# Introgression of Clubroot Resistance from *Brassica oleracea* into *B. napus* Canola through Interspecific Cross

by

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## Abstract

Clubroot disease caused by *Plasmodiophora brassicae* Woronin is a serious threat to canola production in Canada. Most of the available clubroot-resistant canola cultivars carry major resistance genes of the *Brassica* A genome. However, the ineffectiveness of this type of resistance has been reported in Canada. On the other hand, clubroot resistance of the C genome of *B. oleracea* is under quantitative genetic control; this type of resistance is expected to provide a durable resistance in canola. The objective of this thesis research was to investigate the prospects of introgression of the C-genome resistance from *B. oleracea* into *B. napus* canola through interspecific hybridization between these two species. For this, *B. napus* × *B. oleracea* var. *acephala* (clubroot resistant) interspecific hybrids were produced through application of *in vitro* ovule culture technique. Following this approach, 12 hybrids from a total of 175 pollinations were obtained. The $F_1$ plants were self-pollinated for $F_2$ seeds and backcrossed to the *B. napus* parent for $BC_1$ seeds, and the $F_2$ and $BC_1$ populations were self-pollinated to produce $F_3$ and $BC_1F_2$ populations. The $F_1$ plants exhibited high sterility and produced only 0.168 seeds per self-pollination and 0.064 seeds per cross-pollination with the *B. napus* parent. The $F_2$ and $BC_1$ populations were evaluated for agronomic and seed quality traits. These two populations required around 70 and 40 days more time on average to flower as compared with the *B. napus* parent, and plants flowering earlier than the *B. napus* parent could be found in these populations. The average erucic acid in $F_3$ and $BC_1F_2$ seeds, harvested from the $F_2$ and $BC_1$ plants, was 13.4 and 6.8%, and glucosinolate content was 37.4 and 30.3 μmol/g seed, respectively. Nevertheless, zero erucic acid and low glucosinolate plants could be obtained from both populations. Plants exhibiting resistance to pathotype 3H could be found in $F_3$ and $F_4$, but not in the $BC_1F_2$

population. Several $F_3$ plants had nuclear DNA content similar to the *B. napus* parent. In addition to the above-mentioned populations, different advanced generation populations of *B. napus* × *B. oleracea* vars. *capitata* and *gemmifera* (clubroot resistant) interspecific crosses were also evaluated for resistance to pathotypes 3H and 3A. Selection for resistant plants and self-pollination of the selected plants was carried out up to $F_9$ generation, from where a clubroot resistant line was achieved from *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener interspecific cross. *B. oleracea*-specific simple-sequence repeat (SSR) marker alleles could be detected in the clubroot-resistant $F_8$ plants of this cross. However, association between the marker alleles and clubroot resistance could not be established. The results from this thesis research demonstrated the prospects of developing clubroot resistant canola lines from the *B. napus* × *B. oleracea* interspecific crosses.

## Preface

This dissertation is submitted by Zhongyang Zhang for the degree of Master of Science. Zhongyang carried out the experiments including the interspecific crosses, ovule culture, self-pollination and crossings, and evaluation of the populations for seed quality traits, nuclear DNA content, clubroot resistance, and molecular marker analysis. He collected and analysed data from these experiments and prepared the thesis with guidance, comments and suggestions from his supervisor Dr. Habibur Rahman. Final version of the thesis was prepared by incorporating additional suggestions from the examination committee members Drs. Stephen Strelkov and Malinda Thilakarathna.

In addition to this, Dr. Mehdi Farid provided training on interspecific crosses, ovule culture, and flow cytometry analysis. An Vo helped analysis of the seed samples for the seed quality traits, and Drs. Mehdi Farid and Berisso Kebede provided training on SSR marker analysis. Dr. Kebede and Minchien(Coco) Tsai helped in scoring the $F_4$ population of *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor interspecific cross and $F_9$ population of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener cross.

