read in bulk}}{\text{Total number of nucleotide read in bulk}}$$

An SNP-index of 0.5 indicates an equal contribution of both parents in the population. A deviation from the SNP-index of 0.5 indicates a higher number of alleles from one parent than the other for that genomic position. All SNP positions with sequencing read-depth <10 and SNP-index >0.75 for the R-pool and SNP positions with sequencing read-depth <10 and SNP-index <0.25 for the S-pool were excluded while calculating ΔSNP-index. The ΔSNP-index was calculated by subtracting the SNP-index of the R-pool from the SNP-index of the S-pool. For calculation of ΔSNP-index, only homozygous loci were considered. A ΔSNP-index of –1 indicates that the resistant bulk allele is the same as that of the resistant parent while the alternate base is present in the susceptible bulk (Takagi et al. 2013). The nucleotide positions with ΔSNP-index of –1 were considered the causal SNPs for the trait of interest.

4.2.5. Marker-trait association and estimation of trait effect

Based on the position of the genomic regions with high ΔSNP-index, 101 SNP allele-specific primers (AS-primer) were designed using BatchPrimer3 (You et al. 2008) to develop markers associated with resistance (Table S4.1). In addition to this, a total of 46 simple sequence repeat (SSR), seven Indel and seven SCAR markers (Ueno et al. 2012; Kato et al. 2013; Chu et al. 2014; Hobson and Rahman 2016) (Table S4.2) from the genomic regions associated with resistance and the genomic regions carrying TIR-NBS-LRR (Toll/Interleukin 1–Nucleotide Binding Site–Leucine-Rich Repeat) genes *Bra012688*, *Bra012689, Bra019409*, *Bra019410,* and *Bra019413* were used for genotyping the RIL mapping population, following the method described by Hasan and Rahman (2016).

Electrophoretic separation of the PCR products generated by Indel, SCAR, and AS-markers was completed on a 1.0% to 4.0% agarose gel depending on the size of the expected PCR amplicon, stained with SYBR Green1, and imaged with a Typhoon FLA 9500 laser scanner (GE Healthcare, Mississauga, ON, Canada). In genotyping using SSR markers, the forward primer carried the universal M13 sequence 5′-CACGACGTTGTAAAACGAC-3′

(IRD; MWG-Biotech AG, Ebensburg, Germany). In this case, 0.25 µl (10 pM/µl) fluorescently labelled infra-red dyes, *viz.* FAM, VIC, NED, and PET (Applied Biosystems, Foster City, CA, USA), were used in the PCR master mix. Electrophoretic separation and detection of the amplification products was performed using a capillary ABI sequencer 3730 (Applied Biosystems, Foster City, CA, USA) and were scored and analyzed using GeneMapper software v5.0.

4.2.6. Linkage map construction and resistance locus mapping

The parents and the bulks were genotyped with all markers to identify the polymorphic markers, and then these polymorphic markers were used for genotyping the RIL population. The genotypic data were used to develop a genetic map of the chromosome carrying the resistance using JoinMap 4.0 (Van Ooijen 2006). The Kosambi map function (Kosambi 1943) with a recombination frequency of 0.40 was used to achieve the order of the markers on the genetic map. The genetic map was illustrated with MapChart v. 2.32 (Voorrips 2002) using linkage distribution calculated by JoinMap v.4 (Van Ooijen 2006) for better visualization.

Composite Interval Mapping (CIM) analysis of the genotypic and phenotypic data was carried out using QTL IciMapping version 4.2 (Meng et al. 2015) to identify the genomic region carrying the resistance. The parameters set for this analysis were 0.1 cM walking speed, 10 cM window size, and 5 cM for the number of control markers, and a stepwise regression probability of 0.001 (Manichaikul et al. 2009). To declare the location of the resistance gene, the empirical threshold for LOD (p-value < 0.05) was obtained from 1,000 permutations (Churchill and Doerge 1994).

4.2.7. Physical mapping of the markers and putative candidate genes identification

The physical position of the SSR, Indel, SCAR, and nine Kompetitive Allele-Specific PCR (KASP) primers (Table S2) was determined by aligning the forward and reverse primer sequences to RPA_ DH12075 and *B. rapa* cv. Chiifu-401 whole-genome assembly v3.0 using

BLASTn (Zhang et al. 2000) command-line application tool BLAST+ v2.11.0 (Camacho et al. 2009). The position of the markers was determined when forward and reverse primer sequences aligned with the DNA sequence of a chromosome and the length of the fragment between the forward and reverse primer sequences matched with the known or predicted amplicon size. The KASP primers are associated with the CR loci *Rcr2* and *Rcr5* (Huang et al. 2017, 2019). The genomic region between the flanking markers was scanned for annotated genes using JBrowse v1.16.8 (Buels et al. 2016) in the *Brassica* Database (BRAD, http://brassicadb.cn), and the genes found *within these genomic regions of B. rapa* cv. Chiifu-401 v3.0 was hypothesized to be the candidate genes. These putative gene sequences were aligned with the Arabidopsis genome sequence (TAIR, http://www.arabidopsis.org) to identify the homoeologous genes with known functions.

4.3. Results

4.3.1. Inheritance of clubroot resistance of *Brassica rapa* var. *pekinensis* cv. Bilko

The F_2 plants of the cv. Bilko showed a 3:1 segregation (resistant *RR* and *Rr* vs. susceptible *rr*) ($df = 1$, $n = 92$, $\chi^2 = 0.0$, p-value = 1.0) for resistance to *P. brassicae* pathotype 3 (3H). Of the five F_3 families evaluated for resistance, two families were segregating, while the remaining three families showed complete resistance to this pathotype. A 3:1 segregation was also found in the two segregating F_3 families ($df = 1$, $n = 31$, $\chi^2 = 1.82$, p-value = 0.18); thus, the results demonstrated that a major locus is involved in the control of resistance to pathotype 3 (3H) in the Chinese cabbage cv. Bilko. Forty-seven BC_1F_5 *B. napus* RILs derived from *B. napus* × Bilko-CR interspecific cross was evaluated for resistance, where 22 lines were homozygous for resistance, 14 segregating, and 11 were susceptible (*rr*). The homozygous resistant and susceptible lines were used for whole-genome resequencing.

4.3.2. Whole-genome resequencing and mapping sequencing reads to *Brassica napus* genome

The high-throughput Illumina sequencing yielded 213.96, 501.72, 330.52, and 280.92 million 150-bp pair-end raw reads from the RP, SP, R-pool, and S-pool, respectively (Table 4.1), where the RP is *B. rapa* and the SP, R-pool and S-pool are *B. napus*. The maximum sequencing data was obtained from the *B. napus* SP (32.02 Gb) followed by R-pool (22.09 Gb), S-pool (19.1 Gb), and the *B. rapa* RP (14.86 Gb).

A total of 317,231 and 282,991 genome-wide SNPs were identified using the RPA_DH12075 and RPA_Darmor, respectively. Among these, 12,606 SNP loci based on RPA_DH12075 and 11,666 loci based on RPA_Darmor were found homozygous in the two pools; these loci were used to identify the genomic regions associated with clubroot resistance and develop molecular markers associated with the resistance.

4.3.3. Identification of candidate genomic regions for clubroot disease resistance

The SNP-index was plotted for all 10 A-genome chromosomes, and SNP-index plot regression lines were calculated with a 2-Mb interval sliding window and a 50 kb increment. The SNP-index was expected to be distributed randomly around an index of 0.5 across the whole length of the A-genome chromosomes except for the SNPs to be associated with the resistance. A strong deviation in the opposite direction was found between the R- and S-pool for the SNP-index from about 3 – 7 Mb and 23 – 24 Mb regions of A03, indicating the possible involvement of these genomic regions with resistance to pathotype 3 (3H) in the RIL population (Figure S4.1 and S4.2).

A total of 12,606 nucleotide positions with ΔSNP-index score of –1 were identified using the RPA_DH12075, of which 5,338 (42.3%) were from A03; this indicates that this chromosome may carry the CR gene conferring resistance to pathotype 3 (Figure 4.1a). The distribution of these 5,338 SNPs displayed two peaks, one at 4.0 – 7.0 Mb (40% of the A03

SNPs) and the other at 22.5 – 25.0 Mb (36% of the A03 SNPs), providing evidence for possible involvement of these two genomic regions with CR (Figure 4.1b, Figure S3a).

While using the RPA_Darmor, 11,666 nucleotide positions with ΔSNP-index = –1 were identified of which 4,409 (37.8%) were from A03 (Figure 4.1c). The frequency distribution of these SNPs displayed two peaks, one at 3.0 – 6.0 Mb (45% of the A03 SNPs) and the other at 21.0 – 23.5 Mb (36.9% of the A03 SNPs) (Figure 4.1d, Figure S3b). Thus, the alignment of the sequence data with two reference genomes gave similar results and provided evidence for the presence of the CR locus on A03.

4.3.4. Marker development and linkage analysis to map CR locus in *Brassica napus*

A total of 101 SNP-based allele-specific primers (AS-primer) (for each, a common reverse and two allele-specific forward) were developed, and this targeted 62 SNPs from the 4.0 – 7.0 Mb region and 39 SNPs from the 22.5 – 25.0 Mb region. All 101 AS-primers were checked for polymorphism between the *B. rapa* and *B. napus* parents of the RIL population. Of the 101 AS-primer sets, five failed to amplify the parental genotypes. Of the remaining 96 markers, only eight, viz. A03_SNP_4359F1 (4.0 Mb), A03_SNP_4447F1 (4.8 Mb), A03_SNP_4446F1 (4.8 Mb), A03_SNP_4410F2 (21.6 Mb), A03_SNP_4415F1 (22.3 Mb), A03_SNP_4436F1 (23.0 Mb), A03_SNP_4437F1 (23.0 Mb) and A03_SNP_4437F1-4436R (23.0 Mb), produced polymorphic alleles between the parents. Among them, A03_SNP_4415F1, A03_SNP_4436F1 and A03_SNP_4437F1 amplified the alleles of the resistant parent, while A03_SNP_4446F1, A03_SNP_4447F1 and A03_SNP_4437F1-4436R amplified alleles of different sizes between resistant and susceptible parents (Figure 4.2).

The RIL population, comprising of 247 individuals belonging to the 47 families, was genotyped with the eight polymorphic AS-primers and five SSR primer sets. Of the 247 individuals, 88 were derived from 33 RIL families homozygous for resistance or susceptibility, and 159 individuals derived from 14 RIL families showing segregation for resistance to

pathotype 3. Three AS-markers A03_SNP_4437F1-4436R, A03_SNP_4437F1, and A03_SNP_4436F1 showed no recombination (recombination frequency 0%) in the 88 individuals of the 33 homozygous RIL families (Figure 4.2); however, these markers showed 0.4 – 0.8% recombination when all 247 individuals from both homozygous and segregating RIL families were considered (Table 4.2). These three markers are from about 23.0 Mb positions of the RPA_DH12073 assembly. The fourth marker, A03_SNP_4415F1 from about 22.3 Mb position, also showed an association with CR in the homozygous RIL families with 1.1% recombination; however, this marker showed 16.6% recombination when both homozygous and segregating RIL families were considered. The remaining four markers (three from about 4-5 Mb and one from about 22 Mb positions) did not show strong co-segregation; recombination between the marker alleles and resistance varied from 5.7 to 30.7% in the homozygous RIL families and 29.6% to 39.4% when both homozygous and segregating RIL families were considered.

4.3.5. Evaluation of published genetic markers associated with CR

The publicly available markers, especially those reported to be linked to the major CR loci of A03, such as *CRa* and *CRb*, were used to map the CR locus of the Chinese cabbage cv. Bilko introgressed into *B. napus*. Three markers, viz. KB69N08, BGB32 and B1324, reported linked to *CRb* (Kato et al. 2013), showed polymorphism between Bilko-CR and A04-73NA and were included in the partial linkage map of chromosome A03 (Figure 4.3). Among these markers, B1324 and BGB32 showed the strongest association with resistance in the homozygous RIL families (1.1% recombination), followed by the marker KB69N08 (9.1% recombination). However, these three markers did not show a strong co-segregation with resistance when individuals from both homozygous and segregating RIL families were considered in the analysis; in this case, recombination varied from 15.8 to 19.2% (Table 4.2).

Of the seven DNA markers associated with *CRa* (Ueno et al. 2012) and 26 SSR markers (Hobson and Rahman 2016) from the genomic region where the *CRa* is located, two markers, GC1250-2 and A03_12800, were found to be polymorphic between the parents. Among these, GC1250-2 showed 4.5% recombination, while A03_12800 showed 11.4% recombination with CR in the homozygous RIL families (Table 4.2).

4.3.6. Mapping of CR locus

A genetic linkage map of chromosome A03 was constructed using the genotypic data of the polymorphic markers to position the CR locus of Bilko introgressed into *B. napus* (Figure 4.3). This map included 13 markers and covered approximately 45.2 cM, where the mean distance between the markers was 3.48 cM. Composite interval mapping determined the location of the CR locus in between the markers A03_SNP_4415F1 and A03_SNP_4436F1 with a LOD score of 54.9 and explained 100.0% of the total phenotypic variance. The markers A03_SNP_4437F1-4436R, A03_SNP_4437F1 and A03_SNP_4436F1 showing the strongest linkage association with the CR were located in between the flanking markers A03_SNP_4415F1 and B1324 (Figure 4.3a). The marker B1324 was reported to be associated with *CRb* (Kato et al. 2013); BLASTn alignment positioned this marker at 24,594,812 - 24,595,018 bp and 25,529,499 – 25,529,705 bp regions of RPA_DH12075 and *B. rapa* cv. Chiifu-401 v3.0 chromosome A03, respectively (Table 4.2).

The three markers A03_SNP_4437F1-4436R, A03_SNP_4437F1 and A03_SNP_4436F1 are located at 23,046,754 bp, 23,046,784 bp, 23,046,870 bp, respectively, of the *B. napus* RPA_DH12075 consensus sequence, and these markers could also be positioned at a very similar position (23,882,872 bp, 23,882,902 bp, and 23,882,988 bp, respectively) of the *B. rapa* cv. Chiifu-401 whole-genome assembly v3.0 (Figure 4.3b, c). A database search of the genomic region where these three AS-markers are located showed collinearity with the genomic sequence where the *R*-genes *Bra012688* and *Bra012689* are located. BLASTn

alignment positioned *Bra012688* at 23,041,133 – 23,047,049 bp and *Bra012689* at 23,034,612 – 23,040,755 bp of the RPA_DH12075 chromosome A03. The markers A03_SNP_4437F1-4436R, A03_SNP_4437F1 and A03_SNP_4436F1 are, in fact, located within the sequence of *Bra012688* (Figure 4.4). BLASTn search of the *Bra012688* and *Bra012689* sequences in TAIR (http://www.arabidopsis.org) database produced the best hit with *Arabidopsis thaliana* gene *AT4G16890*.1 (Score 1043 and 1290, respectively, with E-value = 0) located at 9,500,271 – 9,505,972 bp of the *A. thaliana* chromosome 4. The *AT4G16890*.1 gene encodes a TIR-NB-LRR (TNL) class disease resistance protein.

4.4. Discussion

Introgression breeding is often applied to introduce a novel trait from an allied species or exotic germplasm into the cultivated crop. It is especially consequential when genetic variation for the trait of interest cannot be found in the primary gene pool, such as CR in oilseed *B. napus*. Generally, this crop is highly susceptible to *P. brassicae*; however, resistance to this pathogen can be found in its diploid progenitor species *B. rapa*. To date, 24 major CR loci have been reported in *B. rapa* (Hasan et al. 2021). Among them, CR of turnip (*B. rapa* var. *rapifera*) line ECD-04, located on chromosome A03 (Diederichsen and Sacristan 1996; Fredua-Agyeman and Rahman 2016), has been introgressed into *B. napus* through resynthesis of this species and used to develop the first clubroot resistant winter canola cv. Mendel (Frauen 1999). The CR locus of Mendel has been used extensively in the breeding of clubroot-resistant spring *B. napus* canola cultivars in Canada (Rahman et al. 2011, 2014b). However, repeated cultivation of canola carrying the same resistance gene opens the door for the rare pathotypes to multiply rapidly, resulting in a shift in pathogen population structure where the rare pathotypes become predominant in a few years, as reported in Canada (Strelkov et al. 2016b; Sedaghatkish et al. 2019; Cao et al. 2020). Therefore, the introgression of additional CR genes

in *B. napus* canola is needed. Only a few research groups attempted to introgress additional CR genes from *B. rapa* into *B. napus* (Lammerink 1970; Hirani et al. 2016).

In this study, introgression of a CR locus from the Chinese cabbage cv. Bilko into a spring *B. napus* canola breeding line has been accomplished through an interspecific cross between these two species. The advanced generation (BC_1F_5) RIL population developed in this study was fully fertile and stabilized into *B. napus* type. Mendelian segregation for resistance to pathotype 3 (3H) was found in the cv. Bilko. Molecular mapping showed that the Bilko CR is located on chromosome A03 and explains 100% of the phenotypic variance for resistance to pathotype 3 (3H) in this RIL population.

Given that A03 carries at least 12 CR loci/QTL (for review, see Hasan et al., 2021), a positional candidate gene mapping approach following QTL-seq analysis has been employed in this study to identify the genomic region associated with resistance and to identify the candidate genes in this region. Most of the *R*-genes identified to date encode nucleotide-binding leucine-rich repeats (NB-LRRs) (Dangl and Jones 2001), including coiled-coil NB-LRRs (CC-NB-LRRs) (Meyers et al. 2003) and Toll interleukin1 receptor NB-LRRs (TIR-NB-LRRs) (Akira and Hemmi 2003). Some *R* genes encode receptor-like kinase (RLKs), transmembrane receptor-like kinases (RLPs), cytoplasmic kinase, and proteins with atypical molecular motifs (Jones and Dangl 2006; Liu et al. 2007; Neik et al. 2017). The *R* genes encoding TIR-NB-LRR class proteins have been reported to confer resistance to clubroot disease in *B. rapa* (Ueno et al. 2012; Hatakeyama et al. 2013, 2017). Seven TNL protein-coding *R*-genes, *viz*. *Bra012689*, *Bra012688*, *Bra012541, Bra012540*, *Bra019413*, *Bra019410* and *Bra019409* (http://brassicadb.cn/#/), have been reported in a 23.8 – 26.0 Mb region of A03 of *B. rapa* cv. Chiifu-401 v3.0 (Cheng et al. 2011; Yu et al. 2014). In this study, BLASTn alignment of this 23.8 – 26.0 Mb region of *B. rapa* cv. Chiifu-401 v3.0 chromosome A03 showed sequence homology with the 23.0 – 25.0 Mb region of RPA_DH12075 chromosome A03 (BLASTn hit

score = 47225, E-value = 0); WGRS identified 36% of the total number SNPs in this region of A03. The 4.0 – 7.0 Mb region of A03 of RPA_DH12075, harboring 40% of the A03 SNPs, was collinear to the 4.0 – 6.0 Mb region of *B. rapa* cv. Chiifu-401 v3.0 chromosome A03 (BLASTn hit score = 69509, E-value = 0). This region of *B. rapa* cv. Chiifu-401 v3.0 harbor leucine-rich repeat (LRR) transmembrane protein kinase putative protein-coding genes, *Bra006717, Bra006774*, *Bra006781,* and invertase/pectin methylesterase inhibit family protein-coding gene *Bra029013*. Taking all this information into account, AS-markers have been designed for the SNPs identified within and around the sequences of 11 putative *R* genes located at about 4.0 – 7.0 Mb and 23.0 – 25.0 Mb region of A03 of the RPA_DH12075.

Among the 101 AS-markers designed from the putative *R*-genes, A03_SNP_4437F1-4436R, A03_SNP_4437F1, and A03_SNP_4436F1 from 23.0 – 25.0 Mb region showed the strongest association with resistance both in the homozygous and the segregating RIL families. These three markers are from the TNL protein-coding gene *Bra012688* with a sequence length of 5,919 nt. BLASTn alignment positioned *Bra012688* to a 23,041,133 - 23,047,049 bp (BLASTn score = 10890, E value = 0.0, missing nucleotides 6/5,919) region of RPA_DH12075 chromosome A03. The *Bra012688* sequence showed collinearity with the *A. thaliana* gene *AT4G16890.1* (BLASTn hit score 12525.08, E-value = 0). The *AT4G16890.1* encodes a Toll Interleukin1 receptor-nucleotide binding-Leucine rich repeat-type (TIR-NB-LRR) protein and is involved in salicylic acid-dependent defence response pathway (Wang et al. 2009a). Plants with the TNL gene constitutively express the pathogenesis-related (PR) protein and have been reported to play an important role in clubroot resistance (Swiderski et al. 2009; Ueno et al. 2012; Hatakeyama et al. 2013, 2017). However, in the present study, the AS-markers A03_SNP_4437F1-4436R, A03_SNP_4437F1, and A03_SNP_4436F1 from *Bra012688* showed a perfect co-segregation with CR in the homozygous RIL population, not in the segregating population. Therefore, this gene might not be the candidate for the CR locus of

Bilko introgressed into *B. napus*. Another TNL gene *Bra012689* (sequence length 6,144 nt), with a similar function as *Bra012688,* is located at about 378 bp upstream of the *Bra012688*. However, WGRS did not identify any SNP within the *Bra012689* to design AS-marker; further investigation will be needed to understand its role in Bilko CR. Nevertheless, the AS-markers developed in this study could be used in marker-assisted breeding for CR in *B. napus* canola.

To date, at least six CR loci, *viz.*, *CRa/CRb, CRq, Rcr1, Rcr2, Rcr4,* and *Rcr5*, have been mapped in the 24.0 – 26.0 Mb genomic region of *B. rapa* cv. Chiifu-401 v3.0 chromosome A03 (for review, see (Hasan et al. 2021)). The locus *CRb* is located between the markers KB59N07 and B1005 and co-segregates with B4701, B4732, B1321, B1324, and B1210 (Kato et al. 2013). The marker B1324 showed a 15.8% recombination in the present study, while KB59N07, B4701, B4732, B1321, B1210, and B1005 did not display polymorphism between the parents. Two markers, KB69N08, and BGB32, flanking the *CRb* (Kato et al. 2013), showed a 16.3% and 19.2% recombination, respectively, with the CR in the RIL population (Table 4.2). The marker GC1250-2, which has been reported to be co-segregating with *CRa* (Ueno et al. 2012), showed 4.5% recombination in the homozygous RIL families. BLASTn alignment positioned the markers B1324, BGB32, GC1250-2 and KB69N08 at about 2,280,155 bp, 1,706,956 bp, 1,546,168 bp and 1,259,287 bp downstream of the *Bra012688* (located between 23,041,133 – 23,047,049 bp), respectively, on the RPA_DH12075. The flanked or co-segregating markers positioned the CR loci *Rcr5, Rcr1*, *Rcr2*, *CRb* and *CRa* at 23,624,345 – 23,738,088 bp, 23,923,266 – 24,738,259 bp, 24,382,485 – 24,618,365 bp, 24,466,585 – 24,754,005 bp and 24,599,545 – 24,779,431 bp respectively, on the RPA_DH12075 chromosome A03 (Figure 4.3b). Based on the occurrence of high recombination of the B1324, BGB32, KB69N08, and GC1250-2 with CR in the RIL population, and physical distance of *Rcr5, Rcr1*, *Rcr2*, *CRb,* and *CRa* associated markers from A03_SNP_4437F1-4436R, A03_SNP_4437F1, and A03_SNP_4436F1 on RPA_DH12075, it can be postulated that the

RIL population carries a CR locus different from the previously reported loci *CRa/CRb, Rcr1, Rcr2,* and *Rcr5*. The difference in physical and genetic position of the Bilko-CR and the previously reported CR loci could also have resulted from genetic recombination between the *B. rapa* and *B. napus* chromosomes. Comparative mapping studies have shown that the A-genome chromosomes of *B. rapa* and A-genome chromosomes of *B. napus* are virtually the same, though a small difference between these two genomes can be seen (Parkin et al. 1995; Parkin and Lydiate 1997), and this difference might have resulted in sequence variation in the progeny of this interspecific cross. High collinearity between A- and C-genomes have been reported as well (Slocum et al. 1990; Song et al. 1991; Parkin et al. 2005), and this can promote recombination between the homoeologous chromosomes (e.g. A03/C03) and thus create genetic variation (Xiong et al. 2011). Therefore, markers for a trait developed in one species may not work well when the trait is introgressed into a different species, especially when the markers are not from the gene itself. Nevertheless, this study demonstrates the potential of introgression of the CR genes from *B. rapa* into *B. napus* through an interspecific cross between these two species and the need to develop molecular markers for use in breeding. Following this approach, additional CR genes can be introgressed from *B. rapa* into *B. napus*.

4.5. Conclusions

This study demonstrated that the single major locus of the Chinese cabbage cv. Bilko conferring resistance to clubroot disease also confer resistance to this disease in oilseed *B. napus*. The lack of strong linkage association of the previously reported *B. rapa*-CR markers in the RIL population, derived from *B. napus* × *B. rapa* interspecific cross, might be due to the difference in the CR loci or due to sequence variation in the chromosome A03 of the RIL population resulted from genetic recombination between the *B. rapa* and *B. napus* chromosomes. This demonstrates the need to develop new molecular markers following the introgression of a gene in the recipient species. The PCR-based AS-markers that have been

developed in this study can be detected by agarose gel electrophoresis and can be used by a wider research community. A similar approach of interspecific hybridization can be carried out for the introgression of additional CR genes into the A-genome of *B. napus* to broaden the genetic base of this crop for resistance to clubroot disease.

4.6. Tables

Table 4.1: Summary of Illumina sequencing of the clubroot resistant *Brassica rapa*(RP) and susceptible *Brassica napus* (SP) parental genotypes and resistant (R-pool) and susceptible (S-pool) bulks of *B. napus* recombinant inbred BC_1F_5 lines derived from (*B. napus* × *B. rapa* var. *pekinensis* cv. Bilko) × *B. napus* interspecific cross

Sample	Illumina sequencing				Number of SNPs		
	Data generated (Gb)	Total reads	Mapped reads	% reads mapped	Total	Homozygous	Heterozygous
RPA_DH12075[1]	14.86	213,957,021	207,426,956	96.95	-	-	-
SP[2]	32.02	501,718,110	497,887,988	99.24	-	-	-
R-pool[2]	22.09	330,516,097	327,427,387	99.07	642,807	194,134	544,977
S-pool[2]	19.10	280,918,410	277,287,488	98.71	667,857	122,880	545,605
RPA_Darmor [c]	14.91	210,901,124	204,724,094	97.07	-	-	-
SP[3]	32.27	492,374,795	489,769,036	99.47	-	-	-
R-pool[4]	22.25	326,700,935	323,876,713	99.14	548,532	176,997	371,533
S-pool[4]	19.14	277,964,943	274,446,921	98.73	580,722	105,838	474,884

[1] Reads of the RP aligned to the *Brassica napus* line DH12075 whole-genome assembly v3.1 (Agriculture and Agri-Food Canada, unpublished) to develop the assembly RPA_DH12075. The RPA_DH12075 was developed based on the SNPs of the RP and *Brassica napus* line DH12075 whole-genome assembly v3.1.

[2] Reads of the SP, R-pool, and S-pool aligned to the RPA_DH12075.

[3] Reads of the RP aligned to the *Brassica napus* cv. Darmor-*bzh* whole-genome assembly v4 (Chalhoub et al. 2014) to develop the assembly RPA_Darmor. The RPA_Darmor was developed based on the SNPs of the RP and *Brassica napus* cv. Darmor-*bzh* whole-genome assembly v4.1

[4] Reads of the SP, R-pool, and S-pool aligned to the RPA_Darmor.

Table 4.2: Physical position and recombination frequency of the markers linked with resistance to *Plasmodiophora brassicae* pathotype 3 in a recombinant inbred BC_1F_5 line population derived from (*B. napus* × *B. rapa* var. *pekinensis* cv. Bilko) × *B. napus* interspecific cross.

Marker	RIL families homozygous for resistance to *P. brassicae* pathotype 3			RIL families homozygous and segregating for resistance to *P. brassicae* pathotype 3			Physical position (nt) on RPA_DHA12075		Genetic position (cM)[a]
	No. of parental type plant	No. of recombinant plant	Recomb. freq. (%)	No. of parental type plant	No. of recombinant plant	Recomb. freq. (%)	Start	End	
A03_SNP_4410F2	61	27	30.7	149	97	39.4	21,625,110	21,625,256	0.0
A03_SNP_4447F1	72	15	17.2	175	71	28.9	4,776,843	4,776,965	15.8
A03_SNP_4446F1	82	6	6.8	175	71	28.9	4,784,561	4,784,851	26.1
A03_SNP_4359F1	83	5	5.7	174	73	29.6	4,087,662	4,087,901	27.2
A03_SNP_4415F1	87	1	1.1	206	41	16.6	22,290,176	22,290,308	32.6
Bra012689	TIR-NB-LRR protein coding *R*-gene						23,034,012	23,040,755	
Bra012688	TIR-NB-LRR protein coding *R*-gene						23,041,133	23,047,049	
A03_SNP_4437F1-4436R	88	0	0.0	245	2	0.8	23,046,754	23,047,150	33.0
A03_SNP_4437F1	88	0	0.0	246	1	0.4	23,046,783	23,047,902	33.0
A03_SNP_4436F1	88	0	0.0	245	2	0.8	23,046,870	23,047,150	33.0
Bra012541	TIR-NB-LRR protein coding *R*-gene.						23,994,754	23,999,208	
B1324[b]	87	1	1.1	208	39	15.8	24,594,812	24,595,018	34.1
BGB32[b]	86	1	1.1	206	40	16.3	24,754,005	24,754,268	34.1
GC1250-2[c]	84	4	4.5	204	43	17.4	24,593,217	24,593,867	37.1
KB69N08[b]	80	8	9.1	198	47	19.2	24,306,336	24,306,532	41.1
A03_12800	78	10	11.4	162	84	34.1	24,599,545	24,599,873	45.2

[a] Genetic position derived by constructing a linkage map using 88 individuals derived from 34 homozygous resistant or susceptible RIL families

[b] Markers reported linked to *CRb* locus (Kato et al. 2013)

[c] Marker reported linked to *CRa* locus (Ueno et al. 2012)

4.7. Figures

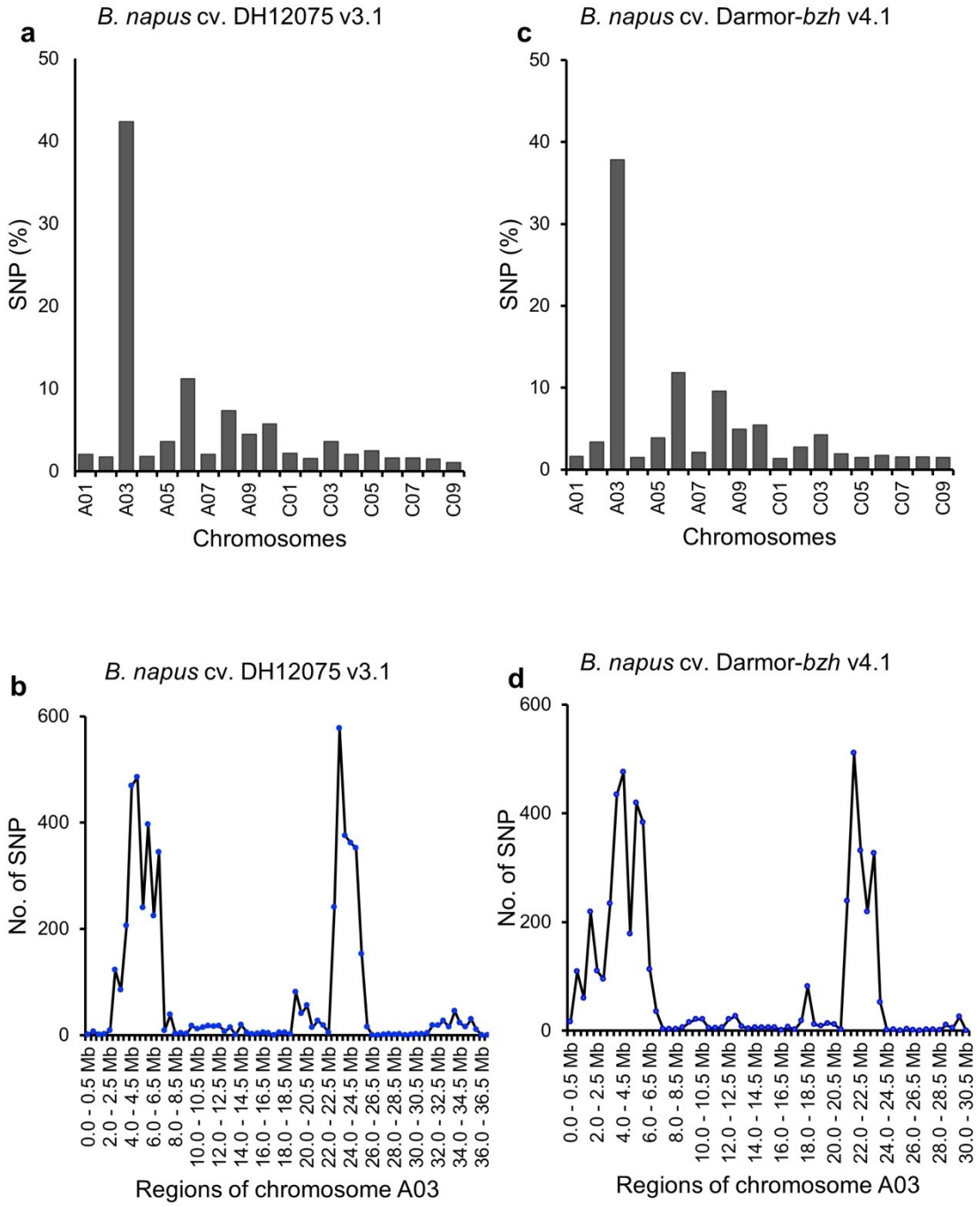

Figure 4.1: Distribution of the SNPs based on the reference genome of *Brassica napus* line DH12075 v3.1 and *B. napus* cv. Darmor-*bzh* v4.1. (a, c) The percentage of the SNPs with ΔSNP index = –1 on the 10 A-genome chromosomes; and (b, d) the distribution of the SNPs with ΔSNP index = –1 on chromosome A03

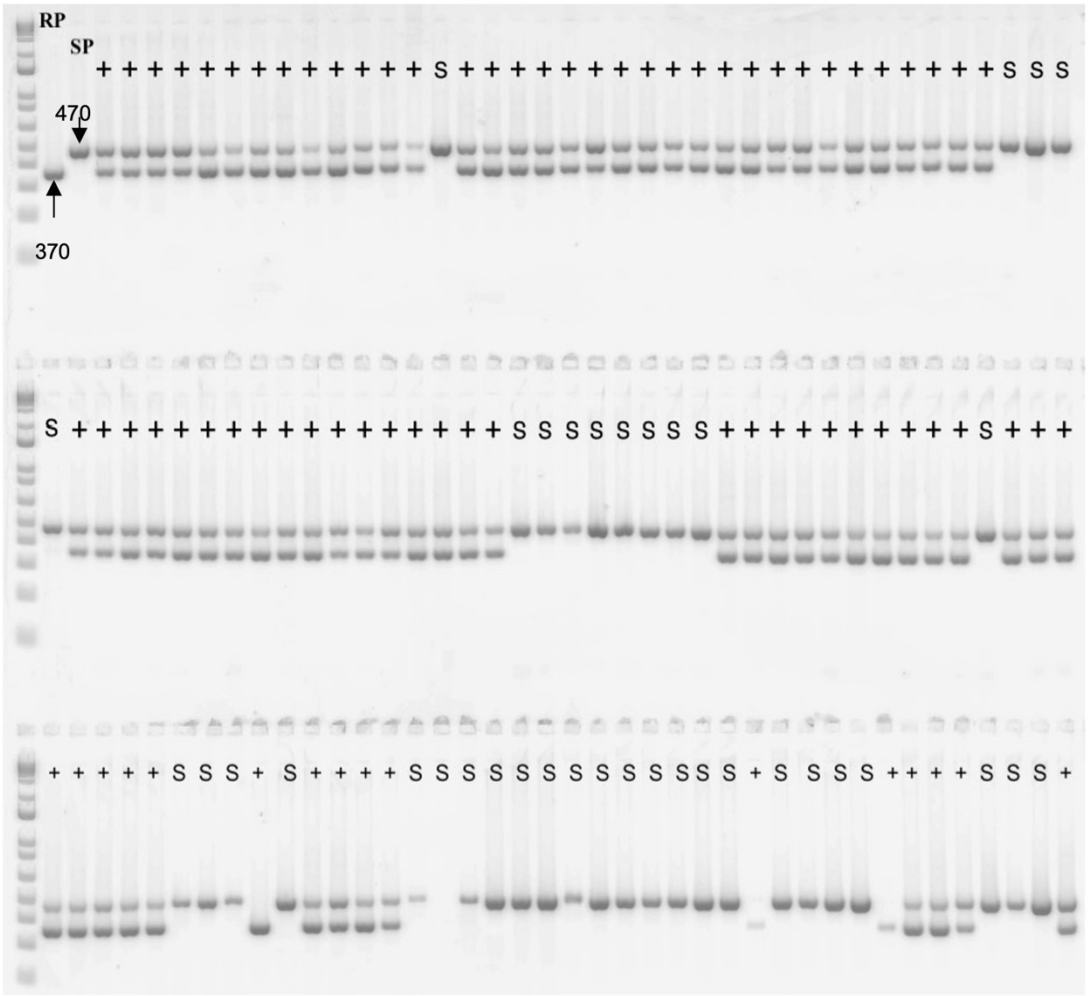

Figure 4.2: Agarose gel electrophoretic separation of PCR amplicons of AS-primer A03_SNP_4437F1-4436R in RIL population derived from (*B. napus* × *B. rapa* var. *pekinensis* cv. Bilko) × *B. napus* interspecific cross. RP = Resistant parent, SP = Susceptible parent, + = resistant RIL plant, S = susceptible RIL plant. The 370 bp allele (R allele) showing co-segregation with resistance.