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## List of Symbols and Abbreviations

| | |
|---|---|
| ± | Plus/minus |
| % | Percent |
| < | Less than |
| = | Equal |
| × | Cross |
| *2n* | Diploid number of chromosomes |
| Σ | Summation |
| $\chi^2$ | chi-square statistic |
| + | Plus |
| AA | *Brassica rapa* genome |
| AACC | *Brassica napus* genome |
| AAFC | Agriculture and Agri-Food Canada |
| A*x* | *x* th chromosome of A *Brassica* genome |
| ANOVA | Analysis of variance |
| *B. nap* | *Brassica napus* |
| *B. ole* | *Brassica oleracea* |
| $BC_1$ | First backcross generation |
| $BC_1F_x$ | *x* th generation of $BC_1$-derived population |
| bp | Base pair |
| °C | Degrees Celsius |
| CC | Coiled-coil or *Brassica oleracea* genome |
| CCD | Canadian Clubroot Differential |
| CR | Clubroot-resistant |
| C*x* | *x* th chromosome of C *Brassica* genome |
| cv. | Cultivar |
| cvs. | Cultivars |
| DAI | Days after inoculation |
| DAP | Days after pollination |
| DSI | Disease severity index |
| ETI | Effector triggered Immunity |
| F*x* | $x^{th}$ generation of $F_1$-derived population |
| g | Gram |
| HSD | Honestly significant difference |
| L | Liter |
| LG | Linkage group |
| LRR | Leucine-rich repeat |
| LZ | Leucine zipper |

| | |
|---|---|
| Mb | Mega base pair |
| mg | Miligram |
| mg/L | Miligram per liter |
| ml | Mililiter |
| NBS | Nucleotide-binding site |
| N-P-K | Nitrogen-phosphorus-potassium |
| *p* | Calculated probability |
| PAMP | Pathogen-associated molecular attern |
| PCR | Polymerase chain reaction |
| Pexel | Plasmodium export element |
| PK | Protein kinase |
| ppm | One part per million |
| PTI | PAMP triggered immunity |
| QTL | Quantitative trait loci |
| rpm | Revolutions per minute |
| RxLR | Arginine-any amino acid-leucine arginine |
| S.E. | Standard Error |
| SSR | Simple-sequence repeat |
| sp. | Species |
| spp. | Multiple species |
| *t* | t-statistic |
| TIR | Toll-interleukin-1 receptor domain |
| var. | Variety |
| vars. | Varieties |
| vs. | Versus |
| µm | Micrometer |
| µmol/g | Micromoles per gram per seed |
| µmol/$m^2$/s | Micromoles per square meter per second |

# Chapter 1 Literature Review

## 1.1 Introduction

*Brassica* oilseed crops, viz. *B. napus*, *B. juncea* and *B. rapa*, constitute the third most economically important source of edible oils in the world after palm and soybean (www.statista.com, retrieved on Oct 2021). Among these, *Brassica napus* canola is the most widely cultivated species; this species is well adapted to temperate regions in the world. The winter growth habit type of this crop is mostly cultivated in Europe, semi-winter type in China, and the spring type is mostly grown in Australia, Canada and China (for review, see Katche et al. 2019). One of the important desirable features of canola oil is the high content of unsaturated fatty acids. The main unsaturated fatty acids of this oil are oleic acid (60%), linoleic acid (20%) and alpha-linolenic acids (10%). This oil is low in saturated fatty acid (7%) which makes it one of the healthiest vegetable oils for human nutrition (for review, see Rakow 2004). The oil with high oleic acid and low saturated fatty acid content makes it ideal to be used to produce biodiesel and lubricant (Jiang et al. 2011; Sharma et al. 2015; Ge et al. 2017) for both hot and cool climates (Sharma et al. 2015). Low glucosinolate content in seed meals is another important feature of this oilseed crop (Rakow 2004). The seed meal leftover after oil extraction contains 20-35% protein with excellent amino acid composition (for review, see Rakow 2004 and Al-shehbaz 2011); therefore, the seed meal is considered as a good protein source for livestock nutrition (Mejicanos et al. 2016; Liu et al. 2018a; Zhong and Adeola 2019).

The world production of *Brassica* oilseeds in 2018 was about 75 million tonnes which were harvested from about 37.6 million hectares of land (www.fao.org/faostat, retrieved on Oct 2021).

Of the different countries producing canola, Canada was the largest producer of this crop in 2018 ─ producing more than 20.3 million tonnes of seeds, and this constituted about 27% of the total production in the world. China was the second-largest producer ─ producing about 13.3 million tonnes, which contributes about 18% of the world's *Brassica* oilseed supply (www.fao.org/faostat, retrieved on Oct 2021). In Canada, canola plays an important role in the economy, especially in the prairie provinces including Alberta, Saskatchewan and Manitoba. It is estimated that the Canadian canola industry contributed about $29.9 billion and provided 207 thousand jobs annually to domestic economics. Of the total canola acreage in Canada, 99.3% of the crop is grown in the prairie provinces (www.canolacouncil.org, retrieved on Oct 2021). More than half of the canola produced in Canada is exported to the United States, China, Japan and Mexico in the forms of seed, oil and seed meal (www.canolacouncil.org, retrieved on Oct 2021). In 2020, the USA was the largest importer of Canadian canola products; this country purchased 51.6% of the total exported oil and 67.4% of the exported seed meal. On the other hand, China was the largest importer of Canadian canola seeds purchasing 21.9% of the total exported seeds (www.canolacouncil.org, retrieved on Oct 2021).

### 1.2 Family Brassicaceae and genus *Brassica*

The family Brassicaceae, also called Cruciferae or mustard family, of the plant kingdom is one of the most economically important plant families in the world. This family includes approximately 310 genera and 3500 species distributed in nearly all continents except Antarctica and plays an indispensable role in human nutrition by providing a variety of vegetables, oils and condiments (for review, see Rakow 2004 and Al-shehbaz 2011). Cruciferous vegetables are high in vitamins, essential minerals, carotenoids, dietary fibers and other phytochemical compounds,

which are beneficial to human health (Šamec et al. 2019). The nutraceutical glucosinolates of the family Brassicaceae have been proved to have an anticarcinogenic property (Abbaoui et al. 2018; Salehi et al. 2021). The inclusion of cruciferous vegetables in the diet can prevent oxidative stress, inflammation, digestive ailments, chronic diseases and cancer (Abbaoui et al. 2018; for review, see Šamec and Salopek-Sondi 2019, Šamec et al. 2019 and Salehi et al. 2021). Apart from these benefits, plant members of the family Brassicaceae have been widely used in biological research. For example, *Arabidopsis thaliana* has been widely used in plant science research due to its small-size (about 135 megabases) genome (https://www.*Arabidopsis*.org/, retrieved on Nov 2021), little-repetitive DNA, and its short life cycle (for review, see Al-shehbaz 2011). The genus *Brassica* has been used to understand the evolution of plant genome and formation of polyploid species (for review, see Paterson et al. 2001). An understanding of the plant genome is important for unraveling the biological basis of different traits for further improvement of our crop plants.

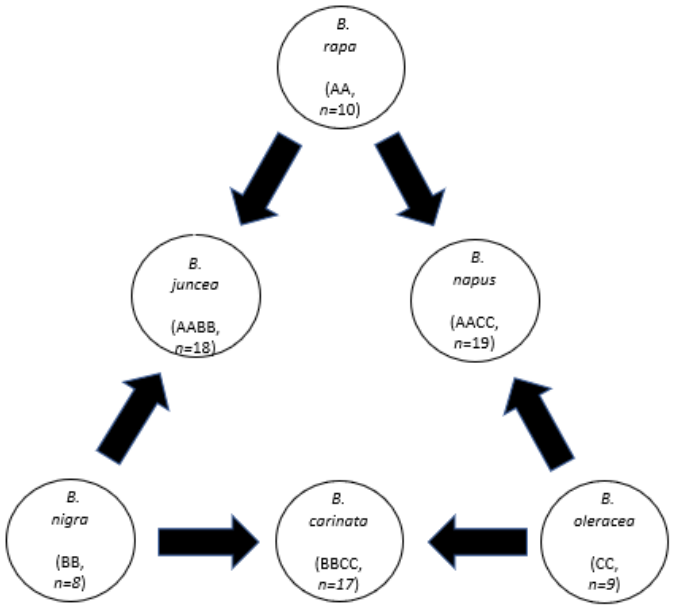
*Brassica,* which includes 37 species, is the most important genus of the family Brassicaceae. Crops of this genus are grown for vegetables, oilseeds, fodder and condiments. The 'cole crops' *B. oleracea* and *B. rapa* include vegetables which constitute an important part of our daily diet. For example, *B. oleracea* genotypes comprises such as kale, collard, cabbage, brussels sprout, cauliflower and broccoli, while *B. rapa* includes turnip, Chinese cabbage and pak choi. *Brassica* oilseed plants, including *B. napus, B. rapa* and *B. juncea*, provide oil for human consumption as well as for industrial use (for review, see Paterson et al. 2001, Rakow 2004, Al-shehbaz 2011). Human kind has a long history of cultivation and use of the plants of the genus *Brassica*. It is believed that cabbage originated in the Mediterranean region about 8,000 years ago, and the

earliest record of the use of *Brassica* plants in India can be found in Sanskrit which can be traced back to about 3,000 BC; some *Brassica* species were also used as medicine in ancient Asia (for review, see Al-shehbaz 2011).