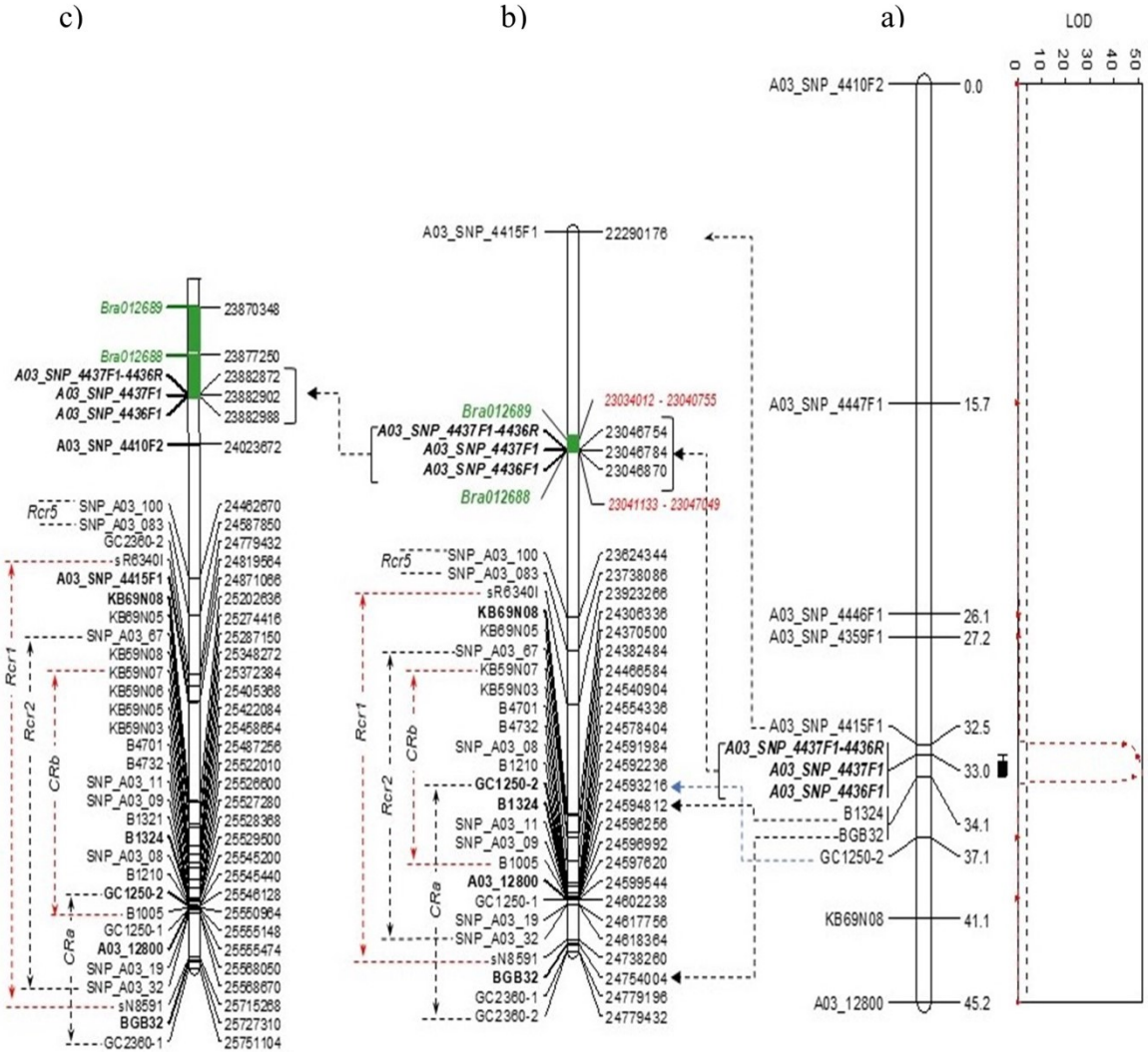

Figure 4.3: (**a**) A partial genetic linkage map of chromosome A03 constructed by using RIL families homozygous for resistant or susceptible phenotypes, and (**b**) its corresponding physical maps of consensus RPA_DH12075 and (**c**) *B. rapa* cv. Chiifu-401 whole-genome assembly v3.0 showing the relative positions of the markers linked to clubroot resistance. The genetic distances are in cM, while the physical distances are in bp. Markers bold-italicized are linked to CR locus in a recombinant inbred BC_1F_5 line population derived from (*B. napus* × *B. rapa* var. *pekinensis* cv. Bilko) × *B. napus* interspecific cross, and resistance derived from the cv. Bilko. Bold-black markers are polymorphic but not linked to CR. Unmarked markers were monomorphic in the population. TNL genes are in green with their physical position. The five CR loci on *B. rapa* chromosome A03 are *CRa* (Ueno et al. 2012)*, CRb* (Piao et al. 2004; Cho et al. 2008; Kato et al. 2013)*, Rcr1* (Chu et al. 2014)*, Rcr2* (Huang et al. 2017) and *Rcr5* (Yu et al. 2017)

Figure 4.4: Physical position of the AS-markers A03_SNP_4437F1 and A03_SNP_4436F1 from TIR-NB-LRR (TNL) protein coding gene *Bra012688*. A03_SNP_4437F1 is located at 23,046,783 bp and A03_SNP_4436F1 is located at 23,046,870 bp position of *Bra012688* sequence of *B. rapa* cv. Chiifu-401 whole-genome assembly v3.0.

4.8. Supplemental materials

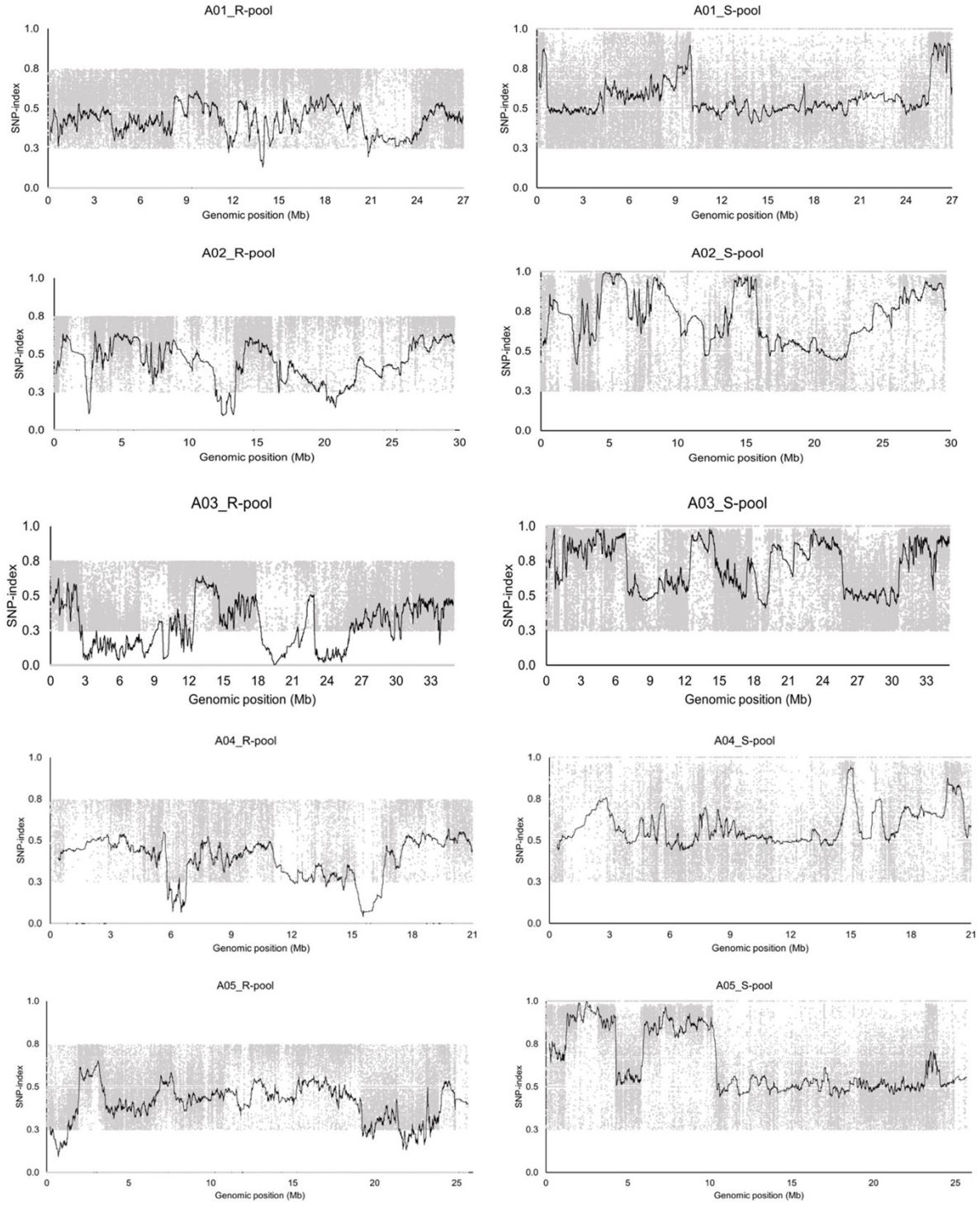

Figure S4.1: SNP index plots for 10 A-genome chromosomes of *Brassica napus* RIL population between resistant (left) and susceptible (right) bulks calculated using RPA_DH12075. Light grey points indicate SNP positions and their SNP indices. Black lines are the regression lines. SNP index plot regression lines were obtained by averaging SNP

indices from a moving window of 255 consecutive SNPs and shifting the window one SNP at a time.

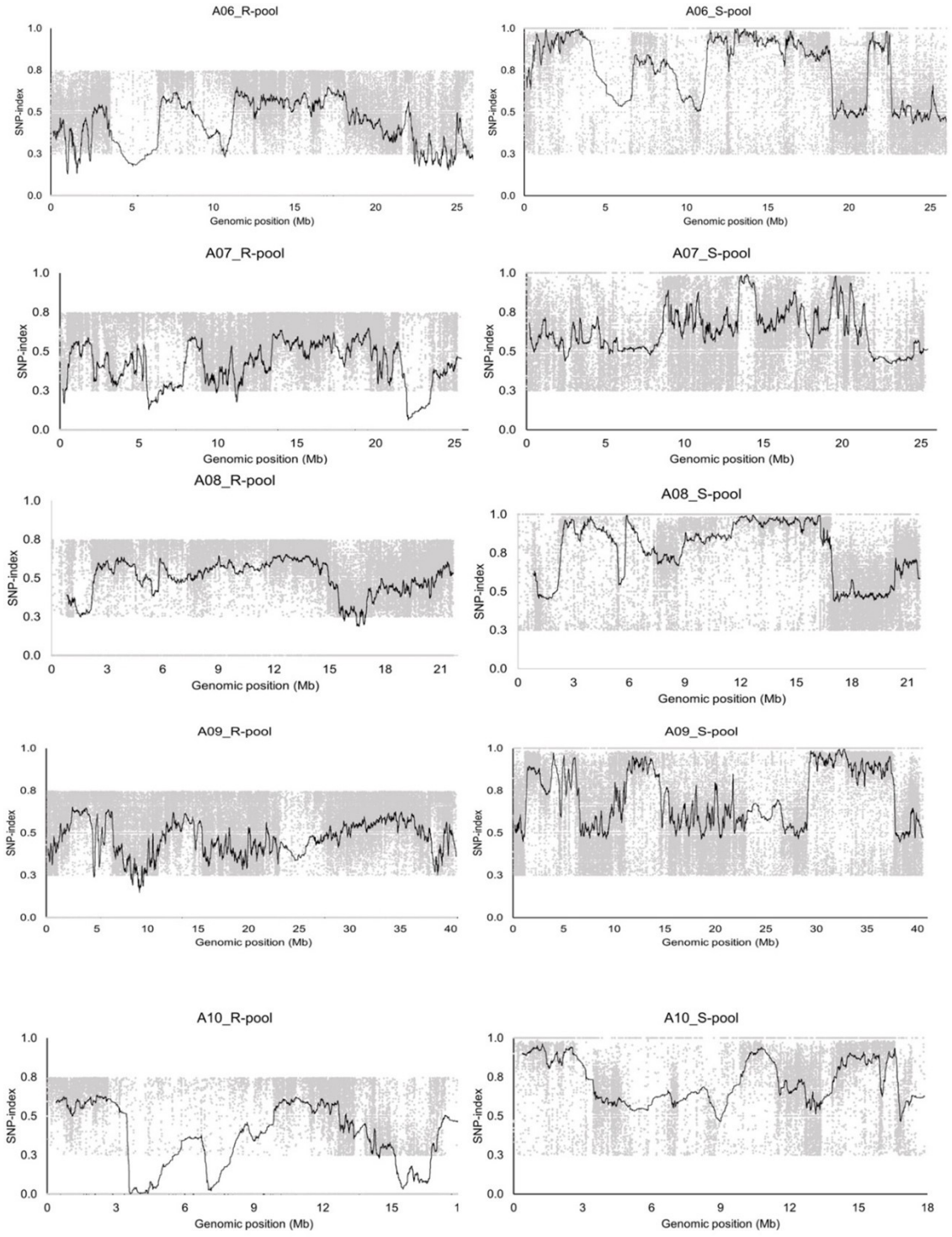

Figure S4.1: Continued

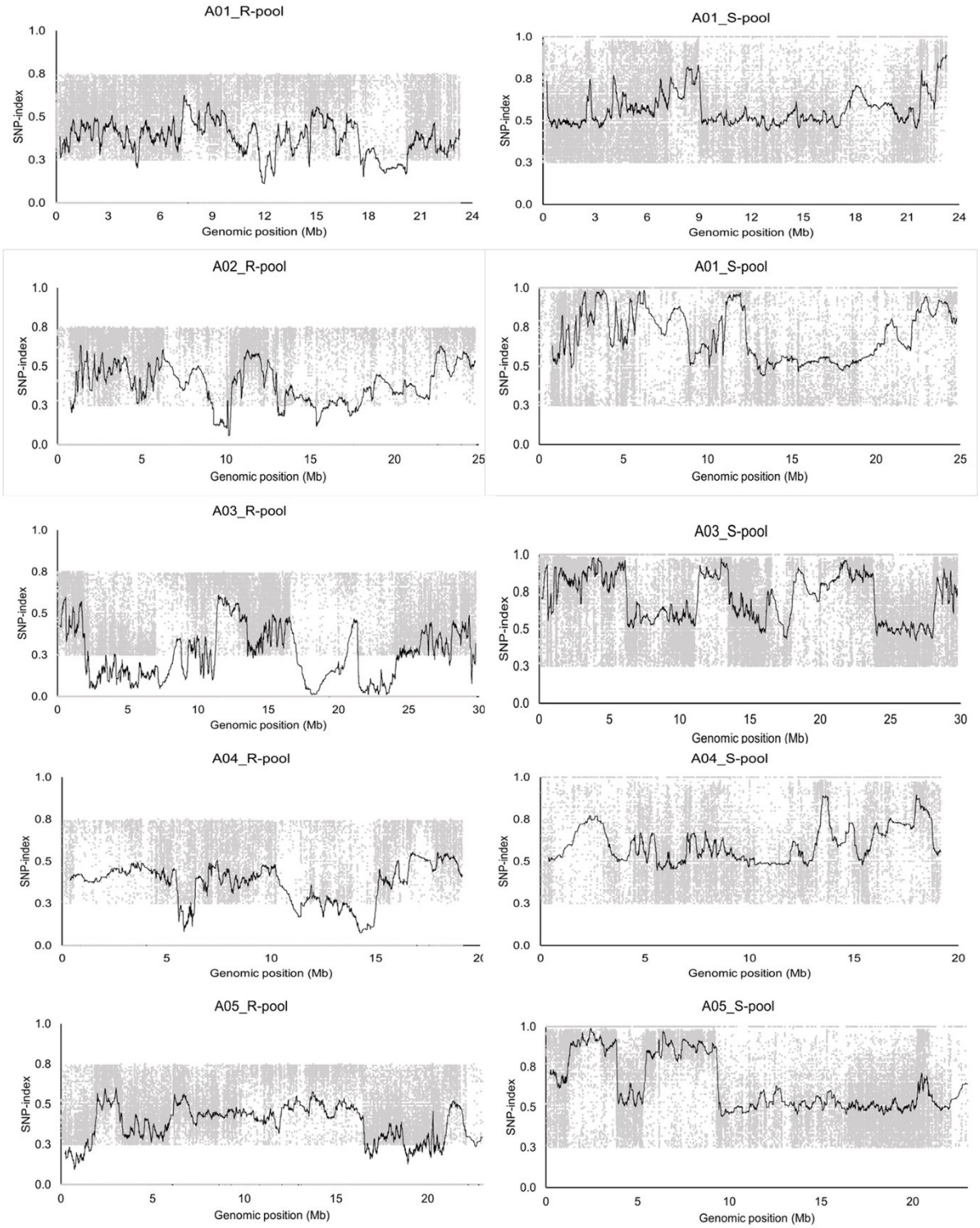

Figure S4.2: SNP index plots for 10 A-genome chromosomes of *Brassica napus* RIL population between resistant (left) and susceptible (right) bulks calculated using RPA_Darmor. Light grey points indicate SNP positions and their SNP indices. Black lines are the regression lines. SNP index plot regression lines were obtained by averaging SNP indices from a moving window of 255 consecutive SNPs and shifting the window one SNP at a time.

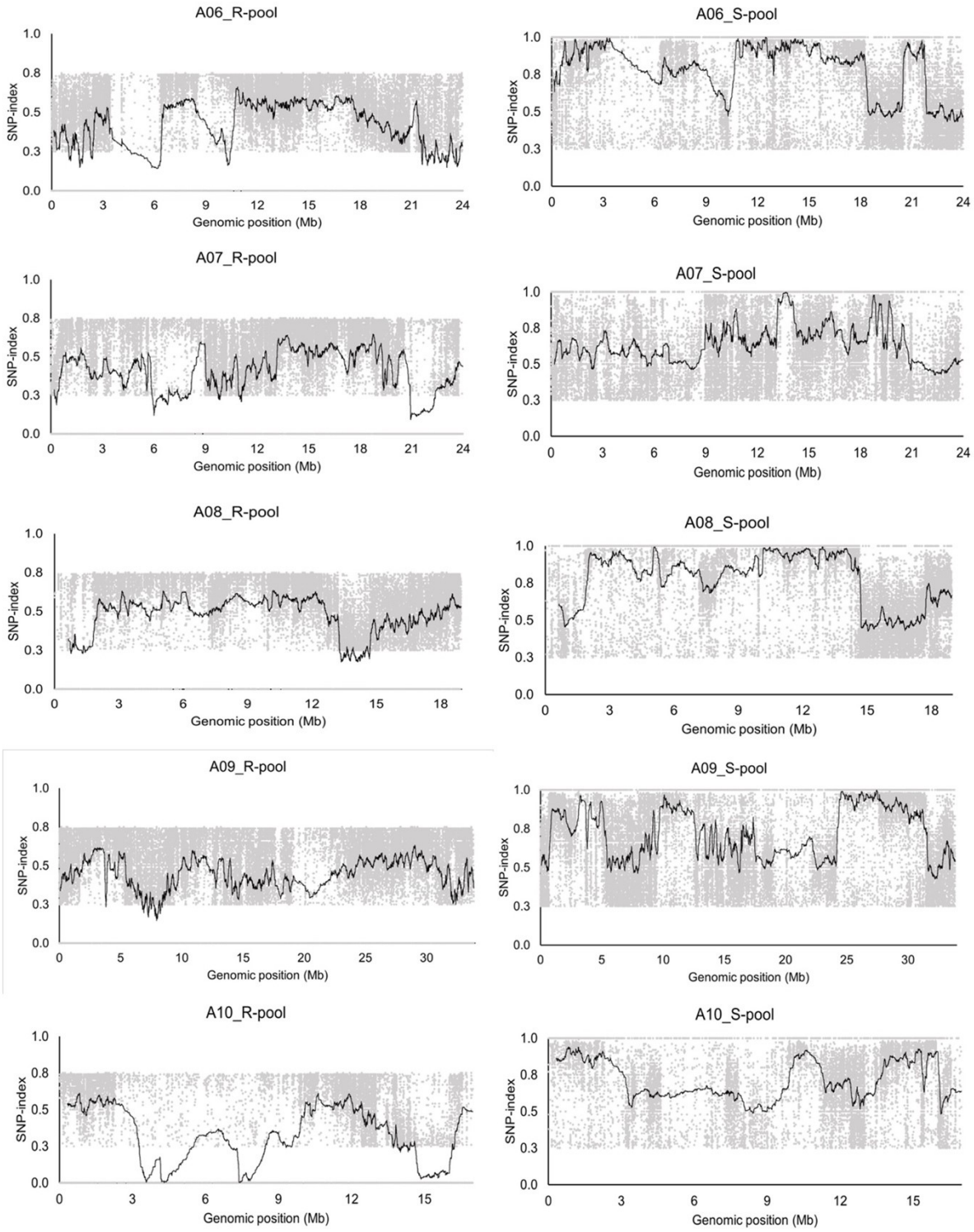

Figure S4.2: Continued

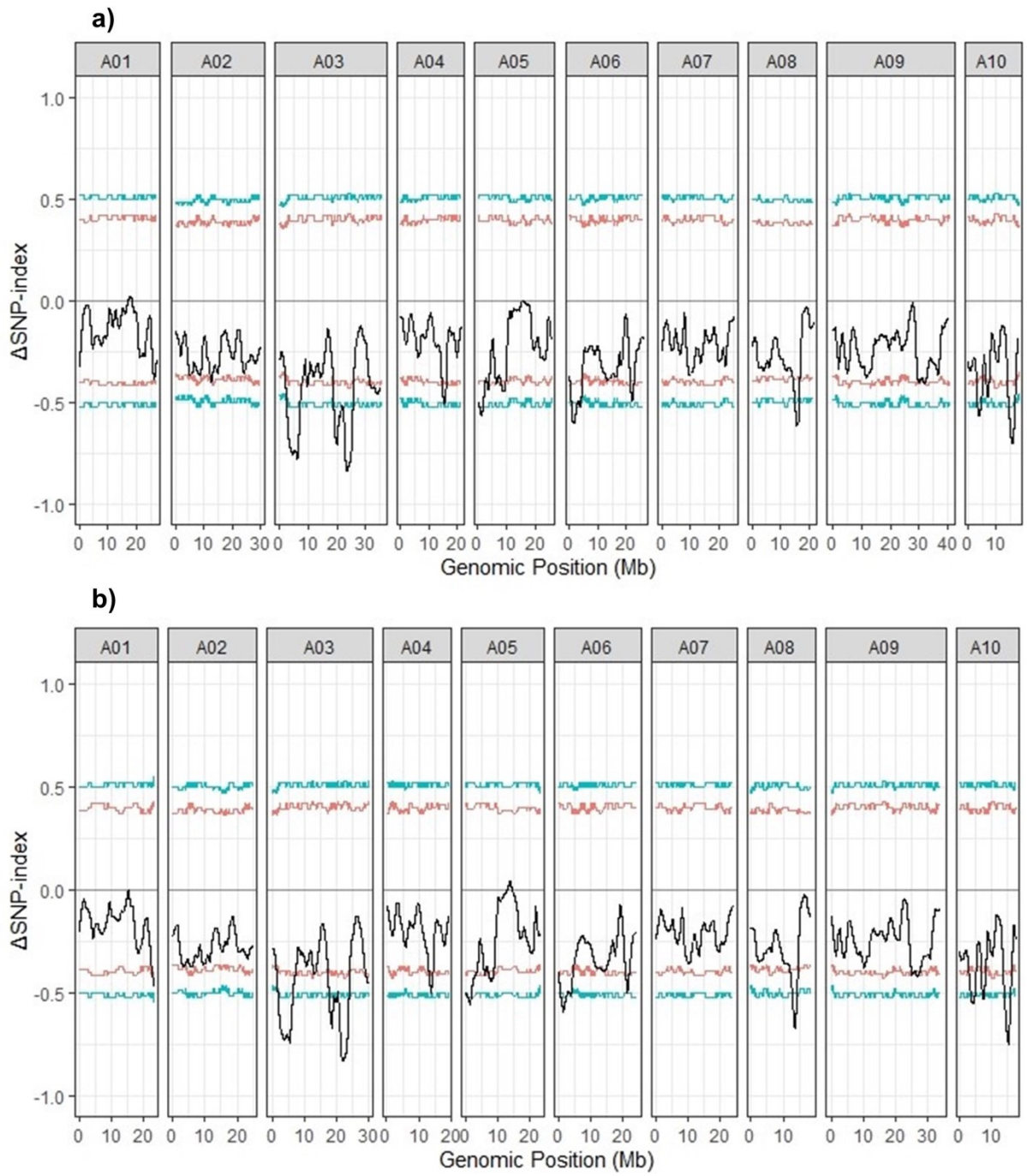

Figure S4.3: ΔSNP index plots of the *Brassica napus* A-genome chromosomes using two resistant-parent genome assemblies **(a)** RPA_DH12075 and **(b)** RPA_Darmor with statistical confidence interval under the null hypothesis of no QTLs (red, $p < 0.05$ and blue $p < 0.01$.

Table S4.1: SNP-based Allele-Specific (AS) markers

Marker	Ref (R) allele	Alt (S) allele	Primer	Sequences (5'-3')	Product size (bp)	Position on RAP_DH12075 (bp)
A03_SNP_4359	A	C	Forward-1	ACGACTGGTATGTTGTACAGATGGA	239	4,087,662
			Forward-2	ACGACTGGTATGTTGTACAGATGGC		
			Reverse	GATCTTTCTCCATAAAGGTTTTCCAGTCT		
A03_SNP_4360	C	T	Forward-1	TAAGAATCACGTTTGTCCTCTTCTTTAGC	213	4,127,027
			Forward-2	TAAGAATCACGTTTGTCCTCTTCTTTAGT		
			Reverse	TCATACAATTGATTTGGCGACTGGTGAA		
A03_SNP_4361	C	T	Forward-1	TAAAAGAGGGTGGTAGAGGTTTTGC	246	4,139,079
			Forward-2	TAAAAGAGGGTGGTAGAGGTTTTGT		
			Reverse	CTAAAGTGCGATTGATTTTATTGTCAAAAT		
A03_SNP_4362	C	A	Forward-1	CAAAAGAGTAGAGAACCAAGGGCAC	140	4,248,680
			Forward-2	CAAAAGAGTAGAGAACCAAGGGCAA		
			Reverse	GAGGAAAGGGAGAGCAGAGGAG		
A03_SNP_4363	T	C	Forward-1	CGTGGATCTCGAGGAACCATCAT	160	4,260,461
			Forward-2	CGTGGATCTCGAGGAACCATCAC		
			Reverse	GTGTTTTTGGAAGGGGTGTTTGTTAATG		
A03_SNP_4364	G	A	Forward-1	TTCCAACTTAAAACCAATTGGCGATAAAG	192	4,291,531
			Forward-2	TTCCAACTTAAAACCAATTGGCGATAAAA		
			Reverse	TCCCATTATCTACGGGATCGATGCT		
A03_SNP_4365	C	A	Forward-1	AGAAAAACCACAACACACATGGCC	165	4,315,364
			Forward-2	AGAAAAACCACAACACACATGGCA		
			Reverse	TGGTCTGCGGCTGCCTATGTC		
A03_SNP_4366	C	A	Forward-1	GAACATAGTAAAGAAGGCTGCAATAAAGC	247	4,321,869
			Forward-2	GAACATAGTAAAGAAGGCTGCAATAAAGA		
			Reverse	CTTGTGTACTCATCAGAAGCACTTGG		
A03_SNP_4367	G	A	Forward-1	CAGATGAACTGCCTGAAGAAACATTG	224	4,351,861
			Forward-2	CAGATGAACTGCCTGAAGAAACATTA		
			Reverse	AGGCAAACTAGTTAGACATTATTTGCTTGTTC		
A03_SNP_4368	A	G	Forward-1	AGTTGCTTTTTAAATGTTTATTTAACATACATACAA	242	4,359,769
			Forward-2	AGTTGCTTTTTAAATGTTTATTTAACATACATACAG		
			Reverse	CAGTTATTGAAAATCAGTTTTTATCTCTACTGAACT		
A03_SNP_4369	T	C	Forward-1	CCTATCACAAAACGATAAAATGTTATAAAGTATT	189	4,375,137
			Forward-2	CCTATCACAAAACGATAAAATGTTATAAAGTATC		
			Reverse	TCGTTACAATTAGGGATTAGACCCGTA		
A03_SNP_4370	C	T	Forward-1	GTAGGACTGGCTACTTTAGTTTGAC	256	4,397,219
			Forward-2	GTAGGACTGGCTACTTTAGTTTGAT		
			Reverse	GGAAAATTAAACCTTTAAACAGATGAACAACAG		
A03_SNP_4371	G	A	Forward-1	TTGGGAGTTTCTGAAATTGATCTACAGAG	257	4,416,061
			Forward-2	TTGGGAGTTTCTGAAATTGATCTACAGAA		
			Reverse	GATTTGTTGTTTTACATACAAACTTATGGATCGA		
A03_SNP_4372	G	A	Forward-1	AAGACTTATAGATGAGCCTGAGG	226	4,425,835
			Forward-2	AAGACTTATAGATGAGCCTGAGA		
			Reverse	GATAACGATTTTTTGGTACATTGTCACACAT		
A03_SNP_4373	T	C	Forward-1	AACAAAATACACACCCGGCCTCT	208	4,450,400
			Forward-2	AACAAAATACACACCCGGCCTCC		
			Reverse	GAACCGATTAGATTAGGCAATCAAAGCTATA		
A03_SNP_4374	G	A	Forward-1	GTTTGACTCGTTGTTCATTATCCCG	212	4,469,113
			Forward-2	GTTTGACTCGTTGTTCATTATCCCA		
			Reverse	AGTTGAATAATTTTAGGAAGGAGGAACTACTTATTT		
A03_SNP_4375	C	T	Forward-1	CGGCTCTTCATCATCATCTTCGTC	175	4,483,109
			Forward-2	CGGCTCTTCATCATCATCTTCGTT		
			Reverse	GATTCCTGGTTTGGCTACAGATCAAGG		
A03_SNP_4376	A	G	Forward-1	TATAGTGCTGGCTTGTATAGAGTTGAA	155	4,497,496
			Forward-2	TATAGTGCTGGCTTGTATAGAGTTGAG		
			Reverse	TCCTCCTTCAGTATATCCTCATCTAG		
A03_SNP_4377	A	C	Forward-1	GGATTGGAATTGTAAGAGCGACTTA	164	4,510,617
			Forward-2	GGATTGGAATTGTAAGAGCGACTTC		
			Reverse	AACTCATCACCAAGCCTGCCATGAA		
A03_SNP_4378	T	C	Forward-1	CAAGAATCACCGTCGTCTAAAGAAAT	145	4,544,244
			Forward-2	CAAGAATCACCGTCGTCTAAAGAAAC		
			Reverse	CTTTAAGTTGATTCATTGATGAGTTATT		

Table S4.1

Marker	Ref (R) allele	Alt (S) allele	Primer	Sequences (5'-3')	Product size (bp)	Position on RAP_DH12075 (bp)
A03_SNP_4379	G	A	Forward-1	ATCTATACTTAAATTAACTACATAGATGGTTAACTAG	179	4,576,126
			Forward-2	ATCTATACTTAAATTAACTACATAGATGGTTAACTAA		
			Reverse	CATTTGTTGTGATAGTATATAGTATGTAACCATATATTT		
A03_SNP_4380	G	A	Forward-1	CAAGGTAGAAAAAAGTCGCAGAAAATAAGG	130	4,604,800
			Forward-2	CAAGGTAGAAAAAAGTCGCAGAAAATAAGA		
			Reverse	CACCAAGGCTGCAGTCAAGAAG		
A03_SNP_4381	A	C	Forward-1	AAGACAATGAGGAAGAAGATCTGTAAGAA	177	4,646,402
			Forward-2	AAGACAATGAGGAAGAAGATCTGTAAGAC		
			Reverse	GATGACTGAAGATATCTTCATTCTTGATTG		
A03_SNP_4382	T	C	Forward-1	CCTTTGCTTCCACATTTCACTCAGT	117	4,652,806
			Forward-2	CCTTTGCTTCCACATTTCACTCAGC		
			Reverse	AAGCTCCTTCACGGAACGGTTCCAA		
A03_SNP_4383	G	A	Forward-1	AAGGGTAGAAATTGAAAAGGCATCGG	125	4,741,301
			Forward-2	AAGGGTAGAAATTGAAAAGGCATCGA		
			Reverse	AGGTCTCTCTCAGAATCTCTGATTTG		
A03_SNP_4384	T	C	Forward-1	CCAAAAGAAACAAGGGCCAATTCTCGAAT	264	4,749,309
			Forward-2	CCAAAAGAAACAAGGGCCAATTCTCGAAC		
			Reverse	ATGTTATAAACATAATCCTAGGCGGGTTAAT		
A03_SNP_4385	G	T	Forward-1	CGTCGCAAGCCCCACCAAACG	136	4,786,755
			Forward-2	CGTCGCAAGCCCCACCAAACT		
			Reverse	CCATTACTTTGGCCTCTCATTTACGAA		
A03_SNP_4386	C	A	Forward-1	TGTCTAGAGAGCTCGTCTTTGTTAC	383	4,797,926
			Forward-2	TGTCTAGAGAGCTCGTCTTTGTTAA		
			Reverse	TGATGTCTAAGAACTTCCATCTGC		
A03_SNP_4387	A	G	Forward-1	ATATGGCATCAGCACTCTGGACA	158	4,930,931
			Forward-2	ATATGGCATCAGCACTCTGGACG		
			Reverse	GCATATAGTGCTAGGTGTTCTCTTCAATT		
A03_SNP_4388	C	T	Forward-1	CGGCTCTTCATCATCATCTTCGTC	161	5,083,200
			Forward-2	CGGCTCTTCATCATCATCTTCGTT		
			Reverse	CTACAGATCAAGGTACGGCACAG		
A03_SNP_4389	T	G	Forward-1	GTCGGCTGCTTCCGAAGCCT	227	5,117,876
			Forward-2	GTCGGCTGCTTCCGAAGCCG		
			Reverse	AGAGCAGCCGAGGCTGAGTAA		
A03_SNP_4390	A	C	Forward-1	CAATGAGGAAGAAGATCTGTAAGAA	247	5,249,246
			Forward-2	CAATGAGGAAGAAGATCTGTAAGAC		
			Reverse	CTTATTGAGACTCTTCTTTCTTTTTATTCCCATATAT		
A03_SNP_4391	G	A	Forward-1	TGCTCTGTGGTTTATGCCAACACAG	187	5,345,994
			Forward-2	TGCTCTGTGGTTTATGCCAACACAA		
			Reverse	TTAAGAATCTCCTCGACCGCCTCG		
A03_SNP_4392	A	G	Forward-1	ATCCATCTCGTTTCATTTGGGAAACAAA	142	5,396,752
			Forward-2	ATCCATCTCGTTTCATTTGGGAAACAAG		
			Reverse	AACACGAGCAGCACGGAGATCC		
A03_SNP_4393	G	A	Forward-1	TGTTGGAGCCGGTGGCGCAGCCG	286	5,524,034
			Forward-2	TGTTGGAGCCGGTGGCGCAGCCA		
			Reverse	CTCTCTTTTTTCTATTGTCTCTTTTACAAT		
A03_SNP_4394	A	G	Forward-1	TGACTCTCCGGTTGTTAGCTTTGCA	263	5,616,163
			Forward-2	TGACTCTCCGGTTGTTAGCTTTGCG		
			Reverse	ATAATTGCTCTGATCAGATCTCACA		
A03_SNP_4395	C	T	Forward-1	GAGCATGCTAAGTTCTTGGGAGTTC	139	5,743,364
			Forward-2	GAGCATGCTAAGTTCTTGGGAGTTT		
			Reverse	CAGTGCAAACTCCATGTAATATAAAGTACAAA		
A03_SNP_4396	T	G	Forward-1	GGAAGAAGACCAATCTCAAACTCAGT	201	5,792,907
			Forward-2	GGAAGAAGACCAATCTCAAACTCAGG		
			Reverse	GTAGTTTCGCCGGGATTCTCATCT		
A03_SNP_4397	G	A	Forward-1	ACTCAGTATGCGTTAAAGGCAAATCAG	241	5,880,482
			Forward-2	ACTCAGTATGCGTTAAAGGCAAATCAA		
			Reverse	CACTCTGCTTCCTAACAATCTCCG		
A03_SNP_4398	G	A	Forward-1	TGCTCTGTGGTTTATGCCAACACAG	183	5,951,044
			Forward-2	TGCTCTGTGGTTTATGCCAACACAA		
			Reverse	GAATCTCCTCGACCGCCTCG		

Table S4.1

Marker	Ref (R) allele	Alt (S) allele	Primer	Sequences (5'-3')	Product size (bp)	Position on RAP_DH12075 (bp)
A03_SNP_4399	C	T	Forward-1	GGGCGTGTTCGTTGGAAAGGGTTC	258	5,960,063
			Forward-2	GGGCGTGTTCGTTGGAAAGGGTTT		
			Reverse	CGTCTTCTACATTTTCCACGTTTGTTC		
A03_SNP_4400	C	T	Forward-1	TTAAGCTTGGGGACTTGATGGGT	156	6,091,817
			Forward-2	TTAAGCTTGGGGACTTGATGGGC		
			Reverse	TTCTCCCCAAGCTAACACTCTTAAGT		
A03_SNP_4401	A	G	Forward-1	GTATCATTGAAATAACATGCTTCGTCGGTA	159	6,414,553
			Forward-2	GTATCATTGAAATAACATGCTTCGTCGGTG		
			Reverse	ATTGACTGTGTAGCCAAAGAAATGTTAGACT		
A03_SNP_4402	C	T	Forward-1	CTATCGGGCGACCTATCACGACGC	216	6,607,292
			Forward-2	CTATCGGGCGACCTATCACGACGT		
			Reverse	CTTCGAGCCGTATGGAAAAATCGTTAA		
A03_SNP_4403	C	A	Forward-1	AAAGGAAAGTTCAATCATGTCTTAATAAGAC	164	6,708,764
			Forward-2	AAAGGAAAGTTCAATCATGTCTTAATAAGAA		
			Reverse	GATTTTTCCTTAAACCGGTTTACTTT		
A03_SNP_4404	T	C	Forward-1	GCCGTGGTGCCTAACATCTCT	196	21,340,889
			Forward-2	GCCGTGGTGCCTAACATCTCC		
			Reverse	TCACCCTCACCACCGCGTCG		
A03_SNP_4405	G	A	Forward-1	TCATTTAAGTAGCAAAACATGTTGGTCTTGG	192	21,345,426
			Forward-2	TCATTTAAGTAGCAAAACATGTTGGTCTTGA		
			Reverse	TGCTCCCATTACAACCACTCCC		
A03_SNP_4406	A	G	Forward-1	CCTTGTTGATGTTTGAGTAATTTTTTGGGTTA	203	21,368,020
			Forward-2	CCTTGTTGATGTTTGAGTAATTTTTTGGGTTG		
			Reverse	GACGAATCAGCCAAATAATATTCAAACGATAG		
A03_SNP_4407	G	A	Forward-1	TAGTTCCGAGGAGCAGAGTCG	139	21,418,150
			Forward-2	TAGTTCCGAGGAGCAGAGTCA		
			Reverse	AGGACATGTTCATTCGGATTGACTTC		
A03_SNP_4408	A	G	Forward-1	TCATATCTATTTTGCGTCTCTATACACATATA	190	21,515,310
			Forward-2	TCATATCTATTTTGCGTCTCTATACACATATG		
			Reverse	ACTTCAAAGTTCAAACCATCTTTGGAGGT		
A03_SNP_4409	G	A	Forward-1	GGTATGAAAATTTATATCCCAAATTGATCTAAAGTTG	198	21,548,011
			Forward-2	GGTATGAAAATTTATATCCCAAATTGATCTAAAGTTA		
			Reverse	GAACATTAGTATACAAGACTTCAGACTAGTT		
A03_SNP_4410	A	C	Forward-1	CCGTGGCAACTGGGGAACA	146	21,625,110
			Forward-2	CCGTGGCAACTGGGGAACC		
			Reverse	TGATTCTTCAGTTACTCTAATGATTGCAACAAA		
A03_SNP_4411	T	C	Forward-1	ATAATGATTTAACGGCGTTT	144	21,815,934
			Forward-2	ATAATGATTTAACGGCGTTC		
			Reverse	TAAAGCCTCGAAATTACGTAAAGGTAGTTTG		
A03_SNP_4412	A	G	Forward-1	CTTAATCCACGGGTGGACGA	179	21,984,701
			Forward-2	CTTAATCCACGGGTGGACGG		
			Reverse	GAATGAAAAGCTAAAAGACTCCACTGATC		
A03_SNP_4413	T	C	Forward-1	GTCGCTATGATTACTATTGGGCTTT	163	22,141,133
			Forward-2	GTCGCTATGATTACTATTGGGCTTC		
			Reverse	TTCATTAGGTGCTTCTGAGAAGGCTAA		
A03_SNP_4414	C	A	Forward-1	GGTGTTCTATGTCATCTTGATTCCCAC	155	22,198,219
			Forward-2	GGTGTTCTATGTCATCTTGATTCCCAA		
			Reverse	TCAAGCTTATCCAACAACATATGTAGCTAAATTAAT		
A03_SNP_4415	G	T	Forward-1	TTTGGATCAGAGCAAGCTGTGATCG	132	22,290,176
			Forward-2	TTTGGATCAGAGCAAGCTGTGATCT		
			Reverse	AAAAAGAAGAAGAATATCAAGAAAACACAATCCA		
A03_SNP_4416	T	C	Forward-1	GCATACGGTAAGATGTACAAGGAT	215	22,473,880
			Forward-2	GCATACGGTAAGATGTACAAGGAC		
			Reverse	ATTCCACCAAGAGGGAGAGAATGGT		
A03_SNP_4417	G	A	Forward-1	TGGTTCGCGAACACAGCACCGAG	111	22,525,769
			Forward-2	TGGTTCGCGAACACAGCACCGAA		
			Reverse	GGTTGATCAAATGCGATTCCGGTTT		
A03_SNP_4418	C	T	Forward-1	GAAGGTGATATTGAGACGGTGGTC	177	22,629,614
			Forward-2	GAAGGTGATATTGAGACGGTGGTT		
			Reverse	AGTTTCTACGTTTTCTGCTGCCACAATA		

Table S4.1

Marker	Ref (R) allele	Alt (S) allele	Primer	Sequences (5'-3')	Product size (bp)	Position on RAP_DH12075 (bp)
A03_SNP_4419	A	G	Forward-1	CTCCATCGCTTGGCAAAACGGA	174	22,896,271
			Forward-2	CTCCATCGCTTGGCAAAACGGG		
			Reverse	GCAGCGGTTAATGGCAATGGGACAA		
A03_SNP_4420	C	T	Forward-1	GTTTTTCACGAACGCCAGAATCTCCTC	191	22,896,436
			Forward-2	GTTTTTCACGAACGCCAGAATCTCCTT		
			Reverse	TGATGAGGTATTTTATTTGTCTCTCA		
A03_SNP_4421	T	G	Forward-1	GATATATATGCAAGCCGAAATACTTTACACG	223	23,077,364
			Forward-2	GATATATATGCAAGCCGAAATACTTTACACT		
			Reverse	GTGGAGCATATATAAATGTATATTGTGGTTTA		
A03_SNP_4422	C	T	Forward-1	GAGGAGACAGGAGTTTCTTCCCC	167	23,168,929
			Forward-2	GAGGAGACAGGAGTTTCTTCCCT		
			Reverse	CCGTATTCTTCTTCTTCCTCCG		
A03_SNP_4423	C	A	Forward-1	GCATCTCTGTAAATGGGCTGCTTCCAA	180	23,206,177
			Forward-2	GCATCTCTGTAAATGGGCTGCTTCCAC		
			Reverse	GGCAAGATATAATCAAGCTGATGAATC		
A03_SNP_4424	A	C	Forward-1	ATGTAGACACCGGCTATCTCATGAA	190	23,217,285
			Forward-2	ATGTAGACACCGGCTATCTCATGAC		
			Reverse	GACCATAGCAACTGGATATTTGTTTCGTT		
A03_SNP_4425	A	G	Forward-1	GACAACGACTCCCTCCTCCTA	242	23,289,108
			Forward-2	GACAACGACTCCCTCCTCCTG		
			Reverse	GGTTTAGAGGAAGCATGTAGTGAAACAAT		
A03_SNP_4426	G	A	Forward-1	ATCAAGGTCTCGGCTTTCTCGATTTTG	247	23,376,148
			Forward-2	ATCAAGGTCTCGGCTTTCTCGATTTTA		
			Reverse	ACATCTCAAAATTTCGAAACTTTCTC		
A03_SNP_4427	C	T	Forward-1	GTGTGTTTTAGTAAAGCGGAGTATGTGATC	337	23,504,823
			Forward-2	GTGTGTTTTAGTAAAGCGGAGTATGTGATT		
			Reverse	TCGCAAGGCAAGACCAACAACATAA		
A03_SNP_4428	T	G	Forward-1	TGTCTCTGAAACTGGGGAGAAGAAAAAT	162	23,771,882
			Forward-2	TGTCTCTGAAACTGGGGAGAAGAAAAAG		
			Reverse	TTATTCAGTTAGGCCTTTTGTAAAGCCA		
A03_SNP_4429	T	C	Forward-1	GAAATGCATACGGTAAGATGTACAAGGAT	231	24,088,478
			Forward-2	GAAATGCATACGGTAAGATGTACAAGGAC		
			Reverse	GAAGTACGACAATTCCACCAAGAGG		
A03_SNP_4430	T	G	Forward-1	ATTTTCAACCCGTTCAATGACGAGTAT	113	4,290,890
			Forward-2	ATTTTCAACCCGTTCAATGACGAGTAG		
			Reverse	GTTATTAACTCAAATGGATTGCTCAACATT		
A03_SNP_4431	A	G	Forward-1	TGTAATACATTTTAATACTGTTTGCATTTGATAGA	173	22,897,748
			Forward-2	TGTAATACATTTTAATACTGTTTGCATTTGATAGG		
			Reverse	CGCTGTATGTGATCCTTTGTCCC		
A03_SNP_4432	G	A	Forward-1	TGTCAAAGACATCAAAACTTCTTTTTCTCTG	176	22,898,143
			Forward-2	TGTCAAAGACATCAAAACTTCTTTTTCTCTA		
			Reverse	TTCAACGATAATTCATTAAAGAAGAAGAGTTT		
A03_SNP_4433	A	G	Forward-1	AGGATTAGAGCAAAACTTACTTCAAAATGAAA	174	23,028,937
			Forward-2	AGGATTAGAGCAAAACTTACTTCAAAATGAAG		
			Reverse	AGATGGTGGATCCTTATCTTGACGC		
A03_SNP_4434	C	T	Forward-1	CCCATCGTTTTCGTGTAATTTACTTTCAC	180	23,049,249
			Forward-2	CCCATCGTTTTCGTGTAATTTACTTTCAT		
			Reverse	TGTGGAAATTTGGATAGACCATATGAGTTA		
A03_SNP_4435	T	G	Forward-1	AGGGCCAAAGAGTACTCGTGAGAT	160	23,047,945
			Forward-2	AGGGCCAAAGAGTACTCGTGAGAG		
			Reverse	TAATAGAAAAAGATGCAAAGACGATAATGCTACA		
A03_SNP_4436	A	G	Forward-1	GGTGATAAGATTAGTGTACCTTAGGA	280	23,046,870
			Forward-2	GGTGATAAGATTAGTGTACCTTAGGG		
			Reverse	GTAGCTACAGCTCTCGTCATTGC		
A03_SNP_4437	A	G	Forward-1	TTGACAACAAAATTCCCTAAGGCTTATGAA	217	23,046,783
			Forward-2	TTGACAACAAAATTCCCTAAGGCTTATGAG		
			Reverse	GGTGAATTACTTTACTTCCCTGTGATG		
A03_SNP_4437F1-4436R	A	G	Forward-1	TTGACAACAAAATTCCCTAAGGCTTATGAA	371	
			Forward-2	TTGACAACAAAATTCCCTAAGGCTTATGAG		
			Reverse	GTAGCTACAGCTCTCGTCATTGC		
A03_SNP_4438	A	G	Forward-1	GTAGGATTAGAGCAAAACTTACTTCAAAATGAAA	150	21,521,484
			Forward-2	GTAGGATTAGAGCAAAACTTACTTCAAAATGAAG		
			Reverse	TGTTTCTCAAACGCTAGCGTTAGAACA		

Table S4.1

Marker	Ref (R) allele	Alt (S) allele	Primer	Sequences (5'-3')	Product size (bp)	Position on RAP_DH12075 (bp)
A03_SNP_4439	A	G	Forward-1	ATTATTTTTCAACACATGTTACCATTGAGGTTA	153	22,277,566
			Forward-2	ATTATTTTTCAACACATGTTACCATTGAGGTTG		
			Reverse	ACAGTCCAAAGAAAAACAAATAAGAAACTGG		
A03_SNP_4440	A	G	Forward-1	GGAAGCACATAGCCGGCGATA	206	23,998,780
			Forward-2	GGAAGCACATAGCCGGCGATG		
			Reverse	AAGATCATGGACTGCAAGAGGACT		
A03_SNP_4441	T	G	Forward-1	AGTTCTCCCCCCTCGCCT	143	24,004,501
			Forward-2	AGTTCTCCCCCCTCGCCG		
			Reverse	GTCGGAGCTCGATTCCTCTTTA		
A03_SNP_4442	G	C	Forward-1	AAGTCCTCTTGCAGTCCATGATCTTG	218	23,998,966
			Forward-2	AAGTCCTCTTGCAGTCCATGATCTTC		
			Reverse	CACGCGCCACTCCTTCACG		
A03_SNP_4443	C	T	Forward-1	TAGAAGAGTAATTAACTTATGTGTTTGCTTTTGTAAC	156	5,902,711
			Forward-2	TAGAAGAGTAATTAACTTATGTGTTTGCTTTTGTAAT		
			Reverse	TCACAAATCATTGAAACCGCAAACCTCT		
A03_SNP_4444	A	G	Forward-1	AATAACAAACAATAACCTA	123	5,287,675
			Forward-2	AATAACAAACAATAACCTG		
			Reverse	ACCAACCTATTGGCGTCTCTTCAC		
A03_SNP_4445	A	T	Forward-1	GGGAGTCGTTGACTTTGGTTCTTCA	258	5,294,966
			Forward-2	GGGAGTCGTTGACTTTGGTTCTTCT		
			Reverse	AGTTTTCACAAATCATTGAAACCGCAAAC		
A03_SNP_4446	G	C	Forward-1	ATCCGATTATCTGTTGAATCAGATTATGTATTG	290	4,784,561
			Forward-2	ATCCGATTATCTGTTGAATCAGATTATGTATTC		
			Reverse	ACTAGGCATTGTGATTCTGGAGCAAA		
A03_SNP_4447	C	G	Forward-1	CGAGGTAGAGCGATGTGGTAAAAAC	122	4,776,843
			Forward-2	CGAGGTAGAGCGATGTGGTAAAAAG		
			Reverse	CGAATCCGGCAGTGCGTGCT		
A03_SNP_4448	T	C	Forward-1	AATCAGATGTGTACAGCTTTGGAGTT	136	5,064,462
			Forward-2	AATCAGATGTGTACAGCTTTGGAGTC		
			Reverse	TGCAGCGTCGGATCTATGGCA		
A03_SNP_4449	T	A	Forward-1	GCTCAACAAGCTAGACGGAGACATT	267	5,063,490
			Forward-2	GCTCAACAAGCTAGACGGAGACATA		
			Reverse	CCAACAAGTAAAAACGCCATGGAGAT		
A03_SNP_4450	C	T	Forward-1	AACTTCGTCTCGCTCCAGACCCTC	148	5,062,806
			Forward-2	AACTTCGTCTCGCTCCAGACCCTT		
			Reverse	AATTGAGAAGTCCCGGAGGGAG		
A03_SNP_4451	C	T	Forward-1	CAAATGAGTTCTTCTTCACCCAAAGC	146	5,062,517
			Forward-2	CAAATGAGTTCTTCTTCACCCAAAGT		
			Reverse	ATGGTGTTGTCGGGGATGGG		
A03_SNP_4452	T	A	Forward-1	AATATTACCTAGCTACAGAAGATAACCGTT	141	5,037,842
			Forward-2	AATATTACCTAGCTACAGAAGATAACCGTA		
			Reverse	ATAGGCGAGTTTGTACCAACATCAC		
A03_SNP_4453	A	G	Forward-1	ATTTTCTCATCTCAACCTATTTCATTTAACCA	143	4,799,569
			Forward-2	ATTTTCTCATCTCAACCTATTTCATTTAACCG		
			Reverse	AATCGCGTGCAGCTGTAACACCAAT		
A03_SNP_4454	C	A	Forward-1	CCCGACATGTTTTTTTCCGATTGAGC	162	4,778,219
			Forward-2	CCCGACATGTTTTTTTCCGATTGAGA		
			Reverse	CTGAGCCGTTAGATCTACTACTCGTT		
A03_SNP_4455	A	G	Forward-1	ACCCTTTCCTCCCTTTCCACGA	170	4,778,193
			Forward-2	ACCCTTTCCTCCCTTTCCACGG		
			Reverse	CTACTACTCGTTCTGATTTCTCAAATTACAAG		
A03_SNP_4456	C	T	Forward-1	TCTAGTTTCCAAATCTTTTGACCAACGC	195	4,311,669
			Forward-2	TCTAGTTTCCAAATCTTTTGACCAACGT		
			Reverse	CAGTGAACGTACAAAACATTCCAAGAGAT		
A03_SNP_4457	C	T	Forward-1	GACTCGTTGTACTTGATTACATCTAGTTTATC	244	4,313,100
			Forward-2	GACTCGTTGTACTTGATTACATCTAGTTTATT		
			Reverse	GCCAAAAGATGTGATAACTTACAAATACTGTAAAT		
A03_SNP_4458	A	C	Forward-1	TTGCTGTAATGTAATGATCGTGTGTGA	252	4,314,467
			Forward-2	TTGCTGTAATGTAATGATCGTGTGTGC		
			Reverse	CATATGAATTTGTTTATAGCAACAATCATGAA		

Table S4.2: Genetic markers linked to previously reported clubroot resistance loci

Primer Name	Primer	Sequences (5'-3')	Product size(bp)	Marker type	Associated with	Source
GC1250-1	Forward	GATCTAGTTTATTATTATTATTGTATCAGG		SCAR	*CRa*	Ueno et al. 2012. DOI:10.1007/s11103-012-9971-5
	Reverse	GAAGTTTAGGTGCACTATCCACTAAA				
GC1250-2	Forward	AATGATCAGAGAGGCAAAACAGAA	650	SCAR	*CRa*	
	Reverse	CAATGATAATCTTACACTAATTAATACAGA				
GC2360-1	Forward	CAGCACCAGCATAACCAGCTACAGTC	906	SCAR	*CRa*	
	Reverse	AGAACTTTGCAAGTGGCTCAGATAAT				
GC2360-2	Forward	AGTTTTGTAATTTTCACCCAAAGTATCA	98	SCAR	*CRa*	
	Reverse	CAGCTGGAGGAGCACTGCAACGGAGAGA				
Cra	Forward	TGAAGAATGCGGGCTACGTCCTCTGAAATC		SCAR	*CRa*	
	Reverse	AGTATCTGAACCGAAGCCCAACTAA				
CRaim-T	Forward	TATATTAATGATAAAGCAGAAGAAGAAA		SCAR	*CRa*	
	Reverse	AATGCGACTGAGAAAGTTGTAG				
craim-Q	Forward	TGAAGAATGCGGGCTACGTCCTCTGAAATC		SCAR	*CRa*	
	Reverse	GAAGTAGATGAACGTGTTTATTTTAGAAA				
KB69N08	Forward	TTACACGGTCCATGAAAAGAT	191	SSR	*CRb*	Kato et al. 2013. DOI:10.1270/jsbbs.63.116
	Reverse	GTTTGGTTACCGAAACAGAAGGAA				
KB69N05	Forward	TCACAACCAAAATGGAATGAC	218	SSR	*CRb*	
	Reverse	GTTTCTCAAGCACCGAGACTCATAA				
KB59N08	Forward	GCATCTTGCAAATTATTTACGTT	204	SSR	*CRb*	
	Reverse	GTTTGACCGTGTTATTGTTGTAGGG				
KB59N07	Forward	ATGTACTCGGGTGTCCCCTAGA	160	SSR	*CRb*	
	Reverse	GTTTGACACGATGAACCAGAC				
KB59N06	Forward	TGAAATTGCAACTCTCAAAATG	211	SSR	*CRb*	
	Reverse	GTTTAGGCTTTCTCCATCAACCACTA				
KB59N05	Forward	AGTCAACGAAACAAAGATATGC	175	SSR	*CRb*	
	Reverse	GTTTCTTTTTCTCCACAAAAGGAGAGC				
KB59N03	Forward	AGGTAAATCCTCAAAAAGCCAT	187	SSR	*CRb*	
	Reverse	GTTTGGCGAAATTCAGTTGACA				
B4701	Forward	AGATTCTTGTTCTTCTCGCTGG	187	Indel	*CRb*	
	Reverse	GTTTACGGAGACCATGAAGGATAATG				
B4732	Forward	ATCTGATGTACCTTTGTGCTGG	226	Indel	*CRb*	
	Reverse	GTTTGTCAATCATTCAAGCTAAGTGG				
B1321	Forward	AGTGAAACAGACTATGATTAATGTTTT	135	Indel	*CRb*	
	Reverse	GTTTCAACTTTGGTATAAGCGTTGAGA				
B1324	Forward	ATAATGGCTTCAAATAGTCAAAA	206	Indel	*CRb*	
	Reverse	GTTTGCATATACACGTTGAGGAAAC				
B1210	Forward	ATTGAAAAGTTGACTCCGTTGA	169	SSR	*CRb*	
	Reverse	GTTTCTTCCTTGAAGTTGCTTCAGCTCTTC				
B1005	Forward	AGGAAGTTGTGGTGTTTTGAA	241	Indel	*CRb*	
	Reverse	GTTTATATCCTCGATCATGGCAGC				
B0902	Forward	AGCCTTGCGTAAAAGCAACTAC	160	SSR	*CRb*	
	Reverse	GTTTGGAATCCGACAAATACATCCAT				
BGA01	Forward	TCTGACTGTTTGTGAAAGCGA	213	SSR	*CRb*	
	Reverse	GTTTAGAGTTTTTGGGTGCAAATGTT				
BGA02	Forward	CAAATTCACAAGTCTTCCTCC	109	SSR	*CRb*	
	Reverse	GTTTACATGCAATTGATGGGAAAA				
BGA06	Forward	GAAATAGCAAAGCTCAAACGG	211	SSR	*CRb*	
	Reverse	GTTTCCAGAAAAGAGATGCAGACAA				
BGA10	Forward	GATTACAAAATTTTCAAAGTGAGA	277	SSR	*CRb*	
	Reverse	GTTTCTCACACTTTCCTTAAATAAAAGCTA				
BGA12	Forward	CCCCCTCTCTTTCCTACTTTC	182	SSR	*CRb*	
	Reverse	GTTTCATTCATTGGTCATAAGGCAA				
BGA15	Forward	CCAAAAACATCAGCTTTCGTA	204	SSR	*CRb*	
	Reverse	GTTTCAGAATTCTTTATGAATAGGTTGC				
BGB29	Forward	TTTCGCTCTACACTTTTCCCC	248	SSR	*CRb*	
	Reverse	GTTTGTTTCTGAGGAGGCTCATT				
BGB32	Forward	TAGATCAACTCCATTACCGCT	263	SSR	*CRb*	
	Reverse	GTTTCCAAGCAAAATATCTACCAGCC				
BGB36	Forward	GCTAACATTGCAGACTTTGCT	195	SSR	*CRb*	
	Reverse	GTTTGATAACCATGCTGTAGCGAG				
BGB41	Forward	TCGCATAAACTAATAAAAATCAAAA	153	SSR	*CRb*	
	Reverse	GTTTGACCCACATGATTAACAA				
KB29N19	Forward	TGAGATCGTCAGCCATTTCTC	240	SSR	*CRb*	
	Reverse	GTTTCCAGTCCGGTTTTTATTACCTT				
KB29N17	Forward	CAGCTCCTTTTTAGGTAACGA	253	Indel	*CRb*	
	Reverse	GTTTGGATTGCAAGTGTTATTTCCA				
KB29N16	Forward	GACTCGACAAGGTATCGATCT	214	Indel	*CRb*	
	Reverse	GTTTGACGCCATTATGACACAACT				
KB29N11	Forward	CTCTCCACCAACACTTCCTAA	152	SSR	*CRb*	
	Reverse	GTTTGAAGCTATCTTAGACCACC				