### 1.3 Genomic relationship of genus *Brassica*

The best-known six species of the genus *Brassica* includes three diploid species *B. rapa* (2*n* = 20; AA), *B. nigra* (2*n* = 16; BB) and *B. oleracea* (2*n* = 18; CC), and three amphidiploid species *B. juncea* (2*n* = 36; AABB), *B. carinata* (2*n* = 34; BBCC) and *B. napus* (2*n* = 38; AACC); the interrelationship between the above-mentioned six species is represented as a triangle which is commonly known as the triangle of U (Figure 1.1) (U, 1935; cited from Chen et al. 2011). In this triangle, the three allotetraploid species are derived from hybridizations between two diploid species, where the three diploid species evolved from a common ancestor (Truco et al. 1996; Chen et al. 2011b). Therefore, exotic genes and alleles of these diploid species can be exploited to improve the amphidiploid species, including *B. napus* canola, through interspecific hybridization. By using the knowledge of the relationships between the *Brassica* genomes, new alleles for many agronomic traits, such as resistance to disease and insect pests, yellow seed color, male sterility, dwarf plant, and drought tolerance, have been introgressed from one to another within *Brassica* species, as well from other genera into *Brassica* (Rahman 2001; Muangprom et al. 2006; Rahman et al. 2011b, 2017; Chamola et al. 2013).

Figure 1.1 Relationship between six species of the genus *Brassica* - the triangle of U (adapted from Nagaharu U 1935)




### 1.4 Biotic and abiotic stresses affecting canola production

The yield of canola can be affected by many abiotic and biotic factors. The abiotic stresses, such as heat, drought, cold, salinity and heavy metal toxicity, can affect the metabolic pathways, which subsequently exert a negative effect on the physiology of the plants (for review, see Lohani et al. 2020). The extreme temperature can damage cell membrane and reduce photosynthetic efficiency (Elferjani and Soolanayakanahally 2018; Megha et al. 2018); yield loss due to heat stress can be up to 85% (Elferjani and Soolanayakanahally 2018). Drought can decrease the content of chlorophyll in leaves due to loss of pigments and reduced efficiency of the thylakoid membranes (for review, see Ahmar et al. 2019). Water stress during the period of anthesis to maturity can lead to a 48% reduction in seed yield and a 44% reduction in oil yield

(Champolivier and Merrien 1996). Salinity can cause a biochemical change in the plants through the accumulation of reactive oxygen species (ROS) (for review, see Ahmar et al. 2019).

Biotic stress in canola can result from infestation by insects, diseases and weeds (www.canolacouncil.org, retrieved on Oct 2021) Among the different diseases of canola, the clubroot disease caused by *Plasmodiophora brassicae* Wornin, brown girdling root rot caused by *Rhizocotonia solani*, sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, blackleg caused by *Leptosphaeria maculans* and verticillium wilt caused by *Verticillium longisporum* are the most important ones, which can cause significant yield loss (Woods et al. 2000; Strelkov et al. 2007; Dunker et al. 2008; Mei et al. 2011; Hwang et al. 2016). Pests, like bertha armyworm (*Mamestra configurata*), cabbage seedpod weevil (*Ceutorhynchus obstrictus*), diamondback moth (*Plutella xylostella*), flea beetles (*Phyllotreta* spp and *Psylliodes punctulata* ), lygus bugs (*Lygus* spp.) and swede midge (*Contarinia nasturtii*) are the most devastating insects to canola in the Canadian prairie provinces (Knop Wright et al. 2006; Knodel et al. 2008; Evenden and Gries 2010; Chen et al. 2011a; de Silva Weeraddana and Evenden 2018). Weeds like cleavers, glyphosate-resistant kochia, Canada thistle, volunteer wheat and barley, wild oats, green foxtail and quackgrass are the strong competitors which can take up space, sunlight and nutrient, and consequently affect canola yield (Aghaalikhani M and Yaghoobi SR 2008; Madden et al. 2021). An integrated pest management approach, including crop scouting, agronomic practices, chemical and biological control, and cultivation of resistant cultivars, has been proposed for sustainable canola production in Canada (www.canolacouncil.org, retrieved on Oct 2021).