Table S4.2

Primer Name	Primer	Sequences (5'-3')	Product size(bp)	Marker type	Associated with	Source
KB29N05	Forward	TACAAGCTCTCAGAGGAGGAA	148	SSR	*CRb*	Kato et al. 2013. DOI:10.1270/jsbbs.63.116
	Reverse	GTTTCAGCTTGACACTCTTGACTTGC				
KB91N13	Forward	GACGGAGACTTTGAGATCTGG	211	SSR	*CRb*	
	Reverse	GTTTCGAGTACTTCCAGAAACACG				
KB91N06	Forward	GAATTTCCTTGTTAGCCAAAT	225	SSR	*CRb*	
	Reverse	GTTTGTGTTTGTTCATTTTCTATTTCAGA				
KB91N03	Forward	TAATCATCGCCACAGATAAGA	228	SSR	*CRb*	
	Reverse	GTTTGTCTCGCATTTTTGGTTTATG				
sR6340I	Forward	ATAGTTGGGAATGTGGCTGC	176	SSR	*Rcr1*	Chu et al. (2014): DOI: 10.1186/1471-2164-15-1166
	Reverse	CGGACACGAAATCAAACCTT				
sN8591	Forward	TTGTGGGCAGGAACAATACA	231	SSR	*Rcr1*	
	Reverse	CTGGACGAGCCAAGCTAATC				
A03_12776	Forward	TTGGCGAAATTCAGTTGACA	183	SSR		Hobson and Rahman (2016); DOI: 10.1139/cjps-2015-0250
	Reverse	CTCAAAAAGCCATCACCACC				
A03_12778	Forward	AGAGCAAGTGGCTTTGGAGA	262	SSR		
	Reverse	TGGAAAAGACATCAACCACG				
A03_12779	Forward	TGGAACCTCCAAAATCTCTAAAA	259	SSR		
	Reverse	CAAGATAAAATTGTCGAAATCAAGTG				
A03_12781	Forward	CGTATATTTTAAAACTGGCCGA	340	SSR		
	Reverse	TTGGCTGATCTAATGGATGG				
A03_12782	Forward	ATGCCCAACTGAAAAGGAAA	342	SSR		
	Reverse	TTATTGATGCCCGAAGTCGT				
A03_12783	Forward	GGCACCTTTCGTCTTTTGTC	331	SSR		
	Reverse	TTCAAAACTTTAAGGTGGTCTCAA				
A03_12785	Forward	CCTGTTCCAGAAATTCAAATCA	305	SSR		
	Reverse	AGTGGGGCTTTGCTTGATAA				
A03_12786	Forward	TGATGTACCTTTGTGCTGGAA	334	SSR		
	Reverse	TGATATTTGGCGAAACCCTC				
A03_12787	Forward	GATTCACGTGCTCGAATGAA	274	SSR		
	Reverse	GGGGAATTCTTAAGGTGGGA				
A03_12788	Forward	GTTGCAGAAACGGGTTTGAT	304	SSR		
	Reverse	GCTCCCTCGTGTTAATGGAA				
A03_12791	Forward	CAATAATTTTTGGCTAATGTGAAA	310	SSR		
	Reverse	AAATTGTGGGTTCGTTTTGG				
A03_12795	Forward	AGCTGACCAACATAGCGAAG	170	SSR		
	Reverse	TGATTAATTAAAGGTTAGTTTTGGTTT				
A03_12797	Forward	CGACACCACTTGGTTGGAAT	350	SSR		
	Reverse	CAGGTTGTTGTCCCTTTGGT				
A03_12798	Forward	GAATGGCAAATGAAAGGGAA	212	SSR		
	Reverse	CGGGTGCAATTTTCCATACT				
A03_12799	Forward	AAAACACAACAAAATCCGGC	316	SSR		
	Reverse	CCTTTGATTTGTGTCCGTCA				
A03_12800	Forward	AAAATTAAACTTTTCGCTTCATAGA	328	SSR		
	Reverse	ACGTCCTCTGGAGAGTTGGA				
A03_12801	Forward	TACTCCCAAATCTGGCTTCG	326	SSR		
	Reverse	TCACACTGGTGACATGCTGA				
A03_1239	Forward	GATGGAGAAGGGCAAAACAA		SSR		
	Reverse	ATGAGGAGGATGGAGCATTG				
A03_1229	Forward	GTCCAAATATGCCATCCCAC		SSR		
	Reverse	AGACTTTCAAACGCGATGGT				

Table S4.2:

Primer Name	Primer	Sequences (5'-3')	Product size(bp)	Marker type	Associated with	Source
SNP_A03_67	SNP_A03_67Fam	GGCAATCTATAAAGTCAGCGAAGCC		KASP	*Rcr2*	Huang et al. (2017); DOI: 10.3389/fpls.2017.01448
	SNP_A03_67Hex	GGCAATCTATAAAGTCAGCGAAGCT				
	SNP_A03_67Re	GCAGATGTGAGCTCTCGGATCTCCTT				
SNP_A03_11	SNP-A03_11Fam	AGTAAGCCATATCCTCACAGCATAG		KASP	*Rcr2*	Used only for BLAST alignment
	SNP-A03_11Hex	CAGTAAGCCATATCCTCACAGCATAT				
	SNP-A03_1Re	GAGGGCAGACATGTCACGGGAT				
SNP_A03_09	SNP-A03_09Fam	AGAACTTGCCTGGGAGGTTACAAAA		KASP	*Rcr2*	
	SNP-A03_09Hex	AGAACTTGCCTGGGAGGTTACAAAT				
	SNP-A03_09Re	AGATCCCATAACCCTCAGTCCCAAA				
SNP_A03_08	SNP-A03_08Fam	GAGACAAAGCAGATGGAGTTGATGAA		KASP	*Rcr2*	
	SNP-A03_08Hex	GAGACAAAGCAGATGGAGTTGATGAT				
	SNP-A03_08Re	CCACCATCTTCTTTATCTTCTAGGTCTTT				
SNP_A03_13	SNP-A03_13Fam	ACACCCTTCCACAATTTCAAGCGT		KASP	*Rcr2*	
	SNP-A03_13Hex	CACCCTTCCACAATTTCAAGCGC				
	SNP-A03_13Re	CTTCATTTAGCTTGCTTTTTCAACGATGAA				
SNP_A03_19	SNP-A03_19Fam	AAAGCTCCAAACATCGTTCCCTTCA		KASP	*Rcr2*	
	SNP-A03_19Hex	AAAGCTCCAAACATCGTTCCCTTCT				
	SNP-A03_19Re	GTGAAGTGGAACCCCGTTGCGAA				
SNP_A03_32	SNP-A03_32Fam	AGCTCCTCAAAGTCTTCCACT		KASP	*Rcr2*	
	SNP-A03_32Hex	CTAGCTCCTCAAAGTCTTCCACG				
	SNP-A03_32Re	AACAGAGATTAGAGAGAAAGTAGATGTGAT				
SNP_ A03_100	SNP_A03_100FAM	TGGCCTTATTTGGATTCCTGCATTTG		KASP	*Rcr5*	Huang et al. (2019): DOI: 10.1007/s11032-019-1038-8 Used only for BLAST alignment
	SNP_A03_100HEX	TGGCCTTATTTGGATTCCTGCATTTA				
	SNP_A03_100Re	GTTTACAGGGAGAATCTGTGGAAGAGCGT				
SNP_A03_83	SNP_A03_83FAM	GGATGGAGGTCTATTTACTGTATGCCAT		KASP	*Rcr5*	
	SNP_A03_83HEX	GGATGGAGGTCTATTTACTGTATGCCAC				
	SNP_A03_83Re	CAGATAGATTGATAGGAGCTGACCCT				

5. Chapter 5: Resynthesis of *Brassica juncea* for resistance to *Plasmodiophora brassicae* pathotype 3 [5]

5.1. Introduction

Brassica juncea (AABB genome; 2*n* = 36) canola, developed from oriental mustard *B. juncea*, is a new oilseed crop in Canada (Woods et al. 1991; Potts et al. 1999). *Brassica juncea* oilseed crop carry many desired traits, such as tolerance to heat (Gunasekera et al. 2006), drought (Wright et al. 1995) and silique shatter (Wang et al. 2007), and resistance to blackleg (Roy 1978) and leaf blight disease (Wechter et al. 2007). Its seed is yellow, which is generally associated with less pigmented coat and reduced fibre content in seed meal (Rashid et al. 1994; Rahman 2001). This crop species yields greater than *B. napus* in heat- and drought-prone areas and in short growing season areas (Burton et al. 1999, Potts et al. 1999). Although *B. juncea* possesses all these desired properties, resistance to *Plasmodiophora brassicae* Woronin, causing clubroot disease, is not available yet (reviewed by Diederichsen et al. 2009; Hasan et al. 2012). Clubroot disease can result in up to 90% yield loss and about 4 - 6% decrease in seed oil content (Pageau et al. 2006).

Resistance to clubroot disease was reported in the two parental species of *B. juncea*, *viz.*, *B rapa* (AA; 2*n* = 20) and *B. nigra* (BB; 2*n* = 16). Turnip type (*B. rapa* ssp. *rapifera*) is the most widely reported form of CR resistant *B. rapa* (Buczacki et al. 1975; Hasan et al. 2012). To date, at least eight clubroot resistance genes were mapped on five chromosomes *viz.*, R1 (≈A01), R2 (≈A02), R3 (≈A03), R6 (≈A06) and R8 (≈A08) (http://www.brassica.info/) in *B. rapa* ssp. *rapifera* (Kuginuki et al. 1997; Matsumoto et al. 1998; Suwabe et al. 2003; Hirai et al. 2004; Piao et al. 2004, 2009; Hayashida et al. 2008). Turnips were used to resynthesize

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[5] A version of this chapter has been published as
Hasan M.J., Rahman H. 2018. Resynthesis of *Brassica juncea* for resistance in *Plasmodiophora brassicae* pathotype 3. Breeding Science **68**(3): 385–391. doi: 10.1270/jsbbs.18010

clubroot resistant polyploid *B. napus* lines and cultivars (Diederichsen and Sacristan 1996). No one has reported the development of clubroot resistant *B. juncea* from its diploid parents yet.

The objective of the present study was to develop a clubroot resistant *B. juncea* line using a resistant *B. rapa* line and to investigate the stability of this resistance in the resynthesized *B. juncea* line.

5.2. Materials and methods

5.2.1. Plant materials

A *B. rapa* line, homozygous for resistance to *P. brassicae* pathotype 3, was used as the female in the interspecific crosses with two susceptible *B. nigra* (BB, 2*n* = 16) accessions CR 2136 and CR 2137 as male. The *B. rapa* line was developed through self-pollination of the *B. rapa* ssp. *rapifera* cv. Gelria (AA; 2*n* = 20). The cv. Gelria carries the clubroot resistance gene *CRa* (Matsumoto et al. 1998; Ueno et al. 2012) and *CRb* (Piao et al. 2004, 2009). The *CRa* and *CRb* genes are located in the same genomic region of chromosome A03 and considered the same gene or clustered together (Kato *et al*. 2013). Seeds of Gelria were obtained from the Green Gene International, Hill Castles, United Kingdom, and the *B. nigra* accessions were obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany. The inbred *B. rapa* line and the *B. nigra* accession CR 2136 were self-incompatible, while the *B. nigra* accession CR 2137 was found to be self-compatible.

5.2.2. Ovule culture

The interspecific cross-derived hybrid ovules were cultured *in vitro* following the technique described by Bennett et al. (2008). For this, the interspecific cross-pollinated siliques at the age of 10 days after pollination (DAP) were harvested, surface sterilized with 7% (w/v) calcium hypochlorite [$Ca(OCl)_2$] solution for 15 minutes and rinsed three times with sterile distilled water. Sterilized siliques were dissected longitudinally, and the fertilized ovules (developed to standard size) were excised, and a small incision was made at the non-micropylar

end before culturing (Ripley and Beversdorf 2003) in a 60 mm x 15 mm size sterile Petri-plate. The liquid medium was composed of Nitsch and Nitsch (1967) medium (*i.e.*, NN13 medium) supplemented with 300 mg/l casein hydrolysate, 200 mg/l glutamine, and 13% sucrose. The medium was adjusted to pH 6.0 and filter sterilized. The Petri dishes containing the cultured ovules were sealed and placed on a shaker set at 90 rpm. After 2–3 weeks on the shaker, the number of torpedo embryos was recorded.

The torpedo-shaped embryos were transferred to solid B5 medium containing 0.1 mg/L GA3, 20 g/L sucrose and 8 g/L agar (Coventry et al. 1988) in a Petri dish (100 x 15 mm) and sealed with parafilm. The Petri dishes were placed at 4°C under the light of 8 h photoperiod for 24 hours and then moved to room temperature (22–25°C) under light [photosynthetic flux density of 30 E (mV) $m^{-2}s^{-1}$ at plant level] of 12 h photoperiod. Embryos were kept on the solid medium for 3–4 weeks until germinated, and roots developed. The plantlets were transplanted to six-inch pots containing soil-free growth medium (Stringam 1971) and placed in a growth chamber (15°/10°C day/night temperature; 16 h photoperiod). The newly transplanted seedlings (S_0) were covered with transparent plastic tubes for three to four days for hardening.

5.2.3. Confirmation of interspecific hybrid

A set of Brassica A- and B-genome linkage group (LG) specific simple sequence repeat (SSR, microsatellite) DNA markers were used to confirm the interspecific hybrid nature of the plants. For this, a total of 29 SSR markers specific to the 10 A-genome linkage groups (A01 to A10) and 36 markers from the eight B-genome linkage groups (B1 to B8) were used. Details of DNA extraction and PCR amplification of the SSR markers were described elsewhere (Hasan and Rahman 2016).

5.2.4. Chromosome doubling and generation of resynthesized *B. juncea* lines

The S_0 plantlets identified as *B. rapa* × *B. nigra* interspecific hybrid were treated with a 0.34% (w/v) aqueous solution of colchicine for chromosome doubling. For this, 25-30 cm tall

plantlets were removed from the pot, and the roots were washed thoroughly under tap water to remove soil and dipped in colchicine solution for 1 h. After treatment, roots were washed thoroughly to remove the trace of colchicine. The plantlets were transplanted to six-inch pots containing Sunshine soil mixture and placed in a growth chamber (18/12 ^{0}C day/night temperature; 16 h photoperiod).

The chromosome-doubled fertile S_0 plants were self-pollinated by treating the bud with a 5% NaCl solution (Tingdong et al. 1992) followed by bag isolation for S_1 seeds. The S_1 families were grown in a glasshouse, self-pollinated by bag isolation, and S_2 seeds were harvested.

5.2.5. Assessment of ploidy level

The ploidy level of the S_2 generation resynthesized *B. juncea* lines and their diploid parents were determined through flow cytometric analysis for nuclear DNA content. One canola quality *B. juncea* breeding line from the Canola Breeding Program of the University of Alberta was used as a reference. For this, approx. 100 mg freshly collected leaf tissue from each plant was chopped with a new single-edged razor blade in a Petri dish containing 1.0 ml ice-cold Partec buffer (DeLaat et al. 1987) for 2 to 3 min at a rate of about 5 chops/sec to release the nuclei. The sample was filtered through a 42-µm-nylon mesh to remove large debris. The nuclear DNA was stained by adding 200-µl of reagent mixture containing 4,6-diamidino-2-phenylindole (DAPI) dye and RNase (19:1 ratio) to the filtered sample. DAPI is a fluorescent stain that binds strongly to A-T-rich regions in DNA; the addition of RNase in the sample degrades RNA and thereby prevents the binding of DAPI to them.

The fluorescent-stained nuclear DNA was analyzed by using an LED (UV band) based CyFlow® Ploidy Analyzer (www.partec.com). The ultra-violet (UV) light of the instrument was adjusted at 365 nm wavelength, and the sample was run at a rate of 20 to 50 nuclei/sec. Data was acquired for a total of 1500 to 2500 nuclei per sample. The DNA ploidy of the samples was calculated by using the following equation (Dolezel et al. 2007);

$$\text{Sample ploidy (integer)} = \frac{\text{Mean position of the G1 sample peak}}{\text{Mean position of the G1 reference peak}} \times \text{Reference ploidy}$$

5.2.6. Evaluation for clubroot resistance

5.2.7. Pathogen isolate:

The resynthesized *B. juncea* lines (S_1 and S_2 plants) and their diploid parental species were evaluated for resistance to the single-spore derived isolates of *P. brassicae*, classified as pathotype 3 based on William's (1966) differentials or as pathotype H based on Canadian Clubroot Differential (Strelkov et al. 2018). Resting spore suspension (inoculums) was prepared from the preserved gall following the protocol described by Strelkov et al. (2007). The concentration of the suspension was adjusted to 10^7 to 10^8 resting spores per ml inoculum.

5.2.8. Resistance test

Seedlings germinated on moistened Whatman filter paper No. 1 were inoculated by dipping the roots in resting spore suspension (Nieuwhof and Wiering 1961). The inoculated seedlings were planted in 3 × 3 × 5 cm (L × W × D) cells filled with Sunshine Professional Natural and Organic Mix # 4 (Sungro Horticulture). The cells were placed in a tray (2 × 4 m) and were grown in a greenhouse at 21 ± 2 °C temperature with a 16-hr photoperiod. After transplanting, 2 mL inoculum was pipetted to each cell to ensure successful inoculation. During the first seven days, the cells were kept saturated with water to ensure sufficient moisture for the development of the pathogen. During this time, HCl solution (10% v/v) @ 20 mL/tray (2 × 4 m) was added each day to ensure acidic soil condition. From the second week, watering was done once a day. Seedlings were evaluated for clubroot resistance at 42 to 45 days after inoculation, and the severity of gall development was rated on a 0 to 3 scale, where 0 = no galling, 1 = one or few tiny galls on lateral roots, 2 = moderate galling on lateral roots, and 3 = severe galling on the lateral or main root.

5.3. Results

5.3.1. Production of resynthesized *Brassica juncea*

A total of 43 interspecific crosses were made, which gave 14 silique carrying fertilized ovules (Table 5.1). Only five siliques of the *B. rapa* cv. Gelria × *B. nigra* CR 2136 cross yielded 34 fertilized ovules, and this translated to 6.8 fertilized ovules/silique. Fifteen (44.1%) of the 34 cultured ovules yielded zygotic embryos, of which 13 (38.2%) grew into plantlets. On the other hand, nine siliques of the *B. rapa* cv. Gelria × *B. nigra* CR 2137 cross yielded 56 ovules translating to 6.2 fertilized ovules/silique; only 21 (37.5%) of the 56 ovules yielded zygotic embryos, of which 17 (30.4%) developed into the plant. All 30 plantlets obtained from the two crosses were treated with colchicine; however, only two (6.67%) plants of *B. rapa* cv. Gelria × *B. nigra* CR 2137 became amphidiploid (AABB). These plants produced fertile pollen and viable seed under self-pollination (Table 5.1). Single silique from each of the two S_0 plants, 1578.001 and 1578.002, produced 13 and seven S_1 seeds, respectively. A total of eight and seven S_1 plants, respectively, of 1578.001 and 1578.002 were grown in a glasshouse, of which three of 1578.001 and four of 1578.002 were self-pollinated by bag isolation for S_2 seeds.

5.3.2. Molecular characterization of resynthesized *Brassica juncea* lines

The interspecific hybrid nature of the S_0 plants was confirmed using SSR markers. For this, 190 SSR markers from the 10 A-genome chromosomes (A01 to A10) were screened, of which 29 showed explicit polymorphism between the A and B genome parental species (Table 5.2). Of the 29 polymorphic markers, 15 amplified the expected alleles in *B. rapa* and showed no amplification in *B. nigra*; these 15 markers also amplified similar size alleles in the resynthesized *B. juncea* plants. The other 14 markers amplified alleles both in *B. rapa* and *B. nigra*, and similar size alleles were also detected in the resynthesized *B. juncea* plants. Based on this marker analysis, it can be anticipated that all 10 A-genome chromosomes of *B. rapa* were present in the resynthesized *B. juncea* plants.

A total of 48 B-genome specific (chromosome B1 to B8) SSR markers were also tested on the two parents; 36 of them amplified alleles only in *B. nigra* parent and in the resynthesized *B. juncea* lines (Table 5.3). This marker analysis confirmed all eight B genome chromosomes of *B. nigra* in the resynthesized *B. juncea* lines.

5.3.3. Ploidy assessment of synthetic amphidiploid

A total of 36 plants belonging to seven S_2 families were analyzed for nuclear DNA content to determine their ploidy level. Of the seven S_2 families, three derived from the S_1 line 1578.001 showed a mean ploidy level of 4.10±0.218, similar to the natural *B. juncea* (Table 5.4). On the other hand, the mean ploidy level of the four S_2 families, derived from the S_1 line 1578.002, was 4.44±0.119, indicating the occurrence of plants with higher chromosome numbers in their population.

5.3.4. Resistance to *Plasmodiophora brassicae*

A total of 15 S_1 plants derived from the two resynthesized *B. juncea* lines (1578.001 and 1578.002) were evaluated for resistance to *P. brassicae* pathotype 3. All 15 plants were completely resistant (disease score 0) to this pathotype. Seven of the 15 S_1 plants were self-pollinated by bag isolation for S_2 seeds. The S_1 plants showed wide variation for seed set − ranging from as low as 30 seed per plant to as high as 515 seed per plant.

A total of 103 plants from seven S_2 families were evaluated against this pathotype. All S_2 plants belonging to three S_2 families of 1578.003, 1578.005 and 1578.008, derived from S_1 family 1578.001, were resistant. However, only 87 to 94% of S_2 plants belonging to the four S_2 families, 1578.004, 1578.006, 1578.007 and 1578.009, derived from the S_1 family 1578.002 were resistant to this pathotype. Thus, resistance was lost in about 6 to 13% of the S_2 plants of these four families during their development through self-pollination (Table 5.5). No significant correlation between seed set on the S1 plants and clubroot resistance in the S_2 families could be found (r = - 0.523, R^2 =0.274; df =5, p <0.05).

5.4. Discussion

The present study demonstrated that a clubroot resistant *B. juncea* line in the S_2 generation could be achieved through the resynthesis of this species by exploiting the resistance available in one of the parental species, *B. rapa*. Theoretically, the allopolyploid resynthesized B. juncea lines were assumed to be homozygous, and the resistance was expected to be inherited stably through the self-pollinated generation. However, loss of resistance occurred in some of the S_2 plants obtained from these experiments (Table 5.5). Several researchers have reported that chromosomes in the resynthesized *Brassica* allopolyploids can undergo meiotic anomalies and homoeologous pairing in their early generations, and this can result in some structural rearrangements, including loss or gain of chromosomes (Udall et al. 2005; Gaeta et al. 2007; Gaeta and Chris Pires 2010; Szadkowski et al. 2011; Xiong et al. 2011). The mechanisms driving the change in chromosome number and structure in the newly formed polyploid is not well understood; this may result from the downsizing of nuclear DNA content, inter- and intra-genomic rearrangements, chromosome breakage and fusion, rDNA change, and loss of repeat sequences (Liu et al. 1998; Leitch and Bennett 2004; Friberg 2005; Han et al. 2005; Xiong et al. 2011; Renny-Byfield et al. 2013; Renny-Byfield and Wendel 2014). According to Xiong et al. (2011), chromosome number in the self-pollinated progeny of a resynthesized *B. napus* ($2n = 38$) plant can vary from $2n = 36$ to 42; in this regard, the occurrence of higher nuclear DNA content in S_2 progeny of the resynthesized *B. juncea* plant 1578.002 agree with the result reported by Xiong et al. (2011). In addition to chromosomal change, allopolyploids can also exhibit a change in gene expression (reviewed by Adams and Wendel 2005; Chen and Ni 2006), which can cause a change in the phenotype. Salmon et al. (2005) found DNA methylation in about 30% of the parental fragments in the allopolyploids of *Spartina spp.* Structural rearrangement of chromosomes in resynthesized *B. napus* can also contribute to the variation of quantitative traits, such as flowering time (Pires et al. 2004). In the case of qualitative traits,

such as self-incompatibility (Rahman 2005) and clubroot resistance (Diederichsen and Sacristan 1996), the stability of the trait has often been seen in the self-pollinated progeny of a resynthesized *B. napus* plant. The clubroot resistance in the resynthesized *B. juncea* lines developed in this research is derived from the *B. rapa* cv. Gelria. Two major clubroot resistance genes, *CRa* and *CRb*, were identified in this cultivar; which of the two genes conferred resistance in these resynthesized *B. juncea* lines, and the reason for the loss of resistance in some of the S_2 plants was beyond the scope of the present study. The loss of resistance might have resulted from the loss of the genomic region carrying the resistance; a further investigation would be needed to resolve this.

The resynthesized *B. juncea* lines obtained in this study showed wide variation for seed set under self-pollination. Poor seed set in a resynthesized allopolyploid is a common phenomenon, especially in their early generations, as reported by Srivastava et al. (2004) in *B. juncea*. Meiotic anomalies in the resynthesized *Brassica* allopolyploids, as discussed above, can result in reduced pollen viability and thus reduced seed set (Ramsey and Schemske 2002). Xiong et al. (2011) found an inverse correlation of seed yield and pollen viability with the increased aneuploidy; they observed the highest fertility in the resynthesized *B. napus* lines carrying the parental chromosomes with a minor change. Self-incompatibility of the parental species may also have contributed to this reduced seed set under self-pollination in the resynthesized *B. juncea* lines developed in this study. Rahman (2005) also reported the effect of the self-incompatibility genes on reduced seed set in resynthesized *B. napus*.

5.5. Tables

Table 5.1: Resynthesis of *Brassica juncea* (AABB, 2*n* = 36) though *in vitro* culture of ovules of *Brassica rapa*(AA, 2*n* = 20) × *Brassica nigra* (BB, 2*n* = 16) interspecific cross

Female	Male	No. pollination	No. silique formed	No. ovule cultured	No. zygotic embryo developed	No. plants transferred	No. synthetic plant obtained	Plant ID
B. rapa ssp. *rapifera* cv. Gelria, p1	*B. nigra* (CR 2136), p1	9	3	20	9	9	0	
B. rapa ssp. *rapifera* cv. Gelria, p3	*B. nigra* (CR 2136), p3	8	2	14	6	4	0	
B. rapa ssp. *rapifera* cv. Gelria, p1	*B. nigra* (CR 2137), p1	11	5	26	13	11	1	1578.001
B. rapa ssp. *rapifera* cv. Gelria, p1	*B. nigra* (CR 2137), p2	15	4	30	8	6	1	1578.002
Total		43	14	90	36	30	2	

Table 5.2: Evaluation of the resynthesized *Brassica juncea* lines by SSR (microsatellite) markers from the ten A-genome linkage groups, including those specific to the A genome of *Brassica rapa*.

Linkage group (LG)	Total no. marker tested	No. marker polymorphic between diploid parents	Primer name	Allele size (bp) in		
				B. rapa ssp. *rapifera* cv. Gelria (AA genome)	*B. nigra* (CR2137) (BB genome)	Resynthesized *B. juncea* (AABB genome)
A01	22	4	sNRA51nm	198	-	198
			sS2136b	123	138	123, 138
			sN11665	276	272	272, 276
			sN11824 (aNP)	384	-	384
A02	24	3	sR12095	349	-	351
			sORE27 (aNP)	213, 239	239	213, 239
			BrSTS-78	158	162	158, 162
A03	15	3	sNRA85	133	162	133, 162
			sN1087(cNP)	471	-	471
			BoGMS1587	282	-	288
A04	31	2	sN2025	155	138	138, 155
			Na12-A01C	135	-	135
A05	15	3	Na10E02	155	-	155
			CB10080	133, 140	146	140, 146
			CB10545	96	-	96
A06	26	4	sN12508II	324	334	324, 334
			sR12156	198	-	198
			sN1958 (bNM)	365	361	365
			sN0904 (a)	234, 247, 255	255	234, 247, 255
A07	14	3	BRAS023	207, 217	-	207, 217
			BnGMS608	158	-	156
			BRMS129	276, 295	276, 284	276, 295
A08	12	2	Na12B05a	191	-	191
			BRMS185	254	-	254
A09	14	3	CB10373A	245	257	245, 257
			Ni4-D09	209	203	203, 209
			BnGMS81	397	-	397
A10	17	2	CB10524	239	-	239
			BRMS244	268	252	252, 268
Total	190	29				

Table 5.3: Evaluation of the resynthesized *Brassica juncea* lines by SSR (microsatellite) markers from the eight B genome linkage groups.

Linkage group (LG)	Total no marker tested	No. markers polymorphic between diploid parents	Primer name	Allele size (bp) in		
				B. rapa ssp. *rapifera* cv. Gelria (AA genome)	*B. nigra* (CR2137) (BB genome)	Resynthesized *B. juncea* (AABB genome)
B1	6	6	sJ3838F	-	289	289
			sJ4933	-	360	360
			sJ84165	-	307	307
			sJ0644	-	457	457
			sJ3891	-	123	123
			sB0563I	-	459	459
B2	6	3	sJ3302RI	-	433	420
			sJ03104	-	405	405
			sB4817R	-	270	270
B3	6	6	sJ3627R	-	308	308
			sB1822	-	282	282
			sB1672	-	208	208
			sJ7046	-	304	304
			sB1990F	-	511	511
			sB1752	-	450	450
B4	6	5	sA0306	-	382	351, 382
			sB0372	-	255	255
			sB214AI	-	401	401
			sB1935A	-	275	275
			sJ8033	-	167	167
B5	6	5	sB3140	-	231	231
			sJ3874I	-	184	184
			sJ6842	-	355	355
			sB2556	-	268	268
			sB3872	-	197	197
B6	6	3	sJ7104	-	346	346
			sJ0338	-	359	359
			sJ0502	-	268	268
B7	6	5	sJ39119I	-	366	366
			sJ13133	-	317	317
			sJ1536	-	231	231
			sB1937	-	280	280
			sJ4633	-	328	328
B8	6	3	sJ34121	-	359	359
			sJ1668I	-	325	325
			sB3739	-	397	397
Total	48	36				

Table 5.4: Ploidy level of the 36 S_2 generations resynthesized *Brassica juncea* plants measured through estimation of nuclear DNA content using a flow cytometer

Family ID	Gen	No. plants tested	Ploidy (Mean ± SE)
Brassica juncea [a]	Inbred	5	4.00 ± 0.083
S_2 derived from 1578.001 (S_1)			
1578.003	S_2	5	4.86 ± 0.254
1578.005	S_2	4	3.41 ± 0.185
1578.008	S_2	4	3.85 ± 0.222
Subtotal		13	4.10 ± 0.218
S_2 derived from 1578.002 (S_1)			
1578.004	S_2	6	4.46 ± 0.069
1578.006	S_2	6	3.93 ± 0.372
1578.007	S_2	4	4.77 ± 0.059
1578.009	S_2	7	4.69 ± 0.097
Subtotal		23	4.44 ± 0.119

[a] Canola quality *Brassica juncea* breeding line from the University of Alberta Canola breeding program

Table 5.5: Resistance in S_1 and S_2 families of resynthesized *Brassica juncea* to *Plasmodiophora brassicae* pathotype 3

Family ID	Gen	No. selfed seed produced	No. plants tested	No. R plant (Score 0)	Number of S plant				Percent resistant plant
					Score 1	Score 2	Score 3	Total S plant	
1578.001	S_1	13	8	8	0	0	0	0	100.0
1578.002	S_1	7	7	7	0	0	0	0	100.0
Subtotal			15	15				0	100.0
S_2 derived from 1578.001									
1578.003	S_2	42	9	9	0	0	0	0	100.0
1578.005	S_2	208	8	8	0	0	0	0	100.0
1578.008	S_2	54	29	29	0	0	0	0	100.0
Subtotal			46	46				0	100.0
S_2 derived from 1578.002									
1578.004	S_2	118	18	17	0	0	1	1	94.4
1578.006	S_2	215	15	13	0	0	2	2	86.7
1578.007	S_2	30	9	8	0	0	1	1	88.9
1578.009	S_2	515	15	13	0	0	2	2	86.7
Subtotal			57	51				6	89.5
Grand Total	S_2		103	97				6	94.2

Note: R = Resistant; S = Susceptible

6. Chapter 6: General discussion

6.1. Discussion

Canola grown in Canada belongs to *Brassica napus* (AACC, 2*n* = 38) and *B. juncea* (AABB, 2*n* = 36). Spring type of *B. napus* canola dominates the industry with more than 98% of the seeded area in Canada. *Brassica juncea* canola is desired in the heat- and drought-prone regions and in short growing season areas for their tolerance to heat (Gunasekera et al. 2006), drought (Wright et al. 1995); this species produce greater yield than *B. napus* canola (Burton et al. 1999, Potts et al. 1999). *Brassica napus* and *B. juncea* evolved through the interspecific hybridization between *B. rapa* (AA, 2*n* = 20) and *B. oleracea* (CC, 2*n* = 18) (Lu et al. 2019) and between *B. rapa* (AA, 2*n* = 20) and *B. nigra* (BB, 2*n* = 16) (U 1935), respectively. Commercially available canola cultivars have a narrow genetic base (Cowling 2007; Fu and Gugel 2010). Evolution of the allopolyploid Brassicas from a limited number of variants of their progenitor species (Chalhoub et al. 2014; Lu et al. 2019), and utilization of zero erucic acid (Downey and Craig 1963; Downey and Harvey 1963) and low glucosinolate (Kondra and Stefansson 1970) genetic materials in repeated cycles of breeding for canola quality traits are some of the reasons for the narrow genetic diversity currently seen in this crop (Cowling 2007; for review, see Rahman 2013; Fu and Gugel 2010). During canola breeding, the undesirable seed constituents, such as erucic acid and glucosinolate contents in seed, have undergone a dramatic reduction, whereas oil content, seed yield, and resistance to diseases have been improved significantly (Knott and Slinkard 1995). Disease resistance generally minimizes the risk of crop failure due to pathogen infestation and, thus, secures crop production. Traditionally, the blackleg disease caused by *Leptosphaeria maculans* and sclerotinia stem rot caused by *Sclerotinia sclerotiorum* were the most important diseases of spring canola. In the last few decades, significant achievements have been made for resistance to blackleg disease (reviewed by Neik et al. 2017; Lv et al. 2020), while sclerotinia stem rot is generally controlled

by spraying fungicides at about 10-50% flowering stage (Bradley et al. 2006). Recently, clubroot disease caused by *P. brassicae* has become a serious threat to canola production in Canada (for review, see (Hwang et al. 2018). Clubroot disease can cause about 30% yield loss in canola (Tewari et al. 2005). Aside from yield loss, this disease decreases seed oil content by about 2-6% (Engqvist 1994; Pageau et al. 2006) and increases the chlorophyll content in seed oil by 50% (Engqvist 1994). Natural oilseed *B. napus* and *B. juncea* lack resistance to clubroot disease (Hasan et al. 2012); therefore, there is a need to introduce resistance to this disease in canola.

Disease resistance loci are often present in unadapted crop germplasm and allied species. In the case of clubroot disease, resistance can be found in the diploid species *B. rapa*, *B. oleracea,* and *B. nigra* (Buczacki et al. 1975; Hasan et al. 2012; Fredua-Agyeman et al. 2019). More than 20 major CR loci and more than 60 QTL have been mapped in the diploid Brassica species, mostly conferring race-specific resistance to *P. brassicae* pathotypes (for review, see Hasan et al. 2021). However, an overestimation of the number of CR loci and QTL cannot be ruled out as these loci have been reported by different researchers and in most cases without using common marker sets. Therefore, naming of the same locus differently by different researchers may have occurred, as has been reported by Kato et al. (2013) and Hatakeyama et al. (2017) the locus *CRa* and *CRb* to be the same.

Nevertheless, only a few of the CR loci, mainly of the A genome of *B. rapa,* have been introgressed into *B. napus* canola (Diederichsen and Sacristan 1996; Frauen 1999; Rahman et al. 2011, 2014b; Hirani et al. 2016). In the case of the amphidiploid species, rutabaga (*B. napus* var. *napobrassica*), a variety of *B. napus*, has been reported to carry resistance to clubroot disease (Karling 1969; Johnston 1970; Ayers and Lelacheur 1972; Buczacki et al. 1975; Hasan et al. 2012; Fredua-Agyeman et al. 2019); it is an important source of CR for use in *B. napus*

canola breeding. The introgression of CR from rutabaga into canola will be much simpler than the introgression of a CR locus from its allied species, such as *B. rapa*.

This thesis research focused on the introgression of rutabaga CR into spring canola and mapping of the resistance using a doubled haploid (DH) population. A doubled haploid (DH) plant is derived from a single microspore where chromosomes of the haploid plants are doubled artificially to get a homozygous diploid plant or line. Therefore, homologous chromosomes of a DH are identical at each locus, and thus, the DH method permits fixing a gene or genes from crosses with wild relatives in a single generation (Cao et al. 2016). In this case, the same DH line can be assessed for resistance to different pathotypes and phenotyping of agronomic and seed quality traits by testing in multiple environments. This was why a DH population was used in this study compared to using a recombinant inbred line (RIL) or other pedigree populations, such as F_2 or backcross (BC). In the DH method, the population passes through a single cycle of meiosis; therefore, the DH lines result from fewer crossovers and, theoretically, contain larger chromosomal blocks than the RILs. In this regard, using a RIL population would provide a greater resolution in genetic mapping; however, the development of a RIL population requires a longer time (five to six generations of self-pollination). Besides, the parent Rutabaga-BF requires vernalization for flowering. In this case, each generation of self-pollination of the F_1 and later generation populations will require additional eight to nine weeks for vernalization. Therefore, developing a RIL population would have a lengthy process, which was not feasible during this thesis research.

Using this DH mapping population, I initially constructed a genetic linkage map of chromosome A08 and mapped the major CR locus. Later, by taking advantage of the high-density SNP markers, I constructed a complete genetic map of 19 *B. napus* chromosomes of the DH-population and further confirmed the CR locus on A08 (*qCR_A8)* and identified an additional CR locus *qCR*_A3 on chromosome A03. This demonstrated the power of using a

complete genetic map to identify the major and minor QTL. The *qCR_A8* confers resistance to *P. brassicae* pathotypes 2, 3, 5, 6, and 8, while the *qCR*_A3 is involved in resistance to pathotype 3. Previous studies have identified at least five CR loci on chromosome A08 conferring mostly race-specific resistance (Suwabe et al. 2003, 2006; Hatakeyama et al. 2013; Laila et al. 2019; Zhu et al. 2019b; Karim et al. 2020). The rutabaga cv. Brookfield also carries resistance to the recently reported virulent *P. brassicae* pathotypes 3A and 5X. Shaikh et al. (2020) reported that more than two loci could be involved in the control resistance to these pathotypes in rutabaga cv. Brookfield, where some of the loci may be involved in the control of resistance to both pathotypes. Further research will be needed to understand whether *qCR*_A3 and *qCR*_A8 are involved in resistance to these new pathotypes.

Rutabaga is genetically distinct from spring canola (Diers and Osborn 1994; Bus et al. 2011); therefore, it may contribute favourable alleles to broaden the genetic base of spring canola for seed yield, agronomic, and seed quality traits (Shiranifar et al. 2020, 2021). However, crossing a crop species to its unadapted germplasm may introduce undesirable alleles (Kjær et al. 1990; Groos et al. 2003; Chen et al. 2005; Suzuki et al. 2012; Summers and Brown 2013; Rahman and Franke 2019), and this can cause a disruption in the desired combinations of the alleles. Zhao et al. (2006), Wei et al. (2014), and Wu et al. (2019) reported a negative association of Sclerotinia stem rot resistance, obtained from exotic germplasm, with flowering time in canola. Therefore, insight into the alleles contributing to agronomic and seed quality and the effect of disease resistance genes on these traits is critical for the rational utilization of an exotic gene pool in canola breeding.

This thesis research identified oil and an erucic acid QTL on the C03 chromosome of rutabaga cv. Brookfield. Erucic acid and oil QTL on C03 has also been reported by several researchers (Qiu et al. 2006; Tang et al. 2019). However, cv. Brookfield QTL allele on chromosome C03 increases about 0.7% oil in spring canola. The occurrence of a single erucic

acid QTL in the rutabaga cv. Brookfield has an important application in canola breeding; the breeding population derived from rutabaga × canola will segregate for one erucic acid locus; thus, a relatively smaller population will be required to select a plant with zero erucic acids in the oil. This thesis research reported a major vernalization QTL on chromosome A02, which also affects flowering time variation. This research demonstrated no association of clubroot resistance of rutabaga with days-to-flowering and seed quality traits.

The single major-gene resistance mostly used in canola can become ineffective due to the emergence of new virulent pathotypes (Strelkov et al. 2016b; Cao et al. 2020). Pyramiding of multiple major CR genes (Matsumoto et al. 2012) or major gene combined with QTL (Tomita et al. 2013; Shah et al. 2019) can confer resistance to multiple *P. brassicae* pathotypes (Matsumoto et al. 2012; Shah et al. 2019) or can result in stronger resistance to a single pathotype (Tomita et al. 2013). Therefore, additional CR genes in Canadian *B. napus* canola are needed to diversify the CR genes in this crop. Gene pyramiding based on phenotypic evaluation is a challenging task and may not be feasible in many cases. The development of gene-specific molecular marker(s) strongly co-segregating with the resistance gene (Ruane and Sonnino 2007) and their use in marker-assisted selection (MAS) can resolve this constraint. Fine mapping of major genes or QTL can be done by taking the advantages of the next-generation sequencing (NGS) technologies and high-density genetic markers (Huang et al. 2017; Pang et al. 2018; Laila et al. 2019; Zhu et al. 2019b; Karim et al. 2020; Dakouri et al. 2021). In this study, I took advantage of the whole-genome sequencing and fine mapped a CR gene of Chinese cabbage cv. Bilko introgressed into *B. napus* canola. This CR gene's introduction into spring *B. napus* canola has been achieved through limited backcrossing (one backcross) followed by self-pollination for five generations. Limited backcrossing was preferred in this case for a quicker recovery of *B. napus* type plants. Besides, Attri and Rahman

(2018) reported that a significant loss of *B. rapa* alleles could occur in early segregating generation populations of *B. napus* × *B. rapa* interspecific cross.

As mentioned earlier, more than 20 major CR loci have been reported in the A-genome; 12 of those loci are from Chinese cabbage (*B. rapa* var. *chinensis/pekinensis*) (for review, see Hasan et al. 2021). Here, I reported the identification of a CR locus from the Chinese cabbage cv. Bilko and mapping this resistance following its introgression into *B. napus* canola. The Bilko-CR locus in *B. napus* is mapped in chromosome A03 region that harbours TNL genes *Bra012688* and *Bra012689*. The SNP-based allele-specific markers A03_SNP_4437F1-4436R, A03_SNP_4436F1, A03_SNP_4437F1, designed from *Bra012688* sequence, co-segregated with this resistance. The TNL gene confers resistance to clubroot disease in *B. rapa* (Ueno et al. 2012; Hatakeyama et al. 2013, 2017). To date, nine CR loci have been mapped on chromosome A03; three loci *viz*., *CRk, CRd,* and *Crr3* in the 15.3 -16.3 Mb region and six loci *viz.*, *CRa/CRb, CRq, Rcr1, Rcr2, Rcr4,* and *Rcr5* in the 24.4 – 26.0 Mb genomic region of chromosome A03 *B. rapa* cv. Chiifu-401 v3.0 (for review, see Hasan et al. 2021). The results of this research positioned the cv. Bilko CR locus in the 23.87 – 23.89 Mb genomic region of *B. rapa* cv. Chiifu-401 v3.0 chromosome A03. Whether Bilko CR locus is one of the CR loci mentioned earlier from the 24.4 – 26.0 Mb genomic region of chromosome A03 or a new locus would need further investigation. Hirani et al. (2016) also introgressed a known CR locus from Chinese cabbage into *B. napus* canola through marker-assisted recurrent backcrossing.

Brassica juncea is a major crop in the hot and low rainfall production regions, including the Indian sub-continent. Clubroot disease has been reported in oilseed *B. juncea* in India (Bhattacharya et al. 2014). However, the lack of clubroot resistance in *B. juncea* is difficult to explain when its two diploid progenitors, *B. rapa* and *B. nigra*, possess resistance to this disease (Hasan et al. 2012; Fredua-Agyeman et al. 2019). Among the different varieties of *B. rapa*, turnip (*B. rapa* ssp. *rapifera*) is most frequently reported to carry clubroot resistance (Buczacki

et al. 1975; Hasan et al. 2012). Turnip has been used in breeding for clubroot resistance in Chinese cabbage (Yoshikawa 1973) and to resynthesize clubroot-resistant *B. napus* (Diederichsen and Sacristan 1996) for the development of winter (Frauen 1999) and spring canola (Rahman et al. 2011) cultivars. My research demonstrated that clubroot resistant *B. juncea* line could be developed through the resynthesis of this species using the resistance available in one of its parental species, *B. rapa*.

Resynthesized *Brassica* allopolyploid chromosomes undergo meiotic anomalies and homoeologous pairing in their early generations; this can cause structural rearrangements in the chromosomes, including loss or gain of chromosomes (Udall et al. 2005; Gaeta et al. 2007; Gaeta and Chris Pires 2010; Szadkowski et al. 2011; Xiong et al. 2011). Chen and Ni (2006) reported changes in gene expression in synthetic allopolyploids, altering plants' phenotypes. Pires et al. (2004) reported structural rearrangement of chromosomes contributing to the variation in quantitative traits, such as flowering time, in resynthesized *B. napus*. On the other hand, stable expression of qualitative traits, such as self-incompatibility (Rahman 2005) and clubroot resistance (Diederichsen and Sacristan 1996), have been reported in self-pollinated progenies of resynthesized *B. napus.* Indeed, clubroot resistance in resynthesized *B. juncea* lines was also inherited almost stably in the self-pollinated generations – only a small percentage (5.8%) of the S_2 *B. juncea* plants showed a loss of resistance. Nonetheless, the clubroot-resistant resynthesized *B. juncea* lines developed in this study can be used to develop clubroot-resistant *B. juncea* canola cultivars.

Thus, the results from this Ph.D. thesis research demonstrated that both rutabaga and Chinese cabbage gene pool could be used to (i) broaden the genetic base for clubroot resistance in *B. napus* canola, (ii) rutabaga carries favourable alleles for some agronomic and seed quality traits of canola, and these alleles can be used to improve this oilseed crop and (iii) clubroot resistance of rutabaga cv. Brookfield, introgressed into *B. napus* canola does not carry linkage

drag for poor agronomic and seed quality traits, and thus, this resistance can safely be used in canola breeding. The results from this Ph.D. thesis research also provide evidence that clubroot resistant *B. juncea* line can be developed by exploiting the resistance available in *B. rapa*.

6.2. Achievements, impact, and future study

In conclusion, this thesis research utilized

I. A DH population derived from clubroot resistant *B. napus* var. *napobrassica* cv. Brookfield × *B. napus* spring canola line A07-29NI cross, and
    i. mapped two CR loci and developed genetic markers for MAS,
    ii. identified genomic regions associated with vernalization requirement, days-to-flowering, seed oil, protein, glucosinolate, and erucic acid contents in rutabaga, and
    iii. demonstrated no effect of clubroot resistance on flowering and seed quality traits.

II. A RIL *B. napus* canola population, derived from *B. napus* spring canola line A04-73NA × *B. rapa* var. *pekinensis* cv. Bilko interspecific cross, and
    i. demonstrated the introgression of the CR locus from Chinese cabbage into spring *B. napus* canola, and
    ii. mapped the CR locus and developed genetic markers for marker-assisted breeding

III. *Brassica juncea* lines, resynthesized from resistant *B. rapa* ssp. *rapifera* cv. Gelria × susceptible *B. nigra* line CR2137 interspecific cross, and
    i. developed clubroot resistant *B. juncea* lines, and
    ii. demonstrated the stability of A-genome resistance in the resynthesized *B. juncea* lines.

Hybrid canola cultivars are mostly grown in western countries. A hybrid cultivar is developed using two genetically distinct homozygous parent lines. Since Brookfield- and Bilko-CR loci are dominantly inherited traits, they can be introgressed separately in one of the parent genotypes to develop a two-gene resistant hybrid cultivar. Among the conventional breeding methods, recurrent backcrossing of the elite parent line followed by the phenotypic selection for resistant plants is commonly practiced to introgress the dominant CR locus from the resistant donor line into the elite parent line without compromising its general/specific combinability (GCA/SCA) value. New breeding lines containing multiple CR loci can also be developed using the DH production approach, as has been reported by (Shaikh et al. 2020), or following pedigree breeding (Rahman et al. 2011). However, marker-assisted selection is the most efficient and cost-effective approach for combining more than one CR loci into a single elite parent line. Genetic markers co-segregating with the resistance with <1 cM genetic distance are used for MAS. However, genetic markers linked to the sequence motifs affecting phenotypic variation (also known as a functional marker) are the most reliable for use in MAS in a plant breeding program that utilizes diverse germplasm.

In this thesis research, recombination between the CR loci, e.g., *qCR_A8* and Bilko-CR, and the associated markers indicated that these markers might not be located within the CR genes; the allelic variation exhibited by the markers may not be due to the variation in sequence motifs affecting the phenotypic variation. Therefore, fine-mapping many of these CR loci will be needed. For example, 12 SNP markers were identified within a 1.0 Mb (10.4 – 11.4 Mb) region of the CR locus of A08 of the rutabaga cv. Brookfield (Figure 3.3, Table S3.4). This suggests the need for a larger mapping population (>500) to identify the sequence motifs affecting the phenotypic variation and develop functional markers. In the case of Bilko-CR, the genetic analysis indicated introgression of this locus into *B. napus* canola, and this locus co-segregated with the AS-markers in the homozygous RIL families. However, a 0.4 – 0.8%

recombination in the segregating RIL families indicates that these markers are not from the candidate CR gene. On the other hand, the loss of resistance in some of the resynthesized S_2 *B. juncea* plants indicated that the genomic regions carrying the CR could be in a state of genomic change, as reported in the case of resynthesized *B. napus* (Gaeta et al. 2007). Taking the results of lack of strong co-segregation of the previously reported A03 CR markers (Ueno et al. 2012; Kato et al. 2013) in the RIL population carrying Bilko-CR and the loss of CR in some of the resynthesized *B. juncea* plants, it is advisable to develop molecular markers for a CR gene following introgression of this gene in the recipient species.

The following studies, utilizing the resources developed and knowledge gained from this thesis research, will further extend our knowledge on the utility of the exotic germplasm and allied species to improve the genetic base of canola for clubroot resistance;

1. Identify candidate genes conferring resistance to *P. brassicae* pathotypes and develop functional markers for use in MAS.
2. Fine map CR loci associated with clubroot resistance in the synthetic *B. juncea* and develop genetic markers for MAS of resistant *B. juncea* canola cultivars.
3. Phenotype the DH and RIL *B. napus* population and synthetic *B. juncea* lines for resistance to the recently reported virulent *P. brassicae* pathotypes and identify candidate genes and develop functional markers.
4. Develop genetic markers associated with QTL for vernalization, flowering time variation, erucic acid, and glucosinolates.
5. Fine map the oil QTL of the rutabaga cv. Brookfield and introgress into elite canola lines.
6. Investigate the value of the Chinese cabbage gene pool for broadening the genetic base of *B. napus* canola using the RIL population derived from spring canola × *Chinese cabbage* interspecific cross.

Literature cited

Abe, A., Kosugi, S., Yoshida, K., Natsume, S., Takagi, H., Kanzaki, H., Matsumura, H., Yoshida, K., Mitsuoka, C., Tamiru, M., Innan, H., Cano, L., Kamoun, S., and Terauchi, R. 2012. Genome sequencing reveals agronomically important loci in rice using MutMap. Nat. Biotechnol. **30**(2): 174–178. doi:10.1038/nbt.2095.

Adams, K.L., and Wendel, J.F. 2005. Polyploidy and genome evolution in plants. Curr. Opin. Plant Biol. **8**(2): 135–141. doi:10.1016/j.pbi.2005.01.001.

Agarwal, A., Kaul, V., Faggian, R., Rookes, J.E., Ludwig-Müller, J., and Cahill, D.M. 2011. Analysis of global host gene expression during the primary phase of the *Arabidopsis thaliana–Plasmodiophora brassicae* interaction. Funct. Plant Biol. **38**(6): 462. doi:10.1071/FP11026.

Agrios, G.N. 2005. Plant Pathology, 5th Edition. *In* 5th edition. Academic Press.

Aist, J.R., and Williams, P.H. 1971. The cytology and kinetics of cabbage root hair penetration by *Plasmodiophora brassicae*. Can. J. Bot. **49**: 2023–2034.

Akira, S., and Hemmi, H. 2003. Recognition of pathogen-associated molecular patterns by TLR family. Immunol. Lett. **85**(2): 85–95. doi:10.1016/S0165-2478(02)00228-6.

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**(1): 96–99. doi:10.1080/07060661.2017.1393696.

Al-Shehbaz, I.A. 2012. A generic and tribal synopsis of the Brassicaceae (Cruciferae). Taxon **61**(5): 931–954. doi:10.1002/tax.615002.

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**(1): 79–93. doi:10.1071/CP17214.

Alberta Clubroot Management Committee. 2010. Alberta clubroot management plan. *In* Government of Alberta. Available from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Alberta+clubroot+management+plan#0

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**(17): 3389–402. doi:10.1093/nar/25.17.3389.

Andersen, E.J., Ali, S., Byamukama, E., Yen, Y., and Nepal, M.P. 2018. Disease resistance

mechanisms in plants. Genes (Basel). **9**(7). doi:10.3390/genes9070339.

Ando, S., Tsushima, S., Kamachi, S., Konagaya, K., and Tabei, Y. 2008. Alternative transcription initiation of the nitrilase gene (*BrNIT2*) caused by infection with *Plasmodiophora brassicae* Woron. in Chinese cabbage (*Brassica rapa* L.). Plant Mol. Biol. **68**(6): 557–569. doi:10.1007/s11103-008-9390-9.

Arends, D., Prins, P., Jansen, R.C., and Broman, K.W. 2010. R/qtl: high-throughput multiple QTL mapping. Bioinformatics **26**(23): 2990–2992. doi:10.1093/bioinformatics/btq565.

Arifuzzaman, M., Mamidi, S., McClean, P., and Rahman, M. 2017. QTL mapping for root vigor and days to flowering in *Brassica napus* L. Can. J. Plant Sci. **97**(1): 99–109. doi:10.1139/CJPS-2016-0048.

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. doi:10.1071/CP17193.

Axtell, M.J., and Meyers, B.C. 2018. Revisiting criteria for plant microRNA annotation in the era of big data. Plant Cell **30**(2): 272–284. doi:10.1105/tpc.17.00851.

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. http://pubs.aic.ca/doi/abs/10.4141/cjps72-155.

Badstöber, J., Ciaghi, S., and Neuhauser, S. 2020. Dynamic cell wall modifications in brassicas during clubroot disease. University of Innsbruck. doi:10.1101/2020.03.02.972901.

Balint-Kurti, P. 2019. The plant hypersensitive response: concepts, control and consequences. Mol. Plant Pathol. **20**(8): 1163–1178. doi:10.1111/mpp.12821.

Barr, D. 1992. Evolution and kingdoms of organisms from the perspective of a mycologist. Mycologia **84**(1): 1–11.

Bartel, D.P. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell **116**(2): 281–297. doi:10.1016/S0092-8674(04)00045-5.

Bauer, W.D., Bateman, D.F., and Whalen, C.H. 1977. Purification of an Endo- β -1,4 Galactanase Produced by Sclerotinia sclerotiorum: Effects on Isolated Plant Cell Walls and Potato Tissue. Phytopathology **77**(7): 862. doi:10.1094/phyto-67-862.

Bennett, R., Thiagarajah, M., King, J., and Rahman, M. 2008. Interspecific cross of *Brassica oleracea* var. alboglabra and *B. napus*: effects of growth condition and silique age on the efficiency of hybrid production, and inheritance of erucic acid in the self-pollinated backcross generation. Euphytica **164**(2): 593–601. doi:10.1007/s10681-008-9788-0.

Bhattacharya, I., Dutta, S., Mondal, S., and Mondal, B. 2014. Clubroot disease on Brassica

crops in India. Can. J. Plant Pathol. **36**(sup1): 154–160. doi:10.1080/07060661.2013.875064.

Blanc, G., Barakat, A., Guyot, R., Cooke, R., and Delseny, M. 2000. Extensive duplication and reshuffling in the Arabidopsis genome. Plant Cell **12**(7): 1093–1101. doi:10.1105/tpc.12.7.1093.

Bolger, A.M., Lohse, M., and Usadel, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30**(15): 2114–2120. doi:10.1093/bioinformatics/btu170.

Boller, T., and Felix, G. 2009. A Renaissance of elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. Annu. Rev. Plant Biol. **60**(1): 379–406. doi:10.1146/annurev.arplant.57.032905.105346.

Boskovic, J., Boskovic, M., Babovic, M., Jerkovic, Z., and Pesic, V. 2001. Pyramiding Strategy for Durable Resistance to Wheat Leaf Rust Pathogen. *In* Developments in Plant Breeding, 9th edition. *Edited by* Z. Bedo and L. Lang. Springer, Dordrecht. pp. 337–343. doi:10.1007/978-94-017-3674-9_43.

Bradley, C.A., Lamey, H.A., Endres, G.J., Henson, R.A., Hanson, B.K., McKay, K.R., Halvorson, M., LeGare, D.G., and Porter, P.M. 2006. Efficacy of fungicides for control of sclerotinia stem rot of canola. Plant Dis. **90**(9): 1129–1134. doi:10.1094/PD-90-1129.

Bradshaw, H.D., Otto, K.G., Frewen, B.E., McKay, J.K., and Schemske, D.W. 1998. Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (Mimulus). Genetics **149**(1): 367–382. doi:10.1093/genetics/149.1.367.

Bradshaw, H.D., Wilbert, S.M., Otto, K.G., and Schemske, D.W. 1995. Genetic mapping of floral traits associated with reproductive isolation in monkeyflowers (Mimulus). Nature **376**(6543): 762–765. doi:10.1038/376762a0.

Bradshaw, J., Gemmell, D., and Wilson, R. 1997. Transfer of resistance to clubroot (*Plasmodiophora brassicae*) to swedes (*Brassica napus* L. var. *napobrassica* Peterm) from *B. rapa*. Ann. Appl. Biol. **130**: 337–348.

Brodmann, D., Schuller, A., Ludwig-Müller, J., Aeschbacher, R.A., Wiemken, A., Boller, T., and Wingler, A. 2002. Induction of trehalase in Arabidopsis plants infected with the trehalose-producing pathogen *Plasmodiophora brassicae*. Mol. Plant-Microbe Interact. **15**(7): 693–700. doi:10.1094/MPMI.2002.15.7.693.

Broman, K.W., Wu, H., Sen, Ś., and Churchill, G.A. 2003. R/qtl: QTL mapping in experimental crosses. Bioinformatics **19**(7): 889–890. doi:10.1093/bioinformatics/btg112.

Buczacki, S., Toxopeus, H., Mattusch, P., Johnston, T., Dixon, G., and Hobolth, L. 1975. Study

of physiologic specialization in *Plasmodiophora brassicae*: Proposals for attempted rationalization through an international approach. Trans. Br. Mycol. Soc. **65**(2): 295–303. British Mycological Society. doi:10.1016/S0007-1536(75)80013-1.

Buels, R., Yao, E., Diesh, C.M., Hayes, R.D., Munoz-Torres, M., Helt, G., Goodstein, D.M., Elsik, C.G., Lewis, S.E., Stein, L., and Holmes, I.H. 2016. JBrowse: a dynamic web platform for genome visualization and analysis. Genome Biol. **17**(1): 66. doi:10.1186/s13059-016-0924-1.

Burns, M.J., Barnes, S.R., Bowman, J.G., Clarke, M.H.E., Werner, C.P., and Kearsey, M.J. 2003. QTL analysis of an intervarietal set of substitution lines in *Brassica napus*: (I) seed oil content and fatty acid composition. Heredity (Edinb). **90**(1): 39–48. doi:10.1038/sj.hdy.6800176.

Burton, W., Pymer, S., Salisbury, P., Kirk, J., and Oram, R. 1999. Performance of Australian canola quality *Brassica juncea* lines. *In* Proceedings of 10th International Rapeseed Congress. pp. 2–7. Available from http://www.regional.org.au/au/gcirc/4/51.htm.

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**(8): 1413–1423. doi:10.1007/s00122-011-1676-7.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., and Madden, T.L. 2009. BLAST+: architecture and applications. BMC Bioinformatics **10**(1): 421. doi:10.1186/1471-2105-10-421.

Campbell, R., and Grethead, A. 1989. Control of clubroot of brassicas by liming. *In* Soilborne plant pathogens: Management of diseases with macro- and microelements. *Edited by* A. Engelhard. APS Press, St. Paul, Minnesota. pp. 90–101.

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. Taylor & Francis. doi:10.1080/07060660909507606.

Cao, T., Manolii, V.P., Zhou, Q., Hwang, S.-F., and Strelkov, S.E. 2020. Effect of canola (*Brassica napus*) cultivar rotation on *Plasmodiophora brassicae* pathotype composition. Can. J. Plant Sci. **100**(2): 218–225. doi:10.1139/cjps-2019-0126.

Cao, T., Srivastava, S., Rahman, M.H., Kav, N.N.V., Hotte, N., Deyholos, M.K., and Strelkov, S.E. 2008. Proteome-level changes in the roots of *Brassica napus* as a result of *Plasmodiophora brassicae* infection. Plant Sci. **174**(1): 97–115. doi:10.1016/j.plantsci.2007.10.002.

Cao, W., Humphreys, G., Fedak, G., Wolfe, D., McCallum, B., Fetch, T., Xue, A., Zhang, L.,

and Chi, D. 2016. Development of aneuploids by means of doubled haploid technology - corn pollination method. *In* Proc Plant and Animal Genome XXIV Conference. San Diego, CA. p. P0257.

Cao, Z., Tian, F., Wang, N., Jiang, C., Lin, B., Xia, W., Shi, J., Long, Y., Zhang, C., and Meng, J. 2010. Analysis of QTLs for erucic acid and oil content in seeds on A8 chromosome and the linkage drag between the alleles for the two traits in *Brassica napus*. J. Genet. Genomics **37**(4): 231–240. doi:10.1016/S1673-8527(09)60041-2.

Chalhoub, B., Denoeud, F., Liu, S., Parkin, I. a. P., Tang, H., Wang, X., Chiquet, J., Belcram, H., Tong, C., Samans, B., Correa, M., Da Silva, C., Just, J., Falentin, C., Koh, C.S., Le Clainche, I., Bernard, M., Bento, P., Noel, B., Labadie, K., Alberti, A., Charles, M., Arnaud, D., Guo, H., Daviaud, C., Alamery, S., Jabbari, K., Zhao, M., Edger, P.P., Chelaifa, H., Tack, D., Lassalle, G., Mestiri, I., Schnel, N., Le Paslier, M.-C., Fan, G., Renault, V., Bayer, P.E., Golicz, a. a., Manoli, S., Lee, T.-H., Thi, V.H.D., Chalabi, S., Hu, Q., Fan, C., Tollenaere, R., Lu, Y., Battail, C., Shen, J., Sidebottom, C.H.D., Wang, X., Canaguier, A., Chauveau, A., Berard, A., Deniot, G., Guan, M., Liu, Z., Sun, F., Lim, Y.P., Lyons, E., Town, C.D., Bancroft, I., Wang, X., Meng, J., Ma, J., Pires, J.C., King, G.J., Brunel, D., Delourme, R., Renard, M., Aury, J.-M., Adams, K.L., Batley, J., Snowdon, R.J., Tost, J., Edwards, D., Zhou, Y., Hua, W., Sharpe, a. G., Paterson, a. H., Guan, C., and Wincker, P. 2014. Early allopolyploid evolution in the post-Neolithic *Brassica napus* oilseed genome. Science (80-.). **345**(6199): 950–953. doi:10.1126/science.1253435.

Chang, A., Lamara, M., Wei, Y., Hu, H., Parkin, I.A.P., Gossen, B.D., Peng, G., and Yu, F. 2019. Clubroot resistance gene *Rcr6* in *Brassica nigra* resides in a genomic region homologous to chromosome A08 in *B. rapa*. BMC Plant Biol. **19**(1): 1–11. BMC Plant Biology. doi:10.1186/s12870-019-1844-5.

Chao, H., Wang, H., Wang, X., Guo, L., Gu, J., Zhao, W., Li, B., Chen, D., Raboanatahiry, N., and Li, M. 2017. Genetic dissection of seed oil and protein content and identification of networks associated with oil content in *Brassica napus*. Sci. Rep. **7**(1): 46295. doi:10.1038/srep46295.

Chen, J., Pang, W., Chen, B., Zhang, C., and Piao, Z. 2016. Transcriptome analysis of *Brassica rapa* near-Isogenic lines carrying clubroot-resistant and -susceptible alleles in response to *Plasmodiophora brassicae* during early infection. Front. Plant Sci. **6**: 1183. doi:10.3389/fpls.2015.01183.

Chen, L.-Q., Qu, X.-Q., Hou, B.-H., Sosso, D., Osorio, S., Fernie, A.R., and Frommer, W.B.

2012. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science (80-.). **335**(6065): 207–211. doi:10.1126/science.1213351.

Chen, W., Li, Y., Yan, R., Xu, L., Ren, L., Liu, F., Zeng, L., Yang, H., Chi, P., Wang, X., Chen, K., Ma, D., and Fang, X. 2019. Identification and characterization of *Plasmodiophora brassicae* primary infection effector candidates that suppress or induce cell death in host and nonhost plants. Phytopathology **109**(10): 1689–1697. doi:10.1094/PHYTO-02-19-0039-R.

Chen, X.M., Luo, Y.H., Xia, X.C., Xia, L.Q., Chen, X., Ren, Z.L., He, Z.H., and Jia, J.Z. 2005. Chromosomal location of powdery mildew resistance gene *Pm16* in wheat using SSR marker analysis. Plant Breed. **124**(3): 225–228. doi:10.1111/j.1439-0523.2005.01094.x.

Chen, Z.J., and Ni, Z. 2006. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays **28**(3): 240–252. doi:10.1097/MPG.0b013e3181a15ae8.Screening.

Cheng, F., Liu, S., Wu, J., Fang, L., Sun, S., Liu, B., Li, P., Hua, W., and Wang, X. 2011. BRAD, the genetics and genomics database for Brassica plants. BMC Plant Biol. **11**(1): 136. BioMed Central Ltd. doi:10.1186/1471-2229-11-136.

Cheng, F., Mandáková, T., Wu, J., Xie, Q., Lysak, M. a, and Wang, X. 2013. Deciphering the diploid ancestral genome of the Mesohexaploid *Brassica rapa*. Plant Cell **25**(5): 1541–1554. doi:10.1105/tpc.113.110486.

Cheng, X., Xu, J., Xia, S., Gu, J., Yang, Y., Fu, J., Qian, X., Zhang, S., Wu, J., and Liu, K. 2009. Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theor. Appl. Genet. **118**(6): 1121–31. doi:10.1007/s00122-009-0967-8.

Chiang, M., and Crête, R. 1970. Inheritance of clubroot resistance in cabbage (*Brassica oleracea* L. var. *capitata* L.). Can. J. Genet. Cytol. **12**: 253–256.

Chisholm, S.T., Coaker, G., Day, B., and Staskawicz, B.J. 2006. Host-microbe interactions: shaping the evolution of the plant immune response. Cell **124**(4): 803–814. doi:10.1016/j.cell.2006.02.008.

Chittem, K., Mansouripour, S.M., and del Río Mendoza, L.E. 2014. First report of clubroot on canola caused by *Plasmodiophora brassicae* in North Dakota. Plant Dis. **98**(10): 1438. doi:10.1094/PDIS-04-14-0430-PDN.

Cho, K., Hong, S., Han, Y., Yoon, B., Ryu, S., and Woo, J. 2008. Resistance in Chinese cabbage using Single-spore Isolates of *Plasmodiophora brassicae* and development of RAPD markers linked to its resistance Gene. J. Crop Sci. Biotechnol. **11**(2): 101–106.

Chu, M., Song, T., Falk, K.C., Zhang, X., Liu, X., Chang, A., Lahlali, R., Mcgregor, L., Gossen, B.D., Yu, F., and Peng, G. 2014. Fine mapping of *Rcr1* and analyses of its effect on transcriptome patterns during infection by *Plasmodiophora brassicae*. BMC Genomics **15**(1): 1–20. doi:10.1186/1471-2164-15-1166.

Churchill, G. a, and Doerge, R.W. 1994. Empirical threshold values for quantitative trait mapping. Genetics **138**(3): 963–371.

Clarke, W.E., Higgins, E.E., Plieske, J., Wieseke, R., Sidebottom, C., Khedikar, Y., Batley, J., Edwards, D., Meng, J., Li, R., Lawley, C.T., Pauquet, J., Laga, B., Cheung, W., Iniguez-Luy, F., Dyrszka, E., Rae, S., Stich, B., Snowdon, R.J., Sharpe, A.G., Ganal, M.W., and Parkin, I.A.P. 2016. A high-density SNP genotyping array for *Brassica napus* and its ancestral diploid species based on optimised selection of single-locus markers in the allotetraploid genome. Theor. Appl. Genet. **129**(10): 1887–1899. doi:10.1007/s00122-016-2746-7.

Collier, S.M., and Moffett, P. 2009. NB-LRRs work a "bait and switch" on pathogens. Trends Plant Sci. **14**(10): 521–9. doi:10.1016/j.tplants.2009.08.001.

Cook, W., and Schwartz, E. 1930. The life-history, cytology and method of infection of *Plasmodiophora brassicae* Woron., the cause of finger-and-toe disease of cabbages and other crucifers. Philos. Trans. R. Soc. London. Ser. B **218**: 283–314.

Coventry, J., Kott, L., and Beversdorf, W.D. 1988. Manual for microspore culture technique for *Brassica napus*. *In* OAC Public. Department of Crop Science Technical Bulletine, University of Guelph, OAC Publication Vol 489.

Cowling, W.A. 2007. Genetic diversity in Australian canola and implications for crop breeding for changing future environments. F. Crop. Res. **104**(1–3): 103–111. doi:10.1016/j.fcr.2006.12.014.

Crisp, P., Crute, I., Sutherland, R., Angell, S., Bloor, K., Burgess, H., and Gordon, P. 1989. The exploitation of genetic resources of *Brassica oleracea* in breeding for resistance to clubroot (*Plasmodiophora brassicae*). Euphytica **42**(215–226).

Crute, I., Phelps, K., Barnes, A., Buczacki, S., and Crisp, P. 1983. The relationship between genotypes of three Brassica species and collections of *Plasmodiophora brassicae*. Plant Pathol. **32**(4): 405–420. doi:10.1111/j.1365-3059.1983.tb02855.x.

Dakouri, A., Lamara, M., Karim, M.M., Wang, J., Chen, Q., Gossen, B.D., Strelkov, S.E., Hwang, S.-F., Peng, G., and Yu, F. 2021. Identification of resistance loci against new pathotypes of *Plasmodiophora brassicae* in *Brassica napus* based on genome-wide association mapping. Sci. Rep. **11**(1): 6599. doi:10.1038/s41598-021-85836-9.

Dakouri, A., Zhang, X., Peng, G., Falk, K.C., Gossen, B.D., Strelkov, S.E., and Yu, F. 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**(1): 1–10. 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): 826–833. doi:10.1038/35081161.

DeLaat, A.M.M., Gohde, W., and Vogelzakg, M.J.D.C. 1987. Determination of ploidy of single plants and plant populations by flow cytometry. Plant Breed. **99**(4): 303–307. doi:10.1111/j.1439-0523.1987.tb01186.x.

Delourme, R., Falentin, C., Huteau, V., Clouet, V., Horvais, R., Gandon, B., Specel, S., Hanneton, L., Dheu, J.E., Deschamps, M., Margale, E., Vincourt, P., and Renard, M. 2006. Genetic control of oil content in oilseed rape (*Brassica napus* L.). Theor. Appl. Genet. **113**(7): 1331–1345. doi:10.1007/s00122-006-0386-z.

Delourme, R., Pilet-Nayel, M.L., Archipiano, M., Horvais, R., Tanguy, X., Rouxel, T., Brun, H., Renard, M., and Balesdent, M.H. 2004. A cluster of major specific resistance genes to *Leptosphaeria maculans* in *Brassica napus*. Phytopathology **94**(6): 578–583. doi:10.1094/PHYTO.2004.94.6.578.

Deora, A., Gossen, B., Walley, F., and McDonald, M. 2011. Boron reduces development of clubroot in canola. Can. J. Plant Pathol. **33**(4): 475–484.

Deora, A., Gossen, B.D., and McDonald, M.R. 2012. Infection and development of *Plasmodiophora brassicae* in resistant and susceptible canola cultivars. Can. J. Plant Pathol. **34**(2): 239–247. doi:10.1080/07060661.2012.681071.

Deora, A., Gossen, B.D., and McDonald, M.R. 2013. Cytology of infection, development and expression of resistance to *Plasmodiophora brassicae* in canola. Ann. Appl. Biol. **163**(1): 56–71. doi:10.1111/aab.12033.

Devos, S., Laukens, K., Deckers, P., Van Der Straeten, D., Beeckman, T., Inzé, D., Van Onckelen, H., Witters, E., and Prinsen, E. 2006. A hormone and proteome approach to picturing the initial metabolic events during *Plasmodiophora brassicae* Infection on arabidopsis. mol. plant-microbe interact. **19**(12): 1431–1443. doi:10.1094/MPMI-19-1431.

Diederichsen, E., Beckmann, J., Schondelmeier, J., and Dreyer, F. 2006. Genetics of clubroot resistance in *Brassica napus* ‘ Mendel .’ Acta Hortic. **706**: 307–312.

Diederichsen, E., Frauen, M., Linders, E., Hatakeyama, K., and Hirai, M. 2009. Status and perspectives of clubroot resistance breeding in crucifer crops. J. Plant Growth Regul.

28(3): 265–281. doi:10.1007/s00344-009-9100-0.

Diederichsen, E., and Sacristan, M. 1996. Disease response of resynthesized *Brassica napus* L . lines carrying different combinations of resistance to *Plasmodiophora brassicae* Wor. Plant Breed. **115**: 5–10.

Diers, B.W., and Osborn, T.C. 1994. Genetic diversity of oilseed *Brassica napus* germplasm based on restriction fragment length polymorphisms. Theor. Appl. Genet. **88**(6–7): 662–668. doi:10.1007/BF01253968.

Dixon, G. 2009. The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. J. Plant Growth Regul. **28**(3): 194–202. doi:10.1007/s00344-009-9090-y.

Dobson, R., Gabrielson, R., Baker, A., and Bennett, L. 1983. Effects of lime particle size and distribution and fertilizer formulation on clubroot disease caused by *Plasmodiophora brassicae*. Plant Dis. **67**(1): 50–52. doi:10.1094/PD-67-50.

Dokken-Bouchard, F., Bouchard, A., Ippolito, J., Peng, G., Strelkov, S., Kirkham, C., and Kutcher, H. 2010. Detection of *Plasmodiophora brassicae* in Saskatchewan, 2008. Can. Plant Dis. Surv. **90**: 126.

Dolezel, J., Greilhuber, J., and Suda, J. 2007. Estimation of nuclear DNA content in plants using flow cytometry. Nat. Protoc. **2**(9): 2233–44. doi:10.1038/nprot.2007.310.

Donald, C., and Porter, I. 2003. Clubroot (*Plasmodiophora brassicae*) an imminent threat to the Australian canola industry. *In* The 13th Biennial Australian Research Assembly on Brassicas. *Edited by* J. Edwards. Tamworth, New South Wales, Australia. pp. 114–118.

Donald, C., and Porter, I. 2009. Integrated control of clubroot. J. Plant Growth Regul. **28**(3): 289–303. doi:10.1007/s00344-009-9094-7.

Doussinault, G., Delibes, A., Sanchez-Monge, R., and Garcia-Olmedo, F. 1983. Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. Nature **303**(5919): 698–700. doi:10.1038/303698a0.

Downey, R., and Craig, B. 1963. Genetic control of fatty acid biosynthesis in rapeseed (*Brassica napus* L.). JAOCS **41**: 475–478.

Downey, R., and Harvey, B. 1963. Methods of breeding for oil quality in rape. Can. J. Plant Sci. **43**(3): 271–275. doi:10.4141/cjps63-054.

Ecke, W., Uzunova, M., and Weißleder, K. 1995. Mapping the genome of rapeseed (*Brassica napus* L.). II. Localization of genes controlling erucic acid synthesis and seed oil content. Theor. Appl. Genet. **91**(6–7): 972–977. doi:10.1007/BF00223908.

Eitas, T.K., and Dangl, J.L. 2010. NB-LRR proteins: pairs, pieces, perception, partners, and

pathways. Curr. Opin. Plant Biol. **13**(4): 472–7. Elsevier Ltd. doi:10.1016/j.pbi.2010.04.007.

Ellis, J.G., Lagudah, E.S., Spielmeyer, W., and Dodds, P.N. 2014. The past, Present and future of breeding rust resistant wheat. Front. Plant Sci. **5**: 641. doi:10.3389/fpls.2014.00641.

Engqvist, L.G. 1994. Distribution of clubroot (*Plasmodiophora brassicae* Wor) in Sweden and the effect of infection on oil content of oilseed rape (*Brassica napus* L.). J. Swedish Seed Assoc. **104**(2): 82–86.

Ernst, T.W., Kher, S., Stanton, D., Rennie, D.C., Hwang, S.F., and Strelkov, S.E. 2019. *Plasmodiophora brassicae* resting spore dynamics in clubroot resistant canola (*Brassica napus*) cropping systems. Plant Pathol. **68**(2): 399–408. doi:10.1111/ppa.12949.

Ewing, B., and Green, P. 1998. Base-calling of automated sequencer traces using Phred. II. Error probabilities. Genome Res. **8**(3): 186–194. doi:10.1101/gr.8.3.186.

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**(2): 91–101. doi:10.1139/gen-2019-0098.

Feechan, A., Kocsis, M., Riaz, S., Zhang, W., Gadoury, D.M., Walker, M.A., Dry, I.B., Reisch, B., and Cadle-Davidson, L. 2015. Strategies for *RUN1* deployment using *RUN2* and *REN2* to manage grapevine powdery mildew informed by studies of race specificity. Phytopathology **105**(8): 1104–1113. doi:10.1094/PHYTO-09-14-0244-R.

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.

Felix, G., Duran, J.D., Volko, S., and Boller, T. 1999. Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J. **18**(3): 265–276. doi:10.1046/j.1365-313X.1999.00265.x.

Feng, J., Hwang, R., Hwang, S.-F., Strelkov, S.E., Gossen, B.D., Zhou, Q.-X., and Peng, G. 2010. Molecular characterization of a serine protease Pro1 from *Plasmodiophora brassicae* that stimulates resting spore germination. Mol. Plant Pathol. **11**(4): 503–512. doi:10.1111/j.1364-3703.2010.00623.x.

Feng, J., Hwang, S.F., and Strelkov, S.E. 2013a. Studies into primary and secondary infection processes by *Plasmodiophora brassicae* on canola. Plant Pathol. **62**(1): 177–183. doi:10.1111/j.1365-3059.2012.02612.x.

Feng, J., Hwang, S.F., and Strelkov, S.E. 2013b. Assessment of gene expression profiles in

primary and secondary zoospores of *Plasmodiophora brassicae* by dot blot and real-time PCR. Microbiol. Res. **168**(8): 518–524. Elsevier GmbH. doi:10.1016/j.micres.2013.02.011.

Ferreira, M.E., Satagopan, J., Yandell, B.S., Williams, P.H., and Osborn, T.C. 1995. Mapping loci controlling vernalization requirement and flowering time in *Brassica napus*. Theor. Appl. Genet. **90**(5): 727–732. doi:10.1007/BF00222140.

Frauen, M. 1999. A new clubroot resistant variety in winter oilseed rape. *In* 10th International Rapeseed Congress. Canberra, Australia. pp. 1–3.

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**(2): 201–213. doi:10.1007/s10681-016-1730-2.

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

Fredua-Agyeman, R., Hwang, S.F., Strelkov, S.E., Zhou, Q., Manolii, V.P., and Feindel, D. 2019. Identification of Brassica accessions resistant to 'old' and 'new' pathotypes of *Plasmodiophora brassicae* from Canada. Plant Pathol. **68**(4): 708–718. doi:10.1111/ppa.12980.

Friberg, H. 2005. Persistence of *Plasmodiophora brassicae*. University of Uppsala.

Friedt, W., and Snowdon, R. 2010. Oil Crops. *In* Handbook of Plant Breeding, 4th edition. *Edited by* J. Vollmann and I. Rajcan. Springer New York, New York, NY. doi:10.1007/978-0-387-77594-4.

Fu, P., Piao, Y., Zhan, Z., Zhao, Y., Pang, W., Li, X., and Piao, Z. 2019. 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.B., and Gugel, R.K. 2010. Genetic diversity of Canadian elite summer rape (*Brassica napus* L.) cultivars from the pre- To post-canola quality era. Can. J. Plant Sci. **90**(1): 23–33. doi:10.4141/CJPS09073.

Fukuoka, S., Saka, N., Mizukami, Y., Koga, H., Yamanouchi, U., Yoshioka, Y., Hayashi, N., Ebana, K., Mizobuchi, R., and Yano, M. 2015. Gene pyramiding enhances durable blast disease resistance in rice. Sci. Rep. **5**: 1–7. doi:10.1038/srep07773.

Gaeta, R.T., and Chris Pires, J. 2010. Homoeologous recombination in allopolyploids: the

polyploid ratchet. New Phytol. **186**(1): 18–28. doi:10.1111/j.1469-8137.2009.03089.x.

Gaeta, R.T., Pires, J.C., Iniguez-Luy, F., Leon, E., and Osborn, T.C. 2007. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell **19**(11): 3403–3417. doi:10.1105/tpc.107.054346.

Gamborg, O., Miller, R., and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. **50**(1): 151–158. doi:10.1016/0014-4827(68)90403-5.

Gaulin, E., Dramé, N., Lafitte, C., Torto-Alalibo, T., Martinez, Y., Ameline-Torregrosa, C., Khatib, M., Mazarguil, H., Villalba-Mateos, F., Kamoun, S., Mazars, C., Dumas, B., Bottin, A., Esquerré-Tugayé, M.T., and Rickauer, M. 2006. Cellulose binding domains of a Phytophthora cell wall protein are novel pathogen-associated molecular patterns. Plant Cell **18**(7): 1766–1777. doi:10.1105/tpc.105.038687.

Ghanbari, M., Paul, M., and Möllers, C. 2020. QTL analysis of shoot elongation before winter in relation to vernalization requirement in the doubled haploid population L16 × Express617 (*Brassica napus* L.). Euphytica **216**(5): 73. doi:10.1007/s10681-020-02604-y.

Gish, W. 2003. WU BLAST. Available from http://blast.wustl.edu.

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.

Gossen, B., McDonald, M., Hwang, S., and Kalpana, K. 2009. Manipulating seeding date to minimize clubroot (*Plasmodiophora brassicae*) damage in canola and vegetable Brassicas. Phytopathology **99**(6): S45.

Graf, A., Schlereth, A., Stitt, M., and Smith, A.M. 2010. Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. Proc. Natl. Acad. Sci. **107**(20): 9458–9463. doi:10.1073/pnas.0914299107.

Gravot, A., Deleu, C., Wagner, G., Lariagon, C., Lugan, R., Todd, C., Wendehenne, D., Delourme, R., Bouchereau, A., and Manzanares-Dauleux, M.J. 2012. Arginase induction represses gall development during clubroot infection in *Arabidopsis*. Plant Cell Physiol. **53**(5): 901–911. doi:10.1093/pcp/pcs037.

Groos, C., Robert, N., Bervas, E., and Charmet, G. 2003. Genetic analysis of grain protein-content, grain yield and thousand-kernel weight in bread wheat. Theor. Appl. Genet. **106**(6): 1032–1040. doi:10.1007/s00122-002-1111-1.

Grsic-Rausch, S., Kobelt, P., Siemens, J.M., Bischoff, M., and Ludwig-Müller, J. 2000. Expression and localization of nitrilase during symptom development of the clubroot

disease in *Arabidopsis*. Plant Physiol. **122**(2): 369–378. doi:10.1104/pp.122.2.369.

Gunasekera, C.P., Martin, L.D., Siddique, K.H.M., and Walton, G.H. 2006. Genotype by environment interactions of Indian mustard (*Brassica juncea* L.) and canola (*Brassica napus* L.) in Mediterranean-type environments. II. Oil and protein concentrations in seed. Eur. J. Agron. **25**: 13–21. doi:10.1016/j.eja.2006.02.001.

Gust, A.A., Biswas, R., Lenz, H.D., Rauhut, T., Ranf, S., Kemmerling, B., Götz, F., Glawischnig, E., Lee, J., Felix, G., and Nürnberger, T. 2007. Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis. J. Biol. Chem. **282**(44): 32338–32348. doi:10.1074/jbc.M704886200.

Gustafsson, M., and Falt, A.-S. 1986. Genetic studies on resistance to clubroot in *Brassica napus*. Ann. Appl. Biol. **108**(2): 409–415. doi:10.1111/j.1744-7348.1986.tb07663.x.

Habekotté, B. 1997. Options for increasing seed yield of winter oilseed rape (*Brassica napus* L.): A simulation study. F. Crop. Res. **54**(2–3): 109–126. doi:10.1016/S0378-4290(97)00041-5.

Han, F., Fedak, G., Guo, W., and Liu, B. 2005. Rapid and repeatable elimination of a parental genome-specific repeat (pGc1R-1a) in newly synthesized wheat allopolyploids. Genetics **170**(3): 1239–1245. doi:10.1534/genetics.104.039263.

Hasan, 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, J., Shaikh, R., Megha, S., Herrmann, D.T., Kebede, B., and Rahman, H. 2021b. Mapping of flowering time, seed quality and clubroot resistance in rutabaga × spring canola populations and their association. Euphytica **217**(8): 160. doi:10.1007/s10681-021-02889-7.

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:10.1270/jsbbs.18010.

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**(10): 805–815. doi:10.1139/gen-2016-0034.

Hasan, M.J., Strelkov, S., Howard, R., 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**(3): 501–515.

doi:10.4141/CJPS2010-006.

Hasan, M.J. 2010. Screening of Brassica germplasm for resistance to *Plasmodiophora brassicae* pathotypes prevalent in Alberta, Canada. MSc thesis, Department of Agricultural, Food and Nutritional Science, University of Alberta.

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 Matsumoto, S. 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., Sakamoto, K., and Matsumoto, E. 2008. Construction of a practical SCAR marker linked to clubroot resistance in chinese cabbage, with intensive analysis of *HC352b* Genes. J. Japanese Soc. Hortic. Sci. **77**(2): 150–154. doi:10.2503/jjshs1.77.150.

He, Y., Fu, Y., Hu, D., Wei, D., and Qian, W. 2018. QTL mapping of seed glucosinolate content responsible for environment in *Brassica napus*. Front. Plant Sci. **9**(June): 891. doi:10.3389/fpls.2018.00891.

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. Breed. **39**(8): 112. doi:10.1007/s11032-019-1021-4.

Henderson, I.R., Liu, F., Drea, S., Simpson, G.G., and Dean, C. 2005. An allelic series reveals essential roles for FY in plant development in addition to flowering-time control. Development **132**(16): 3597–3607. doi:10.1242/dev.01924.

Hirai, M. 2006. Genetic analysis of clubroot resistance in Brassica Crops. Breed. Sci. **56**(3): 223–229. doi:10.1270/jsbbs.56.223.

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): 639–43. doi:10.1007/s00122-003-1475-x.

Hirani, A.H., Gao, F., Liu, J., Fu, G., Wu, C., McVetty, P.B.E., Duncan, R.W., and Li, G. 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. **871**(November). doi:10.3389/fpls.2018.01628.

Hirani, A.H., Gao, F., Liu, J., Fu, G., Wu, C., Yuan, Y., Li, W., Hou, J., Duncan, R., and Li, G. 2016. Transferring clubroot resistance from Chinese cabbage (*Brassica rapa*) to canola (*B. napus*). Can. J. Plant Pathol. **38**(1): 82–90. doi:10.1080/07060661.2016.1141799.

Hobson, N., and Rahman, H. 2016. Genome-wide identification of SSR markers in the Brassica A genome and their utility in breeding. Can. J. Plant Sci. **96**(5): 808–818. doi:10.1139/cjps-2015-0250.

Holtz, M.D., Hwang, S.-F., and Strelkov, S.E. 2018. Genotyping of *Plasmodiophora brassicae* reveals the presence of distinct populations. BMC Genomics **19**(1): 254. doi:10.1186/s12864-018-4658-1.

Horiuchi, S., and Hori, M. 1980. A simple greenhouse technique for obtaining high levels of clubroot incidence. Bull. Chugoku Natl. Agric. Exp. Stn. Ser. E (Environ Div) **17**: 33–35.

Hou, S., Liu, Z., Shen, H., and Wu, D. 2019. Damage-associated molecular pattern-triggered immunity in plants. Front. Plant Sci. **10**: 646. doi:10.3389/fpls.2019.00646.

Hou, S., Yang, Y., Wu, D., and Zhang, C. 2011. Plant immunity evolutionary insights from PBS1, pto and RIN4. Plant Signal. Behav. **6**(6): 794–799. doi:10.4161/psb.6.6.15143.

Howell, P.M., Sharpe, A.G., and Lydiate, D.J. 2003. Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome **46**(3): 454–460. doi:10.1139/g03-028.

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. Breed. **39**(9): 131. doi:10.1007/s11032-019-1038-8.

Huang, Z., Peng, G., Liu, X., Deora, A., Falk, K.C., Gossen, B.D., McDonald, M.R., and Yu, F. 2017. Fine Mapping of a Clubroot Resistance Gene in Chinese Cabbage Using SNP Markers Identified from Bulked Segregant RNA Sequencing. Front. Plant Sci. **8**(August): 1–9. doi:10.3389/fpls.2017.01448.

Hwang, S.F., Ahmed, H.U., Strelkov, S.E., Gossen, B.D., Turnbull, G.D., Peng, G., and Howard, R.J. 2011a. Seedling age and inoculum density affect clubroot severity and seed yield in canola. Can. J. Plant Sci. **91**(1): 183–190. doi:10.4141/CJPS10066.

Hwang, S.F., Ahmed, H.U., Strelkov, S.E., Zhou, Q., Gossen, B.D., McDonald, M.R., Peng, G., and Turnbull, G.D. 2017a. 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., and Strelkov, S.E. 2018. Management of clubroot of canola in Alberta, Canada. *In* 2018 International Clubroot Workshop. Canadian Journal of Plant Pathology, Edmonton,

Alberta, Canada. p. 483. doi:10.1080/07060661.2019.1583478.

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., Strelkov, S.E., Gossen, B.D., Peng, G., and Turnbull, G.D. 2011b. Influence of cultivar resistance and inoculum density on root hair infection of canola (*Brassica napus*) by *Plasmodiophora brassicae*. Plant Pathol. **60**(5): 820–829. doi:10.1111/j.1365-3059.2011.02457.x.

Hwang, S.F., Ahmed, H.U., Zhou, Q., Strelkov, S.E., Gossen, B.D., Peng, G., and Turnbull, G.D. 2012a. Assessment of the impact of resistant and susceptible canola on *Plasmodiophora brassicae* inoculum potential. Plant Pathol. **61**(5): 945–952. doi:10.1111/j.1365-3059.2011.02582.x.

Hwang, S.F., Ahmed, H.U., Zhou, Q., Strelkov, S.E., Gossen, B.D., Peng, G., and Turnbull, G.D. 2014a. Efficacy of Vapam fumigant against clubroot (*Plasmodiophora brassicae*) of canola. Plant Pathol. **63**(6): 1374–1383. doi:10.1111/ppa.12207.

Hwang, S.F., Ahmed, H.U., Zhou, Q., Turnbull, G.D., Strelkov, S.E., Gossen, B.D., and Peng, G. 2015. Effect of host and non-host crops on *Plasmodiophora brassicae* resting spore concentrations and clubroot of canola. Plant Pathol. **64**(5): 1198–1206. doi:10.1111/ppa.12347.

Hwang, S.F., Cao, T., Xiao, Q., Ahmed, H.U., Manolii, V.P., Turnbull, G.D., Gossen, B.D., Peng, G., and Strelkov, S.E. 2012b. Effects of fungicide, seeding date and seedling age on clubroot severity, seedling emergence and yield of canola. Can. J. Plant Sci. **92**(6): 1175–1186. doi:10.4141/CJPS2011-149.

Hwang, S.F., Howard, R.J., Strelkov, S.E., Gossen, B.D., and Peng, G. 2014b. Management of clubroot (*Plasmodiophora brassicae*) on canola (*Brassica napus*) in western Canada. Can. J. Plant Pathol. **36**(sup1): 49–65. doi:10.1080/07060661.2013.863806.

Hwang, S.F., Strelkov, S.E., Ahmed, H.U., Manolii, V.P., Zhou, Q., Fu, H., Turnbull, G., Fredua-Agyeman, R., and Feindel, D. 2017b. Virulence and inoculum density-dependent interactions between clubroot resistant canola (*Brassica napus*) and *Plasmodiophora brassicae*. Plant Pathol. **66**(8): 1318–1328. doi:10.1111/ppa.12688.

Hwang, S.F., Strelkov, S.E., Gossen, B.D., Turnbull, G.D., Ahmed, H.U., and Manolii, V.P. 2011c. Soil treatments and amendments for amelioration of clubroot of canola. Can. J. Plant Sci. **91**(6): 999–1010. doi:10.4141/cjps2011-028.

Ingram, D., and Tommerup, I. 1972. The Life History of *Plasmodiophora brassicae* Woron.

Proc. R. Soc. B Biol. Sci. **180**: 103–112. doi:10.1098/rspb.1972.0008.

Irani, S., Trost, B., Waldner, M., Nayidu, N., Tu, J., Kusalik, A.J., Todd, C.D., Wei, Y., and Bonham-Smith, P.C. 2018. Transcriptome analysis of response to *Plasmodiophora brassicae* infection in the *Arabidopsis* shoot and root. BMC Genomics **19**(1): 23. doi:10.1186/s12864-017-4426-7.

Ito, S., Ichinose, H., Yanagi, C., Tanaka, S., Kameya-Iwaki, M., and Kishi, F. 1999. Identification of an in planta-induced mRNA of *Plasmodiophora brassicae*. J. Phytopathol. **147**(2): 79–82. doi:10.1046/j.1439-0434.1999.147002079.x.

Iwakawa, H., and Tomari, Y. 2015. The functions of microRNAs: mRNA decay and translational repression. Trends Cell Biol. **25**(11): 651–665. doi:10.1016/j.tcb.2015.07.011.

Jahn, L., Mucha, S., Bergmann, S., Horn, C., Staswick, P., Steffens, B., Siemens, J., and Ludwig-Müller, J. 2013. The clubroot pathogen (*Plasmodiophora brassicae*) influences auxin signaling to regulate auxin homeostasis in *Arabidopsis*. Plants **2**(4): 726–749. doi:10.3390/plants2040726.

Javed, N., Geng, J., Tahir, M., McVetty, P.B.E., Li, G., and Duncan, R.W. 2016. Identification of QTL influencing seed oil content, fatty acid profile and days to flowering in *Brassica napus* L. Euphytica **207**(1): 191–211. doi:10.1007/s10681-015-1565-2.

Ji, R., Wang, Y., Wang, X., Liu, Y., Shen, X., and Feng, H. 2018. Proteomic analysis of the interaction between *Plasmodiophora brassicae* and Chinese cabbage (*Brassica rapa* L. ssp. *Pekinensis*) at the initial infection stage. Sci. Hortic. (Amsterdam). **233**: 386–393. doi:10.1016/j.scienta.2018.02.006.

Jia, H., Wei, X., Yang, Y., Yuan, Y., Wei, F., Zhao, Y., Yang, S., Yao, Q., Wang, Z., Tian, B., and Zhang, X. 2017. Root RNA-seq analysis reveals a distinct transcriptome landscape between clubroot-susceptible and clubroot-resistant Chinese cabbage lines after *Plasmodiophora brassicae* infection. Plant Soil **421**(1–2): 93–105. doi:10.1007/s11104-017-3432-5.

Jia, Y.J., Feng, B.Z., Sun, W.X., and Zhang, X.G. 2009. Polygalacturonase, pectate lyase and pectin methylesterase activity in pathogenic strains of *Phytophthora capsici* incubated under different conditions. J. Phytopathol. **157**(10): 585–591. doi:10.1111/j.1439-0434.2008.01533.x.

Jiang, C., Shi, J., Li, R., Long, Y., Wang, H., Li, D., Zhao, J., and Meng, J. 2014. Quantitative trait loci that control the oil content variation of rapeseed (*Brassica napus* L.). Theor. Appl. Genet. **127**(4): 957–968. doi:10.1007/s00122-014-2271-5.

Jiang, H., Li, Z., Liu, J., Shen, Z., Gao, G., Zhang, Q., and He, Y. 2019. Development and evaluation of improved lines with broad-spectrum resistance to rice blast using nine resistance genes. Rice **12**(1). Rice. doi:10.1186/s12284-019-0292-z.

Jiang, J., Fredua-Agyeman, R., Strelkov, S.E., and Hwang, S.-F. 2020. Suppression of Canola (*Brassica napus*) resistance by virulent isolates of *Plasmodiophora brassicae* (Clubroot) during primary infection. Plant Dis. **104**(2): 430–437. doi:10.1094/PDIS-03-19-0659-RE.

Johnston, T. 1970. A new factor for resistance to club root in *Brassica napus* L. Plant Pathol. **19**(4): 156–158. doi:10.1111/j.1365-3059.1970.tb01007.x.

Jones, J.D., and Dangl, J.L. 2006. The plant immune system. Nature **444**(7117): 323–329.

Joshi, R., and Nayak, S. 2010. Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. Biotechnol Mol. Biol. Rev. **5**(3): 51–60.

Julius, B.T., Leach, K.A., Tran, T.M., Mertz, R.A., and Braun, D.M. 2017. Sugar transporters in plants: New Insights and discoveries. Plant Cell Physiol. **58**(9): 1442–1460. doi:10.1093/pcp/pcx090.

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.

Karim, M.M., Dakouri, A., Zhang, Y., Chen, Q., Peng, G., Strelkov, S.E., Gossen, B.D., and Yu, F. 2020. Two clubroot-resistance genes, *Rcr3* and *Rcr9*^{wa}, mapped in *Brassica rapa* using bulk segregant RNA sequencing. Int. J. Mol. Sci. **21**(14): 1–15. doi:10.3390/ijms21145033.

Karling, J. 1969. Plasmodiophorales. *In* 2nd edition. Hafner Publishing Co Ltd, New York.

Kato, T., Hatakeyama, K., Fukino, N., and Matsumoto, S. 2012. Identificaiton of a clubroot resistance locus conferring resistance to a *Plasmodiophora brassicae* classified into pathotype group 3 in Chinese cabbage (*Brassica rapa* L.). Breed. Sci. **62**(3): 282–287. doi:10.1270/jsbbs.62.282.

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): 116–24. doi:10.1270/jsbbs.63.116.

Kenney, A.M., Mckay, J.K., Richards, J.H., and Juenger, T.E. 2014. Direct and indirect selection on flowering time, water-use efficiency (WUE, δ13C), and WUE plasticity to drought in *Arabidopsis thaliana*. Ecol. Evol. **4**(23): 4505–4521. doi:10.1002/ece3.1270.

Kittipol, V., He, Z., Wang, L., Doheny-Adams, T., Langer, S., and Bancroft, I. 2019. Genetic architecture of glucosinolate variation in *Brassica napus*. J. Plant Physiol. **240**: 152988. Elsevier. doi:10.1016/j.jplph.2019.06.001.

Kjær, B., Jensen, H.P., Jensen, J., and Jørgensen, J.H. 1990. Associations between three mlo powdery mildew resistance genes and agronomic traits in barley. Euphytica **46**(3): 185–193. doi:10.1007/BF00027217.

Knott, D.R., and Slinkard, A.E. 1995. Harvest of gold : the history of field crop breeding in Canada. University Extension Press, University of Saskatchewan.

Koboldt, D.C., Zhang, Q., Larson, D.E., Shen, D., McLellan, M.D., Lin, L., Miller, C.A., Mardis, E.R., Ding, L., and Wilson, R.K. 2012. VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res. **22**(3): 568–576. doi:10.1101/gr.129684.111.

Kondra, Z.P., and Stefansson, B.R. 1970. Inheritance of the major glucosinolates of rapeseed (*Brassica napus*) meal. Can. J. Plant Sci. **50**(6): 643–647. doi:10.4141/cjps70-122.

Kosambi, D.D. 1943. The estimation of map distances from recombination values. Ann. Eugen. **12**(1): 172–175. doi:10.1111/j.1469-1809.1943.tb02321.x.

Kroll, T.K., Lacy, G.H., and Moore, L.D. 1983. A quantitative description of the colonization of susceptible and resistant radish plants by *Plasmodiophora brassicae*. J. Phytopathol. **108**(2): 97–105. doi:10.1111/j.1439-0434.1983.tb00568.x.

Kuang, H., Wei, F., Marano, M.R., Wirtz, U., Wang, X., Liu, J., Shum, W.P., Zaborsky, J., Tallon, L.J., Rensink, W., Lobst, S., Zhang, P., Tornqvist, C.E., Tek, A., Bamberg, J., Helgeson, J., Fry, W., You, F., Luo, M.C., Jiang, J., Robin Buell, C., and Baker, B. 2005. The R1 resistance gene cluster contains three groups of independently evolving, type I R1 homologues and shows substantial structural variation among haplotypes of *Solanum demissum*. Plant J. **44**(1): 37–51. doi:10.1111/j.1365-313X.2005.02506.x.

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

Kunze, G., Zipfel, C., Robatzek, S., Niehaus, K., Boller, T., and Felix, G. 2004. The N terminus of bacterial elongation factor Tu elicits innate immunity in Arabidopsis plants. Plant Cell **16**(12): 3496–3507. doi:10.1105/tpc.104.026765.

Lagercrantz, U., and Lydiate, D. 1996. Comparative genome mapping in Brassica. Genetics **144**(4): 1903–1910.

Laila, R., Park, J.I., Robin, A.H.K., Natarajan, S., Vijayakumar, H., Shirasawa, K., Isobe, S., Kim, H.T., and Nou, I.S. 2019. Mapping of a novel clubroot resistance QTL using ddRAD-seq in Chinese cabbage (*Brassica rapa* L.). BMC Plant Biol. **19**(1): 1–9. doi:10.1186/s12870-018-1615-8.

Lammerink, J. 1967. The inheritance of clubroot resistance in *Brassica napus* L. New Zeal. J.

Agric. Res. **10**(1): 109–115. doi:10.1080/00288233.1967.10423081.

Lammerink, J. 1970. Inter-specific transfer of clubroot resistance from Brassica campestris L. to *B. napus* L. New Zeal. J. Agric. Res. **13**(1): 105–110. doi:10.1080/00288233.1970.10421200.

Lan, M., Li, G., Hu, J., Yang, H., Zhang, L., Xu, X., Liu, J., He, J., and Sun, R. 2019. iTRAQ-based quantitative analysis reveals proteomic changes in Chinese cabbage (*Brassica rapa* L.) in response to *Plasmodiophora brassicae* infection. Sci. Rep. **9**(1): 12058. doi:10.1038/s41598-019-48608-0.

Laperche, A., Aigu, Y., Jubault, M., Ollier, M., Guichard, S., Glory, P., Strelkov, S., Gravot, A., and Manzanares-Dauleux, M. 2017. Clubroot resistance QTL are modulated by nitrogen input in *Brassica napus*. Theor. Appl. Genet. **130**(4): 669–684. doi:10.1007/s00122-016-2842-8.

Laroche, A., Frick, M., Graf, R.J., Larsen, J., and Laurie, J.D. 2019. Pyramiding disease resistance genes in elite winter wheat germplasm for Western Canada. Crop J. **7**(6): 739–749. doi:10.1016/j.cj.2019.08.005.

Laurens, F., and Thomas, G. 1993. Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassica oleracea* ssp. *acephala*). Hereditas **119**: 253–262.

Leclerc, M., Clément, J.A.J., Andrivon, D., and Hamelin, F.M. 2019. Assessing the effects of quantitative host resistance on the life-history traits of sporulating parasites with growing lesions. Proc. R. Soc. B Biol. Sci. **286**(1912): 20191244. doi:10.1098/rspb.2019.1244.

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.

Lee, J., Izzah, N.K., Choi, B.-S., Joh, H.J., Lee, S.-C., Perumal, S., Seo, J., Ahn, K., Jo, E.J., Choi, G.J., Nou, I.-S., Yu, Y., and Yang, T.-J. 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.

Leister, D. 2004. Tandem and segmental gene duplication and recombination in the evolution of plant disease resistance genes. Trends Genet. **20**(3): 116–122. doi:10.1016/j.tig.2004.01.007.

Leitch, I., and Bennett, M. 2004. Genome downsizing in polyploid plants. Biol. J. Linn. Soc. **82**(4): 651–663. doi:10.1111/j.1095-8312.2004.00349.x.

Lemarié, S., Robert-Seilaniantz, A., Lariagon, C., Lemoine, J., Marnet, N., Jubault, M., Manzanares-Dauleux, M.J., and Gravot, A. 2015. Both the Jasmonic Acid and the

Salicylic Acid Pathways contribute to resistance to the biotrophic clubroot agent *Plasmodiophora brassicae* in *Arabidopsis*. Plant Cell Physiol. **56**(11): 2158–2168. doi:10.1093/pcp/pcv127.

Lemoine, R., Camera, S. La, Atanassova, R., Dédaldéchamp, F., Allario, T., Pourtau, N., Bonnemain, J.-L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J., and Durand, M. 2013. Source-to-sink transport of sugar and regulation by environmental factors. Front. Plant Sci. **4**: 272. doi:10.3389/fpls.2013.00272.

Li, C. 2016. Combining selective pressures to enhance the durability of disease resistance genes. Front. Plant Sci. **7**(1916). doi:10.3389/fpls.2016.01916.

Li, F., Chen, B., Xu, K., Wu, J., Song, W., Bancroft, I., Harper, A.L., Trick, M., Liu, S., Gao, G., Wang, N., Yan, G., Qiao, J., Li, J., Li, H., Xiao, X., Zhang, T., and Wu, X. 2014. Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.). DNA Res. **21**(4): 355–367. doi:10.1093/dnares/dsu002.

Li, H., and Durbin, R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics **25**(14): 1754–1760. doi:10.1093/bioinformatics/btp324.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R. 2009. The sequence Alignment/Map format and SAMtools. Bioinformatics **25**(16): 2078–2079. doi:10.1093/bioinformatics/btp352.

Li, H., Li, X., Xuan, Y., Jiang, J., Wei, Y., and Piao, Z. 2018. Genome wide identification and expression profiling of SWEET genes family reveals its role during *Plasmodiophora brassicae*-induced formation of clubroot in *Brassica rapa*. Front. Plant Sci. **9**: 207. doi:10.3389/fpls.2018.00207.

Li, H., Ye, G., and Wang, J. 2007a. A modified algorithm for the improvement of Composite Interval Mapping. Genetics **175**(1): 361–374. doi:10.1534/genetics.106.066811.

Li, J., Ding, J., Zhang, W., Zhang, Y., Tang, P., Chen, J.Q., Tian, D., and Yang, S. 2010a. Unique evolutionary pattern of numbers of gramineous NBS-LRR genes. Mol. Genet. Genomics **283**(5): 427–438. doi:10.1007/s00438-010-0527-6.

Li, L., Long, Y., Li, H., and Wu, X. 2020. Comparative Transcriptome analysis reveals key pathways and hub genes in rapeseed during the early stage of *Plasmodiophora brassicae* infection. Front. Genet. **10**: 1275. doi:10.3389/fgene.2019.01275.

Li, L., Luo, Y., Chen, B., Xu, K., Zhang, F., Li, H., Huang, Q., Xiao, X., Zhang, T., Hu, J., Li, F., and Wu, X. 2016. A genome-wide association study reveals new loci for resistance to

clubroot disease in *Brassica napus*. Front. Plant Sci. **7**: 1483. doi:10.3389/fpls.2016.01483.

Li, N., Han, X., Feng, D., Yuan, D., and Huang, L.-J. 2019. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense: Do we understand what they are whispering? Int. J. Mol. Sci. **20**(3): 671. doi:10.3390/ijms20030671.

Li, X., Quigg, R.J., Zhou, J., Xu, S., Masinde, G., Mohan, S., and Baylink, D.J. 2006. A critical evaluation of the effect of population size and phenotypic measurement on QTL detection and localization using a large F2 murine mapping population. Genet. Mol. Biol. **29**(1): 166–173. doi:10.1007/11760146_15.

Li, Y., Zhang, Q., Zhang, J., Wu, L., Qi, Y., and Zhou, J.-M. 2010b. Identification of MicroRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. Plant Physiol. **152**(4): 2222–2231. doi:10.1104/pp.109.151803.

Li, Y.Q., Yang, S.H., Yang, H.J., and Hua, J. 2007b. The TIR-NB-LRR gene *SNC1* is regulated at the transcript level by multiple factors. Mol. Plant-Microbe Interact. **20**(11): 1449–1456. doi:Doi 10.1094/Mpmi-20-11-1449.

Lichter, R. 1982. Induction of haploid plants from isolated pollen of *Brassica napus*. Zeitschrift für Pflanzenphysiologie **105**(5): 427–434. doi:http://dx.doi.org/10.1016/j.bbr.2011.03.031.

Lindhout, P. 2002. The perspectives of polygenic resistance in breeding for durable disease resistance. Euphytica **124**(2): 217–226. doi:10.1023/A:1015686601404.

Liu, B., Vega, J.M., Segal, G., Abbo, S., Rodova, M., and Feldman, M. 1998. Rapid genomic changes in newly synthesized amphiploids of *Triticum* and *Aegilops*. I. Changes in low-copy noncoding DNA sequences. Genome **41**(2): 272–277. doi:10.1139/g98-011.

Liu, J., Hirani, A.H., Li, Z., Wu, C., McVetty, P.B.E., Daayf, F., and Li, G. 2016a. QTL controlling glucosinolate content in seeds of *Brassica napus* L. Aust. J. Crop Sci. **10**(2): 152–160.

Liu, J., Liu, X., Dai, L., and Wang, G. 2007. Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. J. Genet. Genomics **34**(9): 765–776. doi:10.1016/S1673-8527(07)60087-3.

Liu, S., Fan, C., Li, J., Cai, G., Yang, Q., Wu, J., Yi, X., Zhang, C., and Zhou, Y. 2016b. A genome-wide association study reveals novel elite allelic variations in seed oil content of *Brassica napus*. Theor. Appl. Genet. **129**(6): 1203–1215. doi:10.1007/s00122-016-2697-z.

Liu, Y., Xu, A., Liang, F., Yao, X., Wang, Y., Liu, X., Zhang, Y., Dalelhan, J., Zhang, B., Qin,

M., Huang, Z., and Shaolin, L. 2018. Screening of clubroot-resistant varieties and transfer of clubroot resistance genes to *Brassica napus* using distant hybridization. Breed. Sci. **68**(2): 258–267. doi:10.1270/jsbbs.17125.

Ljung, K. 2013. Auxin metabolism and homeostasis during plant development. Development **140**(5): 943–950. doi:10.1242/dev.086363.

De Lorenzo, G., Brutus, A., Savatin, D.V., Sicilia, F., and Cervone, F. 2011. Engineering plant resistance by constructing chimeric receptors that recognize damage-associated molecular patterns (DAMPs). FEBS Lett. **585**(11): 1521–1528. doi:10.1016/j.febslet.2011.04.043.

Lovelock, D.A., Donald, C.E., Conlan, X.A., and Cahill, D.M. 2013. Salicylic acid suppression of clubroot in broccoli (*Brassicae oleracea* var. *italica*) caused by the obligate biotroph *Plasmodiophora brassicae*. Australas. Plant Pathol. **42**(2): 141–153. doi:10.1007/s13313-012-0167-x.

Lowe, A., Jones, A., Raybould, A., Trick, M., Moule, C., and Edwards, K. 2002. Transferability and genome specificity of a new set of microsatellite primers among Brassica species of the U triangle. Mol. Ecol. Notes **2**(1): 7–11. doi:10.1046/j.1471-8286.2002.00126.x.

Lowe, A., Moule, C., Trick, M., and Edwards, K. 2004. Efficient large-scale development of microsatellites for marker and mapping applications in Brassica crop species. Theor. Appl. Genet. **108**(6): 1103–12. doi:10.1007/s00122-003-1522-7.

Lu, K., Wei, L., Li, X., Wang, Y., Wu, J., Liu, M., Zhang, C., Chen, Z., Xiao, Z., Jian, H., Cheng, F., Zhang, K., Du, H., Cheng, X., Qu, C., Qian, W., Liu, L., Wang, R., Zou, Q., Ying, J., Xu, X., Mei, J., Liang, Y., Chai, Y.R., Tang, Z., Wan, H., Ni, Y., He, Y., Lin, N., Fan, Y., Sun, W., Li, N.N., Zhou, G., Zheng, H., Wang, X., Paterson, A.H., and Li, J. 2019. Whole-genome resequencing reveals *Brassica napus* origin and genetic loci involved in its improvement. Nat. Commun. **10**(1): 1–12. doi:10.1038/s41467-019-09134-9.

Lu, S., Sun, Y.-H., Amerson, H., and Chiang, V.L. 2007. MicroRNAs in loblolly pine (*Pinus taeda* L.) and their association with fusiform rust gall development. Plant J. **51**(6): 1077–1098. doi:10.1111/j.1365-313X.2007.03208.x.

Ludwig-Müller, J. 2009a. Plant defence – what can we learn from clubroots? Australas. Plant Pathol. **38**(4): 318. doi:10.1071/AP09020.

Ludwig-Müller, J. 2009b. Glucosinolates and the clubroot disease: defense compounds or auxin precursors? Phytochem. Rev. **8**(1): 135–148. doi:10.1007/s11101-008-9096-2.

Ludwig-Müller, J. 2014. Auxin homeostasis, signaling, and interaction with other growth

hormones during the clubroot disease of Brassicaceae. Plant Signal. Behav. **9**(4): e28593. doi:10.4161/psb.28593.

Ludwig-Müller, J., Prinsen, E., Rolfe, S.A., and Scholes, J.D. 2009. Metabolism and plant hormone action during clubroot disease. J. Plant Growth Regul. **28**(3): 229–244. doi:10.1007/s00344-009-9089-4.

Lv, H., Fang, Z., Yang, L., Zhang, Y., and Wang, Y. 2020. An update on the arsenal: mining resistance genes for disease management of Brassica crops in the genomic era. Hortic. Res. **7**(1): 34. doi:10.1038/s41438-020-0257-9.

Lysak, M.A., Cheung, K., Kitschke, M., and Bureš, P. 2007. Ancestral chromosomal blocks are triplicated in brassiceae species with varying chromosome number and genome size. Plant Physiol. **145**(2): 402–410. doi:10.1104/pp.107.104380.

Lysak, M.A., Koch, M.A., Pecinka, A., and Schubert, I. 2005. Chromosome triplication found across the tribe Brassiceae. Genome Res. **44**(4): 516–525. doi:10.1101/gr.3531105.

Maekawa, T., Kufer, T.A., and Schulze-Lefert, P. 2011. NLR functions in plant and animal immune systems: so far and yet so close. Nat. Immunol. **12**(9): 817–826. doi:10.1038/ni.2083.

Malinowski, R., Truman, W., and Blicharz, S. 2019. Genius architect or clever thief—How *Plasmodiophora brassicae* reprograms host development to establish a pathogen-oriented physiological sink. Mol. Plant-Microbe Interact. **32**(10): 1259–1266. doi:10.1094/MPMI-03-19-0069-CR.

Manichaikul, A., Moon, J.Y., Sen, Ś., 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 LP – 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**(5–6): 885–891. doi:10.1007/s001220051557.

Manzanares-Dauleux, M., Divaret, I., Baron, F., and Thomas, G. 2001. Assessment of biological and molecular variability between and within field isolates of *Plasmodiophora brassicae*. Plant Pathol. **50**(2): 165–173. doi:10.1046/j.1365-3059.2001.00557.x.

Mathesius, U. 2001. Flavonoids induced in cells undergoing nodule organogenesis in white clover are regulators of auxin breakdown by peroxidase. J. Exp. Bot. **52**(suppl 1): 419–426. doi:10.1093/jexbot/52.suppl_1.419.

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 Soc. Hortic. Sci. **81**(2): 184–190. doi:10.2503/jjshs1.81.184.

Matsumoto, E., Yasui, C., Ohi, M., and Tsukada, M. 1998. Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Euphytica **104**: 79–86.

McCouch, S.R., Cho, Y., Yano, M., Paul, E., Blinstrub, M., Morishima, H., and Kinosita, T. 1997. Report on QTL nomenclature. Rice Genet. Newsl. **14**: 11–13.

McDonald, M.R., Sharma, K., Gossen, B.D., Deora, A., Feng, J., and Hwang, S.F. 2014. The role of primary and secondary infection in host response to *Plasmodiophora brassicae*. Phytopathology **104**(10): 1078–1087. doi:10.1094/PHYTO-07-13-0189-R.

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., and DePristo, M.A. 2010. The genome analysis toolkit: A mapreduce framework for analyzing next-generation DNA sequencing data. Genome Res. **20**(9): 1297–1303. doi:10.1101/gr.107524.110.

Meglécz, E., Costedoat, C., Dubut, V., Gilles, A., Malausa, T., Pech, N., and Martin, J.-F. 2010. QDD: a user-friendly program to select microsatellite markers and design primers from large sequencing projects. Bioinformatics **26**(3): 403–404. doi:10.1093/bioinformatics/btp670.

Mei, J., Guo, Z., Wang, J., Feng, Y., Ma, G., Zhang, C., Qian, W., and Chen, G. 2019. Understanding the resistance mechanism in *Brassica napus* to clubroot caused by *Plasmodiophora brassicae*. Phytopathology **109**(5): 810–818. doi:10.1094/PHYTO-06-18-0213-R.

Méndez-Vigo, B., Picó, F.X., Ramiro, M., Martínez-Zapater, J.M., and Alonso-Blanco, C. 2011. Altitudinal and climatic adaptation is mediated by flowering traits and *FRI* , *FLC* , and *PHYC* Genes in *Arabidopsis*. Plant Physiol. **157**(4): 1942–1955. doi:10.1104/pp.111.183426.

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., Chin, D.B., Shen, K. a, Sivaramakrishnan, S., Lavelle, D.O., Zhang, Z., and Michelmore, R.W. 1998. The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell **10**(11): 1817–1832.

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.

Michelmore, R.W., and Meyers, B.C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. **8**(11): 1113–1130. doi:10.1101/gr.8.11.1113.

Michelmore, R.W., Paran, I., and Kesseli, R. V. 1991. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc. Natl. Acad. Sci. U. S. A. **88**(21): 9828–32. doi:10.1073/pnas.88.21.9828.

Miedes, E., Vanholme, R., Boerjan, W., and Molina, A. 2014. The role of the secondary cell wall in plant resistance to pathogens. Front. Plant Sci. **5**: 358. doi:10.3389/fpls.2014.00358.

Mok, D.W., and Mok, M.C. 2001. Cytokinin metabolism and action. Annu. Rev. Plant Physiol. Plant Mol. Biol. **52**(1): 89–118. doi:10.1146/annurev.arplant.52.1.89.

Monosi, B., Wisser, R.J., Pennill, L., and Hulbert, S.H. 2004. Full-genome analysis of resistance gene homologues in rice. Theor. Appl. Genet. **109**(7): 1434–47. doi:10.1007/s00122-004-1758-x.

Moriguchi, K., Kimizuka-Takagi, C., Ishii, K., and Nomura, K. 1999. A genetic map based on RAPD, RFLP, Isozyme, morphological markers and QTL analysis for clubroot resistance in *Brassica oleracea*. Breed. Sci. **49**: 257–265.

Morrison, M.J., Harker, K.N., Blackshaw, R.E., Holzapfel, C.J., and O'Donovan, J.T. 2016. Canola yield improvement on the Canadian Prairies from 2000 to 2013. Crop Pasture Sci. **67**(4): 245–252. Available from https://doi.org/10.1071/CP15348.

Mundt, C. 1990. Probability of mutation to multiple virulence and durability of resistance genes pyramids. Phytopathology **80**(3): 221–223. Available from http://www.apsnet.org/publications/phytopathology/backissues/Documents/1990Articles/Phyto80n03_221.PDF [accessed 30 May 2013].

Murakami, H., Tsushima, S., Kuroyanagi, Y., and Shishido, Y. 2002. Reduction of resting spore density of *Plasmodiophora brassicae* and clubroot disease severity by liming. Soil Sci. plant Nutr. **48**(5): 685–691.

Myers, D., Campbell, R., and Greathead, A. 1981. Clubroot of brassicas in California: soils respond differently to lime for clubroot control. Phytopathology **71**(9): 1005–1006.

Nagaoka, T., Doullah, M.A.U., Matsumoto, S., Kawasaki, S., Ishikawa, T., Hori, H., and Okazaki, K. 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**(7): 1335–1346. doi:10.1007/s00122-010-1259-z.

Nandety, R.S., Caplan, J.L., Cavanaugh, K., Perroud, B., Wroblewski, T., Michelmore, R.W., and Meyers, B.C. 2013. The role of TIR-NBS and TIR-X proteins in plant basal defense responses. Plant Physiol. **162**(3): 1459–72. doi:10.1104/pp.113.219162.

Neik, T.X., Barbetti, M.J., and Batley, J. 2017. Current status and challenges in identifying disease resistance genes in *Brassica napus*. Front. Plant Sci. **8**: 1788. doi:10.3389/fpls.2017.01788.

Nejat, N., and Mantri, N. 2018. Emerging roles of long non-coding RNAs in plant response to biotic and abiotic stresses. Crit. Rev. Biotechnol. **38**(1): 93–105. doi:10.1080/07388551.2017.1312270.

Newman, M.A., Daniels, M.J., and Dow, J.M. 1995. Lipopolysaccharide from *Xanthomonas campestris* induces defense-related gene expression in *Brassica campestris*. doi:10.1094/MPMI-8-0778.

Newman, M.A., Sundelin, T., Nielsen, J.T., and Erbs, G. 2013. MAMP (microbe-associated molecular pattern) triggered immunity in plants. Front. Plant Sci. **4**: 139. doi:10.3389/fpls.2013.00139.

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.

Ni, Z., Kim, E., Ha, M., Lackey, E., Liu, J., Zhang, Y., Sun, Q., and Chen, Z.J. 2009. Altered circadian rhythms regulate growth vigour in hybrids and allopolyploids. Nature **457**(7227): 327–31. doi:10.1038/nature07523.

Nieuwhof, M., and Wiering, D. 1961. Testing cabbage plants for clubroot resistance. Euphytica **10**(2): 191–200. doi:10.1007/BF00022210.

Niks, R.E., Qi, X., and Marcel, T.C. 2015. Quantitative resistance to biotrophic filamentous plant pathogens: concepts, misconceptions, and mechanisms. Annu. Rev. Phytopathol. **53**(1): 445–470. doi:10.1146/annurev-phyto-080614-115928.

Nikzad, A., Kebede, B., Pinzon, J., Bhavikkumar, J., Yang, R.-C., and Rahman, H. 2019. Potential of the C genome of different variants of *Brassica oleracea* for the improvement of agronomic and seed quality traits of *B. napus* canola. Crop Sci. **59**(6): 2608–2620. doi:10.2135/cropsci2019.05.0304.

Ning, Y., Wang, Y., Fang, Z., Zhuang, M., Zhang, Y., Lv, H., Liu, Y., Li, Z., and Yang, L. 2019. Comparative transcriptome analysis of cabbage (*Brassica oleracea* var. *capitata*) infected by *Plasmodiophora brassicae* reveals drastic defense response at secondary

infection stage. Plant Soil **443**(1–2): 167–183. doi:10.1007/s11104-019-04196-6.

Nitsch, C., and Nitsch, J. 1967. The induction of flowering in vitro in stem segments of *Plumbago indica* L. I. The production of vegetative buds. Planta **72**(4): 355–370. doi:10.1007/BF00390146.

Van Ooijen, J. 2006. JoinMap4, Software for the calculation of genetic linkage maps in experimental populations. Kyazma, BV, Wageningen, Netherlands.

Osborne, B.G. 2000. Near-Infrared Spectroscopy in Food Analysis. *In* Encyclopedia of Analytical Chemistry. *Edited by* R.A. Meyers. John Wiley & Sons, Ltd, Chichester, UK. pp. 1–14. doi:10.1002/9780470027318.a1018.

Oxley, S. 2007, July. Clubroot disease of oilseed rape and other brassica crops. The Scottish Agricultural College, West Mains Roads, Edinburgh EH9 3JG.

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.

Pandey, M.K., Khan, A.W., Singh, V.K., Vishwakarma, M.K., Shasidhar, Y., Kumar, V., Garg, V., Bhat, R.S., Chitikineni, A., Janila, P., Guo, B., and Varshney, R.K. 2017. QTL-seq approach identified genomic regions and diagnostic markers for rust and late leaf spot resistance in groundnut (*Arachis hypogaea* L.). Plant Biotechnol. J. **15**(8): 927–941. doi:10.1111/pbi.12686.

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. **9**(May): 1–9. doi:10.3389/fpls.2018.00653.

Park, W.J., Kriechbaumer, V., Müller, A., Piotrowski, M., Meeley, R.B., Gierl, A., and Glawischnig, E. 2003. The nitrilase ZmNIT2 converts Indole-3-Acetonitrile to Indole-3-Acetic Acid. Plant Physiol. **133**(2): 794–802. doi:10.1104/pp.103.026609.

Parkin, I., Gulden, S., Sharpe, A., Lukens, L., Trick, M., Osborn, T., and Lydiate, D. 2005. Segmental structure of the *Brassica napus* genome based on comparative analysis with *Arabidopsis thaliana*. Genetics **171**(2): 765–781. doi:10.1534/genetics.105.042093.

Parkin, I., and Lydiate, D. 1997. Conserved patterns of chromosome pairing and recombination in *Brassica napus* crosses. Genome **40**(4): 496–504.

Parkin, I.A., Sharpe, A.G., Keith, D.J., and Lydiate, D.J. 1995. Identification of the A and C genomes of amphidiploid *Brassica napus* (oilseed rape). Genome **38**(6): 1122–1131. doi:10.1139/g95-149.

Päsold, S., Siegel, I.N.A., Seidel, C., Ludwig-Müller, J., Pasold, S., Siegel, I.N.A., Seidel, C.,

and Ludwig -Muller, J. 2010. Flavonoid accumulation in *Arabidopsis thaliana* root galls caused by the obligate biotrophic pathogen *Plasmodiophora brassicae*. Mol. Plant Pathol. **11**(4): 545–562. doi:10.1111/j.1364-3703.2010.00628.x.

Peer, W.A., and Murphy, A.S. 2007. Flavonoids and auxin transport: modulators or regulators? Trends Plant Sci. **12**(12): 556–563. doi:10.1016/j.tplants.2007.10.003.

Peng, G., Falk, K.C., Gugel, R.K., Franke, C., Yu, F., James, B., Strelkov, S.E., Hwang, S.-F., and McGregor, L. 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., Gossen, B.D., and Strelkov, S.E. 2014b. Crop rotation, cultivar resistance, and fungicides/biofungicides for managing clubroot (*Plasmodiophora brassicae*) on canola. Can. J. Plant Pathol. **36**(sup1): 99–112. doi:10.1080/07060661.2013.860398.

Peng, G., Pageau, D., Strelkov, S.E., Gossen, B.D., Hwang, S.-F., and Lahlali, R. 2015. A >2-year crop rotation reduces resting spores of *Plasmodiophora brassicae* in soil and the impact of clubroot on canola. Eur. J. Agron. **70**(August): 78–84. doi:10.1016/j.eja.2015.07.007.

Peng, L., Zhou, L., Li, Q., Wei, D., Ren, X., Song, H., Mei, J., Si, J., and Qian, W. 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.

Pérez-López, E., Hossain, M.M., Tu, J., Waldner, M., Todd, C.D., Kusalik, A.J., Wei, Y., and Bonham-Smith, P.C. 2020. Transcriptome analysis identifies *Plasmodiophora brassicae* secondary infection effector candidates. J. Eukaryot. Microbiol.: 337–351. doi:10.1111/jeu.12784.

Pérez-López, E., Waldner, M., Hossain, M., Kusalik, A.J., Wei, Y., Bonham-Smith, P.C., and Todd, C.D. 2018. Identification of *Plasmodiophora brassicae* effectors — a challenging goal. Virulence **9**(1): 1344–1353. doi:10.1080/21505594.2018.1504560.

Peschansky, V.J., and Wahlestedt, C. 2014. Non-coding RNAs as direct and indirect modulators of epigenetic regulation. Epigenetics **9**(1): 3–12. doi:10.4161/epi.27473.

Piao, Z., Deng, Y., Choi, S., Park, Y., and Lim, Y. 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**(3): 252–264. doi:10.1007/s00344-009-9093-8.

Pilet-Nayel, M.L., Moury, B., Caffier, V., Montarry, J., Kerlan, M.C., Fournet, S., Durel, C.E., and Delourme, R. 2017. Quantitative resistance to plant pathogens in pyramiding strategies for durable crop protection. Front. Plant Sci. **8**: 1838. doi:10.3389/fpls.2017.01838.

Piquemal, J., Cinquin, E., Couton, F., Rondeau, C., Seignoret, E., Doucet, I., Perret, D., Villeger, M.-J., Vincourt, P., and Blanchard, P. 2005. Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theor. Appl. Genet. **111**(8): 1514–23. doi:10.1007/s00122-005-0080-6.

Pires, J., Zhao, J., Schranz, M., Leon, E., Quijada, P., Lukens, L., and Osborn, T. 2004. Flowering time divergence and genomic rearrangements in resynthesized Brassicapolyploids (Brassicaceae). Biol. J. Linn. Soc. **82**: 675–688.

Potts, D.A., Rakow, G.W., and Males, D.R. 1999. Canola quality *Brassica juncea*, a new oilseed crop for the Canadian prairies. *In* Proceedings of 10th International Rapeseed Congress. Canberra, Australia. Available from http://www.regional.org.au/au/gcirc/4/70.htm.

Qiu, D., Morgan, C., Shi, J., Long, Y., Liu, J., Li, R., Zhuang, X., Wang, Y., Tan, X., Dietrich, E., Weihmann, T., Everett, C., Vanstraelen, S., Beckett, P., Fraser, F., Trick, M., Barnes, S., Wilmer, J., Schmidt, R., Li, J., Li, D., Meng, J., and Bancroft, I. 2006. A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. Theor. Appl. Genet. **114**(1): 67–80. doi:10.1007/s00122-006-0411-2.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.r-project.org/.

Rafiqi, M., Ellis, J.G., Ludowici, V.A., Hardham, A.R., and Dodds, P.N. 2012. Challenges and progress towards understanding the role of effectors in plant–fungal interactions. Curr. Opin. Plant Biol. **15**(4): 477–482. doi:10.1016/j.pbi.2012.05.003.

Rahman, H. 2013. Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Can. J. Plant Sci. **5**(January): 363–373. doi:10.4141/CJPS2012-074.

Rahman, H. 2017. UA AlfaGold Clearfield herbicide-tolerant spring *Brassica napus* canola developed from winter × spring canola cross. Can. J. Plant Sci. **97**(1): 144–146. doi:10.1139/CJPS-2016-0028.

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., and Franke, C. 2019. Association of fusarium wilt susceptibility with clubroot resistance derived from winter *Brassica napus* L. 'Mendel.' Can. J. Plant Pathol. **41**(1): 60–64. Taylor & Francis. doi:10.1080/07060661.2018.1505780.

Rahman, H., and Kebede, B. 2021. Mapping of seed quality traits in the C genome of *Brassica napus* by using a population carrying genome content of *B. oleracea* and their effect on other traits. Plant Genome: e20078. doi:10.1002/tpg2.20078.

Rahman, H., Kebede, B., Zimmerli, C., and Yang, R.-C. 2014a. Genetic study and QTL mapping of seed glucosinolate content in *Brassica rapa* L. Crop Sci. **54**(2): 537–543. doi:10.2135/cropsci2013.06.0391.

Rahman, H., Peng, G., Yu, F., Falk, K.C., Kulkarni, M., and Selvaraj, G. 2014b. Genetics and breeding for clubroot resistance in Canadian spring canola (*Brassica napus* L.). Can. J. Plant Pathol. **36**(sup1): 122–134. doi:10.1080/07060661.2013.862571.

Rahman, H., Shakir, A., and Jakir Hasan, M. 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.

Rahman, M. 2001. Production of yellow-seeded *Brassica napus* through interspecic crosses. Plant Breed. **120**: 463–472.

Rahman, M.H. 2005. Resynthesis of *Brassica napus* L. for self-incompatibility: self-incompatibility reaction, inheritance and breeding potential. Plant Breed. **124**(1): 13–19. doi:10.1111/j.1439-0523.2004.01045.x.

Raman, H., Raman, R., Eckermann, P., Coombes, N., Manoli, S., Zou, X., Edwards, D., Meng, J., Prangnell, R., Stiller, J., Batley, J., Luckett, D., Wratten, N., and Dennis, E. 2013. Genetic and physical mapping of flowering time loci in canola (*Brassica napus* L.). Theor. Appl. Genet. **126**(1): 119–32. doi:10.1007/s00122-012-1966-8.

Ramsey, J., and Schemske, D.W. 2002. Neopolyploidy in flowering plants. Annu. Rev. Ecol. Syst. **33**(1): 589–639. Annual Reviews. doi:10.1146/annurev.ecolsys.33.010802.150437.

Ramzi, N., Kaczmarek, J., and Jedryczka, M. 2018. Identification of clubroot resistance sources from world gene bank accessions. *In* IOBC-WPRS Bulletin. Integrated Control in Oilseed Crops. pp. 144–147.

Rana, D., Boogaart, T., O'Neill, C.M., Hynes, L., Bent, E., Macpherson, L., Park, J.Y., Lim, Y.P., and Bancroft, I. 2004. Conservation of the microstructure of genome segments in *Brassica napus* and its diploid relatives. Plant J. **40**(5): 725–733. doi:10.1111/j.1365-

313X.2004.02244.x.

Rashid, A., Rakow, G., and Downey, R.K. 1994. Development of yellow seeded *Brassica napus* through interspecific crosses. Plant Breed. **112**(2): 127–134. doi:10.1111/j.1439-0523.1994.tb00660.x.

Renny-Byfield, S., Kovarik, A., Kelly, L.J., Macas, J., Novak, P., Chase, M.W., Nichols, R.A., Pancholi, M.R., Grandbastien, M.-A., and Leitch, A.R. 2013. Diploidization and genome size change in allopolyploids is associated with differential dynamics of low- and high-copy sequences. Plant J. **74**(5): 829–839. doi:10.1111/tpj.12168.

Renny-Byfield, S., and Wendel, J.F. 2014. Doubling down on genomes: Polyploidy and crop plants. Am. J. Bot. **101**(10): 1711–1725. doi:10.3732/ajb.1400119.

Rhoades, M.W., Reinhart, B.J., Lim, L.P., Burge, C.B., Bartel, B., and Bartel, D.P. 2002. Prediction of plant microRNA targets. Cell **110**(4): 513–520. doi:10.1016/S0092-8674(02)00863-2.

Ripley, V., and Beversdorf, W. 2003. Development of self-incompatible *Brassica napus*: (I) introgression of S-alleles from *Brassica oleracea* through interspecific hybridization. Plant Breed. **122**(1): 1–5. doi:10.1046/j.1439-0523.2003.00780.x.

Rocherieux, J., Glory, P., Giboulot, A., Boury, S., Barbeyron, G., Thomas, G., and Manzanares-Dauleux, M.J. 2004. Isolate-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*. Theor. Appl. Genet. **108**(8): 1555–63. doi:10.1007/s00122-003-1580-x.

Rolfe, S.A., Strelkov, S.E., Links, M.G., Clarke, W.E., Robinson, S.J., Djavaheri, M., Malinowski, R., Haddadi, P., Kagale, S., Parkin, I.A.P., Taheri, A., and Borhan, M.H. 2016. The compact genome of the plant pathogen *Plasmodiophora brassicae* is adapted to intracellular interactions with host *Brassica* spp. BMC Genomics **17**(1): 1–15. BMC Genomics. doi:10.1186/s12864-016-2597-2.

Ron, M., and Avni, A. 2004. The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. Plant Cell **16**(6): 1604 LP – 1615. doi:10.1105/tpc.022475.

Rotmistrovsky, K., Jang, W., and Schuler, G.D. 2004. A web server for performing electronic PCR. Nucleic Acids Res. **32**(WEB SERVER ISS.): 108–112. doi:10.1093/nar/gkh450.

Roy, N.N. 1978. A study on disease variation in the populations of an interspecific cross of *Brassica juncea* L. x *B. napus* L. Euphytica **27**(1): 145–149. doi:10.1007/BF00039129.

Ruane, J., and Sonnino, A. 2007. Marker-assisted selection as a tool for genetic improvement of crops, livestock, forestry and fish in developing countries: an overview of the issues.

In Marker-Assisted Selection: Current status and future perspectives in crops, livestock, forestry and fish. *Edited by* E. Guimarães, J. Ruane, B. Scherf, A. Sonnino, and J. Dargie. Food and Agriculture Organization (FAO) of the United Nations, Rome, Italy. pp. 5–13.

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): 759–67. doi:10.1007/s00122-008-0817-0.

Salmon, A., Ainouce, M.L., and Wendel, J.F. 2005. Genetic and epigenetic consequences of recent hybridization and polyploidy in Spartina (Poaceae). Mol. Ecol. **14**(4): 1163–1175. doi:10.1111/j.1365-294X.2005.02488.x.

SAS Institute Inc. 2011. SAS® 9.3 Software. SAS Institute Inc., Cary, NC.

Sasidharan, R., Voesenek, L.A., and Pierik, R. 2011. Cell wall modifying proteins mediate plant acclimatization to biotic and abiotic stresses. CRC. Crit. Rev. Plant Sci. **30**(6): 548–562. doi:10.1080/07352689.2011.615706.

Schatzki, J., Ecke, W., Becker, H.C., and Möllers, C. 2014. Mapping of QTL for the seed storage proteins cruciferin and napin in a winter oilseed rape doubled haploid population and their inheritance in relation to other seed traits. Theor. Appl. Genet. **127**(5): 1213–1222. doi:10.1007/s00122-014-2292-0.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nat. Biotechnol. **18**(2): 233–234. doi:10.1038/72708.

Schuler, G.D. 1997. Sequence mapping by electronic PCR. Genome Res. **7**(5): 541–550. doi:10.1101/gr.7.5.541.

Schuller, A., Kehr, J., and Ludwig-Müller, J. 2014. Laser microdissection coupled to transcriptional profiling of *Arabidopsis* roots inoculated by *Plasmodiophora brassicae* indicates a role for brassinosteroids in clubroot formation. Plant Cell Physiol. **55**(2): 392–411. doi:10.1093/pcp/pct174.

Sedaghatkish, A., Gossen, B.D., Yu, F., Torkamaneh, D., and McDonald, M.R. 2019. Whole-genome DNA similarity and population structure of *Plasmodiophora brassicae* strains from Canada. BMC Genomics **20**(1): 744. doi:10.1186/s12864-019-6118-y.

Shah, N., Sun, J., Yu, S., Yang, Z., Wang, Z., Huang, F., Dun, B., Gong, J., Liu, Y., Li, Y., Li, Q., Yuan, L., Baloch, A., Li, G., Li, S., and Zhang, C. 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. Breed. **39**(10–11): 153. doi:10.1007/s11032-019-1075-3.

Shaikh, R., Farid, M., and Rahman, H. 2020. Inheritance of resistance to the newly identified *Plasmodiophora brassicae* pathotypes in *Brassica napus* L. Can. J. Plant Pathol.: 1–11. doi:10.1080/07060661.2020.1823483.

Sharma Poudel, R., Al-Hashel, A.F., Gross, T., Gross, P., and Brueggeman, R. 2018. Pyramiding *rpg4*- and *Rpg1*-mediated stem rust resistance in barley requires the *Rrr1* gene for both to function. Front. Plant Sci. **871**(December): 1–17. doi:10.3389/fpls.2018.01789.

Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., Minami, E., Okada, K., Yamane, H., Kaku, H., and Shibuya, N. 2010. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. Plant J. **64**(2): 204–214. doi:10.1111/j.1365-313X.2010.04324.x.

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., 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**(2): 305–319. doi:10.1111/pbr.12895.

Shriram, V., Kumar, V., Devarumath, R.M., Khare, T.S., and Wani, S.H. 2016. MicroRNAs as potential targets for abiotic stress tolerance in plants. Front. Plant Sci. **7**: 817. doi:10.3389/fpls.2016.00817.

Siemens, J., Gonzales, M.-C., Wolf, S., Hofmann, C., Greiner, S., Du, Y., Rausch, T., Roitsch, T., and Ludwig-Muller, J. 2011. Extracellular invertase is involved in the regulation of clubroot disease in *Arabidopsis thaliana*. Mol. Plant Pathol. **12**(3): 247–262. doi:10.1111/j.1364-3703.2010.00667.x.

Siemens, J., Graf, H., Bulman, S., In, O., and Ludwig-Müller, J. 2009. Monitoring expression of selected *Plasmodiophora brassicae* genes during clubroot development in *Arabidopsis thaliana*. Plant Pathol. **58**(1): 130–136. doi:10.1111/j.1365-3059.2008.01943.x.

Siemens, J., Keller, I., Sarx, J., Kunz, S., Schuller, A., Nagel, W., Schmülling, T., Parniske, M., and Ludwig-Müller, J. 2006. Transcriptome analysis of *Arabidopsis* clubroots indicate a key role for cytokinins in disease development. Mol. Plant-Microbe Interact. **19**(5): 480–494. doi:10.1094/MPMI-19-0480.

Slocum, M.K.M., Figdore, S.S., Kennard, W.C., Suzuki, J.Y., and Osborn, T.C.T. 1990.

Linkage arrangement of restriction fragment length polymorphism loci in *Brassica oleracea*. Theor. Appl. Genet. **80**(1): 57–64. doi:10.1007/BF00224016.

Some, A., Manzanares, M., 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.

Song, K.M., Suzuki, J.Y., Slocum, M.K., Williams, P.M., and Osborn, T.C. 1991. A linkage map of *Brassica rapa* (syn. *campestris*) based on restriction fragment length polymorphism loci. Theor. Appl. Genet. **82**(3): 296–304. doi:10.1007/BF02190615.

Song, X., Li, Y., Cao, X., and Qi, Y. 2019. MicroRNAs and their regulatory roles in plant–environment interactions. Annu. Rev. Plant Biol. **70**(1): 489–525. doi:10.1146/annurev-arplant-050718-100334.

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.

Srivastava, A., Mukhopadhyay, A., Arumugam, N., Gupta, V., Verma, J., Pental, D., and Pradhan, A. 2004. Resynthesis of *Brassica juncea* through interspecific crosses between *B. rapa* and B. nigra. Plant Breed. **123**: 204–207.

Stanke, M., Steinkamp, R., Waack, S., and Morgenstern, B. 2004. AUGUSTUS: a web server for gene finding in eukaryotes. Nucleic Acids Res. **32**(Web Server issue): W309-12. doi:10.1093/nar/gkh379.

Strelkov, S., Manolii, V., Cao, T., Xue, S., and Hwang, S. 2007. Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta, Canada. J. Phytopathol. **155**(11–12): 706–712. doi:10.1111/j.1439-0434.2007.01303.x.

Strelkov, S., Manolii, V., Harding, M., Sieusahai, G., Hwang, S., Shi, Y., and Lawson, A. 2016a. Occurrence and spread of clubroot on canola in Alberta in 2015. *In* Canadian Plant Disease Survey. pp. 163–165.

Strelkov, S.E., Hwang, S.-F., Manolii, V.P., Cao, T., and Feindel, D. 2016b. Emergence of new virulence phenotypes of *Plasmodiophora brassicae* on canola (*Brassica napus*) in Alberta, Canada. Eur. J. Plant Pathol. **145**(3): 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., Peng, G., Gossen, B.D., Mcdonald, M.R., and Feindel, D. 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., 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.

Stringam, G.R. 1971. Genetics of four hypocotyl mutants in *Brassica campestris* L. J. Hered. **62**(4): 248–250.

Stringam, G.R., Degenhardt, D.F., Thiagarajah, M.R., and Bansal, V.K. 2000. Hi-Q summer rape. Can. J. Plant Sci. **80**(4): 835–836. doi:10.4141/P00-044.

Summanwar, A., Basu, U., Rahman, H., and Kav, N. 2019. Identification of lncRNAs responsive to infection by *Plasmodiophora brassicae* in clubroot-susceptible and -resistant *Brassica napus* lines carrying resistance introgressed from rutabaga. Mol. Plant-Microbe Interact. **32**(10): 1360–1377. doi:10.1094/MPMI-12-18-0341-R.

Summers, R.W., and Brown, J.K.M. 2013. Constraints on breeding for disease resistance in commercially competitive wheat cultivars. Plant Pathol. **62**(S1): 115–121. doi:10.1111/ppa.12165.

Sung, S., and Amasino, R.M. 2004. Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein *VIN3*. Nature **427**(6970): 159–164. doi:10.1038/nature02195.

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**(6–7): 1092–1098. doi:10.1007/s00122-002-0875-7.

Suwabe, K., Suzuki, G., Nunome, T., Hatakeyama, K., Mukai, Y., Fukuoka, H., and Matsumoto, S. 2012. Microstructure of a *Brassica rapa* genome segment homoeologous to the resistance gene cluster on *Arabidopsis* chromosome 4. Breed. Sci. **62**(2): 170–7. doi:10.1270/jsbbs.62.170.

Suwabe, K., Tsukazaki, H., Iketani, H., Hatakeyama, K., Fujimura, M., Nunome, T., Fukuoka, H., Matsumoto, S., and Hirai, M. 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., Nunome, T., Fukuoka, H., Hirai, M., and Matsumoto, S. 2006. Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics **173**(1): 309–19. doi:10.1534/genetics.104.038968.

Suzuki, T., Sato, M., and Takeuchi, T. 2012. Evaluation of the effects of five QTL regions on Fusarium head blight resistance and agronomic traits in spring wheat (*Triticum aestivum* L.). Breed. Sci. **62**(1): 11–17. doi:10.1270/jsbbs.62.11.

Swiderski, M.R., Birker, D., and Jones, J.D.G. 2009. The TIR domain of TIR-NB-LRR

resistance proteins is a signaling domain involved in cell death induction. Mol. Plant. Microbe. Interact. **22**(2): 157–65. doi:10.1094/MPMI-22-2-0157.

Szadkowski, E., Eber, F., Huteau, V., Lodé, M., Coriton, O., Jenczewski, E., and Chèvre, A.M. 2011. Polyploid formation pathways have an impact on genetic rearrangements in resynthesized *Brassica napus*. New Phytol. **191**(3): 884–894. doi:10.1111/j.1469-8137.2011.03729.x.

Takagi, H., Abe, A., Yoshida, K., Kosugi, S., Natsume, S., Mitsuoka, C., Uemura, A., Utsushi, H., Tamiru, M., Takuno, S., Innan, H., Cano, L.M., Kamoun, S., and Terauchi, R. 2013. QTL-seq: Rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. Plant J. **74**(1): 174–183. doi:10.1111/tpj.12105.

Tang, M., Zhang, Y., Liu, Y., Tong, C., Cheng, X., Zhu, W., Li, Z., Huang, J., and Liu, S. 2019. Mapping loci controlling fatty acid profiles, oil and protein content by genome-wide association study in *Brassica napus*. Crop J. **7**(2): 217–226. Elsevier B.V. doi:10.1016/j.cj.2018.10.007.

Tewari, J., Strelkov, S., Orchard, D., Hartman, M., Lange, R., and Turkington, T. 2005. Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Can. J. Plant Pathol. **27**(1): 143–144. doi:10.1080/07060660509507206.

Ting, J.P.-Y., Willingham, S.B., and Bergstralh, D.T. 2008. NLRs at the intersection of cell death and immunity. Nat. Rev. Immunol. **8**(5): 372–379. doi:10.1038/nri2296.

Tlngdong, F., Ping, S., Xiaoniu, Y., and Guangsheng, Y. 1992. Overcoming Self-Incompatibility of *Brassica napus* by Salt (NaC1) Spray. Plant Breed. **109**(3): 255–258. Blackwell Publishing Ltd. doi:10.1111/j.1439-0523.1992.tb00181.x.

Tomita, H., Shimizu, M., Asad-ud Doullah, M., Fujimoto, R., and Okazaki, K. 2013. Accumulation of quantitative trait loci conferring broad-spectrum clubroot resistance in *Brassica oleracea*. Mol. Breed. **32**(4): 889–900. doi:10.1007/s11032-013-9918-9.

Toxopeus, H., and Janssen, A. 1975. Clubroot resistance in turnip II. The 'slurry' screening method and clubroot races in the Netherlands. Euphytica **24**(3): 751–755. doi:10.1007/BF00132914.

Toyofuku, K., Kasahara, M., and Yamaguchi, J. 2000. Characterization and expression of monosaccharide transporters (OsMSTs) in Rice. Plant Cell Physiol. **41**(8): 940–947. doi:10.1093/pcp/pcd016.

Tremblay, N., Bélec, C., Coulombe, J., and Godin, C. 2005. Evaluation of calcium cyanamide and liming for control of clubroot disease in cauliflower. Crop Prot. **24**(9): 798–803. doi:10.1016/j.cropro.2004.12.013.

Trick, M., Long, Y., Meng, J., and Bancroft, I. 2009. Single nucleotide polymorphism (SNP) discovery in the polyploid *Brassica napus* using Solexa transcriptome sequencing. Plant Biotechnol. J. **7**(4): 334–346. doi:10.1111/j.1467-7652.2008.00396.x.

Tudor, E.H., Jones, D.M., He, Z., Bancroft, I., Trick, M., Wells, R., Irwin, J.A., and Dean, C. 2020. QTL-seq identifies *BnaFT.A02* and *BnaFLC.A02* as candidates for variation in vernalization requirement and response in winter oilseed rape (*Brassica napus*). Plant Biotechnol. J. **18**(12): 2466–2481. doi:10.1111/pbi.13421.

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

Udall, J.A., Quijada, P.A., and Osborn, T.C. 2005. Detection of chromosomal rearrangements derived from homeologous recombination in four mapping populations of *Brassica napus* L. Genetics **169**(2): 967–979. doi:10.1534/genetics.104.033209.

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.

Ugajin, T., Takita, K., Takahashi, H., Muraoka, S., Tada, T., Mitsui, T., Hayakawa, T., Ohyama, T., and Hori, H. 2003. Increase in indole-3-acetic acid (IAA) level and nitrilase activity in turnips induced by *Plasmodiophora brassicae* Infection. Plant Biotechnol. **20**(3): 215–220. doi:10.5511/plantbiotechnology.20.215.

Umemoto, N., Kakitani, M., Iwamatsu, A., Yoshikawa, M., Yamaoka, N., and Ishida, I. 1997. The structure and function of a soybean β-glucan-elicitor-binding protein. Proc. Natl. Acad. Sci. U. S. A. **94**(3): 1029–1034. doi:10.1073/pnas.94.3.1029.

Underwood, W. 2012. The Plant Cell Wall: A dynamic barrier against pathogen invasion. Front. Plant Sci. **3**: 85. doi:10.3389/fpls.2012.00085.

USDA. 2019. Rutabagas, raw. Available from https://fdc.nal.usda.gov/fdc-app.html#/food-details/168454/nutrients [accessed 22 March 2020].

Vales, M.I., Schön, C.C., Capettini, F., Chen, X.M., Corey, A.E., Mather, D.E., Mundt, C.C., Richardson, K.L., Sandoval-Islas, J.S., Utz, H.F., and Hayes, P.M. 2005. Effect of population size on the estimation of QTL: A test using resistance to barley stripe rust. Theor. Appl. Genet. **111**(7): 1260–1270. doi:10.1007/s00122-005-0043-y.

Venter, F. 1970. Methods for determining fungitoxicity of calcium cyanamide in vitro. J. Plant Dis. Prot. **77**(9): 494–497.

Verma, S.S., Rahman, M.H., Deyholos, M.K., Basu, U., and Kav, N.N. V. 2014. Differential expression of miRNAs in *Brassica napus* root following infection with *Plasmodiophora*

brassicae. PLoS One **9**(1): e86648. doi:10.1371/journal.pone.0086648.

Vision, T.J., Brown, D.G., and Tanksley, S.D. 2000. The origins of genomic duplications in Arabidopsis. Science (80-.). **290**(5499): 2114–2117. doi:10.1126/science.290.5499.2114.

Vo, K., Schwartzenberg, Kruse, S., Reski, R., Moffatt, B., and Laloue, M. 1998. Cloning and characterization of an adenosine kinase from Physcomitrella involved in cytokinin metabolism. Plant J. **13**(2): 249–257. doi:10.1046/j.1365-313X.1998.00011.x.

Voorrips, R., Jongerius, M., and Kanne, H. 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.

Voorrips, R.E. 1995. *Plasmodiophora brassicae* : aspects of pathogenesis and resistance in *Brassica oleracea*. Euphytica **83**: 139–146.

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., and Visser, D.L. 1993. Examination of resistance to clubroot in accessions of *Brassica oleracea* using a glasshouse seedling test. Netherlands J. Plant Pathol. **99**(5–6): 269–276. doi:10.1007/BF01974308.

Wagner, G., Laperche, A., Lariagon, C., Marnet, N., Renault, D., Guitton, Y., Bouchereau, A., Delourme, R., Manzanares-Dauleux, M.J., Gravot, A., and Hancock, R. 2019. Resolution of quantitative resistance to clubroot into QTL-specific metabolic modules. J. Exp. Bot. **70**(19): 5375–5390. doi:10.1093/jxb/erz265.

Walerowski, P., Gündel, A., Yahaya, N., Truman, W., Sobczak, M., Olszak, M., Rolfe, S., Borisjuk, L., and Malinowski, R. 2018. Clubroot disease stimulates early steps of phloem differentiation and recruits SWEET sucrose transporters within developing galls. Plant Cell **30**(12): 3058–3073. doi:10.1105/tpc.18.00283.

Walker, J.C. 1939. Resistance to clubroot in varieties of turnip and rutabaga. J. Agric. Res. **59**(11): 815–827.

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**: 710–719.

Wallenhammar, A.-C., Johnsson, L., and Gerhardson, B. 1999. Clubroot resistance and yield loss in spring oilseed turnip rape and spring oilseed rape. *In* Proceedings of 10th International Rapeseed Congress. *Edited by* N. Wratten and P. Salisbury. Canberra, Australia. pp. 2–7.

Wallenhammar, A., Almquist, C., Söderström, M., and Jonsson, A. 2011. In-field distribution

of *Plasmodiophora brassicae* measured using quantitative real-time PCR. Plant Pathol. **61**(1): 16–28. doi:10.1111/j.1365-3059.2011.02477.x.

Walters, D.R., and McRoberts, N. 2006. Plants and biotrophs: a pivotal role for cytokinins? Trends Plant Sci. **11**(12): 581–586. doi:10.1016/j.tplants.2006.10.003.

Wang, R., Ripley, V.L., and Rakow, G. 2007. Pod shatter resistance evaluation in cultivars and breeding lines of *Brassica napus*, *B. juncea* and *Sinapis alba*. Plant Breed. **126**(6): 588–595. doi:10.1111/j.1439-0523.2007.01382.x.

Wang, S., Yu, F., Zhang, W., Tang, J., Li, J., Yu, L., Wang, H., and Jiang, J. 2019. Comparative transcriptomic analysis reveals gene expression changes during early stages of *Plasmodiophora brassicae* infection in cabbage (*Brassica oleracea* var. capitata L.). Can. J. Plant Pathol. **41**(2): 188–199. doi:10.1080/07060661.2019.1567592.

Wang, X., Wang, H., Wang, J., Sun, R., Wu, J., Liu, S., Bai, Y., Mun, J.-H., Bancroft, I., Cheng, F., Huang, S., Li, X., Hua, W., Wang, J., Wang, X., Freeling, M., Pires, J.C., Paterson, A.H., Chalhoub, B., Wang, B., Hayward, A., Sharpe, A.G., Park, B.-S., Weisshaar, B., Liu, B., Li, B., Liu, B., Tong, C., Song, C., Duran, C., Peng, C., Geng, C., Koh, C., Lin, C., Edwards, D., Mu, D., Shen, D., Soumpourou, E., Li, F., Fraser, F., Conant, G., Lassalle, G., King, G.J., Bonnema, G., Tang, H., Wang, H., Belcram, H., Zhou, H., Hirakawa, H., Abe, H., Guo, H., Wang, H., Jin, H., Parkin, I.A.P., Batley, J., Kim, J.-S., Just, J., Li, J., Xu, J., Deng, J., Kim, J.A., Li, J., Yu, J., Meng, J., Wang, J., Min, J., Poulain, J., Wang, J., Hatakeyama, K., Wu, K., Wang, L., Fang, L., Trick, M., Links, M.G., Zhao, M., Jin, M., Ramchiary, N., Drou, N., Berkman, P.J., Cai, Q., Huang, Q., Li, R., Tabata, S., Cheng, S., Zhang, S., Zhang, S., Huang, S., Sato, S., Sun, S., Kwon, S.-J., Choi, S.-R., Lee, T.-H., Fan, W., Zhao, X., Tan, X., Xu, X., Wang, Y., Qiu, Y., Yin, Y., Li, Y., Du, Y., Liao, Y., Lim, Y., Narusaka, Y., Wang, Y., Wang, Z., Li, Z., Wang, Z., Xiong, Z., and Zhang, Z. 2011. The genome of the mesopolyploid crop species *Brassica rapa*. Nat. Genet. **43**(10): 1035–1039. doi:10.1038/ng.919.

Wang, Y., Bao, Z., Zhu, Y., and Hua, J. 2009a. Analysis of temperature modulation of plant defense against biotrophic microbes. Mol. Plant-Microbe Interact. **22**(5): 498–506. doi:10.1094/MPMI-22-5-0498.

Wang, Z., Gerstein, M., and Snyder, M. 2009b. RNA-Seq: a revolutionary tool for transcriptomics. Nat. Rev. Genet. **10**(1): 57–63. doi:10.1038/nrg2484.

Warwick, S., Francis, A., and Al-Shehbaz, I. 2006. Brassicaceae: Species checklist and database on CD-Rom. Plant Syst. Evol. **259**(2–4): 249–258. doi:10.1007/s00606-006-0422-0.

Webster, M., and Dixon, G. 1991. Boron, pH and inoculum concentration influencing colonization by *Plasmodiophora brassicae*. Mycol. Res. **95**(1): 74–79. British Mycological Society. doi:10.1016/S0953-7562(09)81363-4.

Wechter, W.P., Farnham, M.W., Smith, J.P., and Keinath, A.P. 2007. Identification of resistance to peppery leaf spot among *Brassica juncea* and *Brassica rapa* plant introductions. Hort Sci. **42**(5): 1140–1143.

Wei, D., Mei, J., Fu, Y., Disi, J.O., Li, J., and Qian, W. 2014. Quantitative trait loci analyses for resistance to *Sclerotinia sclerotiorum* and flowering time in *Brassica napus*. Mol. Breed. **34**(4): 1797–1804. doi:10.1007/s11032-014-0139-7.

Wei, X., Xu, W., Yuan, Y., Yao, Q., Zhao, Y., Wang, Z., Jiang, W., and Zhang, X. 2016. Genome-wide investigation of microRNAs and their targets in *Brassica rapa* ssp. *pekinensis* root with *Plasmodiophora brassicae* infection. Hortic. Plant J. **2**(4): 209–216. doi:10.1016/j.hpj.2016.11.004.

Werner, K., Friedt, W., and Ordon, F. 2005. Strategies for Pyramiding resistance genes against the Barley Yellow Mosaic Virus Complex (*BaMMV, BaYMV, BaYMV-2*). Mol. Breed. **16**(1): 45–55. doi:10.1007/s11032-005-3445-2.

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): 363–372. doi:10.1007/s00122-007-0674-2.

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

Woods, D., Capcara, J., and Downey, R. 1991. The potential of mustard (*Brassica juncea* (L.) Coss) as an edible oil crop on the Canadian Prairies. Can. J. Plant Sci. **98**: 195–198.

Woronin, M. 1878. *Plasmodiophora brassicae*, Urheber der Kohlpflanzen-Hernie. Jahrb Wissenschaftl Bot **11**: 548–578.

Wright, P.R., Morgan, J.M., Jessop, R.S., and Cass, A. 1995. Comparative adaptation of canola (*Brassica napus*) and Indian mustard (*B. juncea*) to soil water deficits: yield and yield components. F. Crop. Res. **42**(1): 1–13. doi:10.1016/0378-4290(95)00013-G.

Wu, J., Chen, P., Zhao, Q., Cai, G., Hu, Y., Xiang, Y., Yang, Q., Wang, Y., and Zhou, Y. 2019. Co-location of QTL for Sclerotinia stem rot resistance and flowering time in *Brassica napus*. Crop J. **7**(2): 227–237. Elsevier B.V. doi:10.1016/j.cj.2018.12.007.

Xin, M., Wang, Y., Yao, Y., Xie, C., Peng, H., Ni, Z., and Sun, Q. 2010. Diverse set of microRNAs are responsive to powdery mildew infection and heat stress in wheat (*Triticum aestivum* L.). BMC Plant Biol. **10**(1): 123. doi:10.1186/1471-2229-10-123.

Xiong, Z., Gaeta, R., and Pires, J. 2011. Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. Proc. Natl. Acad. Sci. U. S. A. **108**(19): 7908–7913. doi:10.1073/pnas.1014138108.

Xu, L., Yang, H., Ren, L., Chen, W., Liu, L., Liu, F., Zeng, L., Yan, R., Chen, K., and Fang, X. 2018. Jasmonic acid-mediated aliphatic glucosinolate metabolism is involved in clubroot disease development in *Brassica napus* L. Front. Plant Sci. **9**: 750. doi:10.3389/fpls.2018.00750.

Xue, S., Cao, T., Howard, R., Hwang, S., and Strelkov, S. 2008. Isolation and variation in virulence of single-spore isolates of *Plasmodiophora brassicae* from Canada. Plant Dis. **92**(3): 456–462.

Yoshikawa, H. 1973. Breeding for clubroot resistance of crucifer crops in Japan. Jarq-japan Agric. Res. Q. **17**(1): 6–11.

Yoshikawa, H. 1993. Studies on breeding of clubroot resistance in cole [Cruciferae] crops. Bull. Natl. Res. Inst. Veg. Ornam. Plants Tea. Ser. A **7**: 1–165.

You, F.M., Huo, N., Gu, Y., Luo, M., Ma, Y., Hane, D., Lazo, G.R., Dvorak, J., and Anderson, O.D. 2008. BatchPrimer3: A high throughput web application for PCR and sequencing primer design. BMC Bioinformatics **9**: 253. doi:10.1186/1471-2105-9-253.

Yu, F., Zhang, X., Huang, Z., Chu, M., Song, T., Falk, K.C., Deora, A., Chen, Q., Zhang, Y., McGregor, L., Gossen, B.D., McDonald, M.R., and Peng, G. 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**(1): 1–11. Springer US. doi:10.1038/s41598-017-04903-2.

Yu, J., Tehrim, S., Zhang, F., Tong, C., Huang, J., Cheng, X., Dong, C., Zhou, Y., Qin, R., Hua, W., and Liu, S. 2014. Genome-wide comparative analysis of NBS-encoding genes between Brassica species and *Arabidopsis thaliana*. BMC Genomics **15**(1): 3. doi:10.1186/1471-2164-15-3.

Yu, K., Wang, X., Li, W., Sun, L., Peng, Q., Chen, F., Zhang, W., Guan, R., and Zhang, J. 2019. Identification and physical mapping of QTLs associated with flowering time in *Brassica napus* L. Euphytica **215**(10): 1–16. doi:10.1007/s10681-019-2480-8.

Yu, Z. 2019. Characterization of rutabaga for genetic diversity and as a source of clubroot

resistance. University of Alberta. MS thesis, Department of Agricultural Food and Nutritional Science.

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. p. 41.

Zhang, H., Feng, J., Hwang, S.F., Strelkov, S.E., Falak, I., Huang, X., and Sun, R. 2016a. Mapping of clubroot (*Plasmodiophora brassicae*) resistance in canola (*Brassica napus*). Plant Pathol. **65**(3): 435–440. doi:10.1111/ppa.12422.

Zhang, H., Feng, J., Zhang, S.S., Zhang, S.S., Li, F., Strelkov, S.E., Sun, R., and Hwang, S.F. 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, T., Zhao, Z., Zhang, C., Pang, W., Choi, S.R., Lim, Y.P., and Piao, Z. 2014. Fine genetic and physical mapping of the *CRb* gene conferring resistance to clubroot disease in *Brassica rapa*. Mol. Breed. **34**(3): 1173–1183. doi:10.1007/s11032-014-0108-1.

Zhang, W., Wang, S., Yu, F., Tang, J., Shan, X., Bao, K., Yu, L., Wang, H., Fei, Z., and Li, J. 2019a. Genome-wide characterization and expression profiling of *SWEET* genes in cabbage (*Brassica oleracea* var. *capitata* L.) reveal their roles in chilling and clubroot disease responses. BMC Genomics **20**(1): 93. doi:10.1186/s12864-019-5454-2.

Zhang, W., Wang, S., Yu, F., Tang, J., Yu, L., Wang, H., and Li, J. 2019b. Genome-wide identification and expression profiling of Sugar Transporter Protein (STP) family genes in Cabbage (*Brassica oleracea* var. *capitata* L.) reveals their involvement in clubroot disease responses. Genes (Basel). **10**(1): 71. doi:10.3390/genes10010071.

Zhang, X., Liu, Y., Fang, Z., Li, Z., Yang, L., Zhuang, M., Zhang, Y., and Lv, H. 2016b. Comparative Transcriptome Analysis between Broccoli (*Brassica oleracea* var. *italica*) and Wild Cabbage (*Brassica macrocarpa* Guss.) in response to *Plasmodiophora brassicae* during different infection stages. Front. Plant Sci. **7**: 1929. doi:10.3389/fpls.2016.01929.

Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. 2000. A greedy algorithm for aligning DNA sequences. J. Comput. Biol. **7**(1–2): 203–214. doi:10.1089/10665270050081478.

Zhao, J., Dimov, Z., Becker, H.C., Ecke, W., and Möllers, C. 2008. Mapping QTL controlling fatty acid composition in a doubled haploid rapeseed population segregating for oil content. Mol. Breed. **21**(1): 115–125. doi:10.1007/s11032-007-9113-y.

Zhao, J., Huang, J., Chen, F., Xu, F., Ni, X., Xu, H., Wang, Y., Jiang, C., Wang, H., Xu, A.,

Huang, R., Li, D., and Meng, J. 2012. Molecular mapping of *Arabidopsis thaliana* lipid-related orthologous genes in *Brassica napus*. Theor. Appl. Genet. **124**(2): 407–421. doi:10.1007/s00122-011-1716-3.

Zhao, J., Kulkarni, V., Liu, N., Del Carpio, D.P., Bucher, J., and Bonnema, G. 2010. *BrFLC2* (*FLOWERING LOCUS C*) as a candidate gene for a vernalization response QTL in *Brassica rapa*. J. Exp. Bot. **61**(6): 1817–1825. doi:10.1093/jxb/erq048.

Zhao, J., Udall, J.A., Quijada, P.A., Grau, C.R., Meng, J., and Osborn, T.C. 2006. Quantitative trait loci for resistance to *Sclerotinia sclerotiorum* and its association with a homeologous non-reciprocal transposition in *Brassica napus* L. Theor. Appl. Genet. **112**(3): 509–516. doi:10.1007/s00122-005-0154-5.

Zhao, Y., Bi, K., Gao, Z., Chen, T., Liu, H., Xie, J., Cheng, J., Fu, Y., and Jiang, D. 2017. Transcriptome Analysis of *Arabidopsis thaliana* in Response to *Plasmodiophora brassicae* during early infection. Front. Microbiol. **8**: 673. doi:10.3389/fmicb.2017.00673.

Zhu, H., Li, X., Xi, D., Zhai, W., Zhang, Z., and Zhu, Y. 2019a. Integrating long noncoding RNAs and mRNAs expression profiles of response to *Plasmodiophora brassicae* infection in Pakchoi (*Brassica campestris* ssp. *chinensis* Makino). PLoS One **14**(12): e0224927. doi:10.1371/journal.pone.0224927.

Zhu, H., Zhai, W., Li, X., and Zhu, Y. 2019b. Two QTLs controlling clubroot resistance identified from Bulked Segregant Sequencing in Pakchoi (*Brassica campestris* ssp. *chinensis* Makino). Sci. Rep. **9**(1): 1–9. doi:10.1038/s41598-019-44724-z.

Zipfel, C. 2014. Plant pattern-recognition receptors. Trends Immunol. **35**(7): 345–351. doi:10.1016/j.it.2014.05.004.