### 1.5 Interspecific hybridization for transfer of traits from *B. oleracea* into *B. napus*

Interspecific crosses between *B. oleracea* and *B. napus* have been performed by several researchers to transfer genes or alleles from *B. oleracea* into *B. napus* as well as to broaden the genetic base of *B. napus* canola (Bennett et al. 2008, 2012; Rahman et al. 2011a, 2015, 2017, 2018). For example, Rahman et al. (2015, 2017 and 2018) crossed *B. napus* canola with Chinese kale *B. oleracea* var. *alboglabra* and introgressed an early-flowering allele from var. *alboglabra* into canola, which demonstrated the possibility of introgression of genes from *B. oleracea* into *B. napus* species. To attain viable progenies from this interspecific cross, Bennett et al. (2008) investigated the optimal stage of ovule culture for the production of *B. napus* × *B. oleracea* interspecific hybrid. They found that ovule culture at 16 days after pollination (DAP) yielded the greatest number of hybrid embryos when *B. oleracea* was used as the female parent and the plants were grown at 25°/15°C (day/night) temperature; when using *B. napus* as the female parent and the plants were grown at 15°/10°C (day/night) temperature, ovule culture at 14 DAP generated the greatest number of interspecific hybrid plats. The $F_1$ plants generally show high sterility due to anomaly of the chromosomes in meiosis. However, fertile euploid *B. napus* plants can be recovered from the progeny of this interspecific cross (Li et al. 2014b; Rahman et al. 2015). Interestingly, the self-pollinated progeny of the digenomic triploid (ACC) derived from *B. napus* × *B. oleracea* crosses often stabilize into amphidiploid *B. napus* type ($2n = 38$), and the reconstituted *B. napus* plants were found to carry alleles of the C genome of *B. oleracea* (Bennett et al. 2012; Rahman et al. 2015; Iftikhar et al. 2018).

In addition to introgression of specific genes from *B. oleracea* into *B. napus*, this interspecific cross can also be used to broaden the genetic base of *B. napus* canola. The narrow genetic base

in modern canola cultivars resulted from the bottleneck during its evolution, and later from intensive breeding for the canola-quality traits (for review, see Rahman 2013). Of the two genomes of *B. napus*, the genetic base of the C genome is narrower than its A genome (Bus et al. 2011). In this regard, novel *B. napus* lines derived from *B. napus* × *B. oleracea* interspecific cross would be a valuable resource for canola breeding.

### 1.6 Clubroot disease and management

Among the different diseases of *Brassica* oilseed crops, clubroot disease caused by the obligate parasite *P. brassicae* Woronin is one of the most devastating. Potentially, all members of the family Brassicaceae can be subjected to infection and become the host of this pathogen; therefore, this disease has become a major threat to cruciferous crop production worldwide (Dixon 2009a). In the last few decades, clubroot has spread rapidly and threatened the production of *Brassica* oilseed crops in several countries, including Australia, Canada, China, Czech Republic, Denmark, France, Germany, Japan, Sweden, Poland United Kingdom and the United States (for review, see Dixon 2009a and Řičařová et al. 2016; Strelkov et al. 2016). In Canada, clubroot disease in the canola field was first reported in 2003 in central Alberta (Tewari et al. 2005) and the incidence of this disease rapidly increased (Strelkov et al. 2018). This disease has been reported to cause about 30% yield loss in canola (Tewari et al. 2005); complete crop loss has also been reported in extreme cases (for review, see Hwang et al. 2012; Strelkov and Hwang 2014).

*Plasmodiophora brassicae* belongs to the order Plasmodiophorida, which was historically classified as a fungus due to its capacity to produce spores and cause plant disease. Recently,

based on analysis of small subunit ribosomal RNA genes and other protein-coding genes, the order Plasmodiophorida have been categorized into the protist supergroup Rhizaria under the phylum Cercozoa (for review, see Hwang et al. 2012). The life cycle of *P. brassicae* can be broadly divided into resting spore, primary infection and secondary infection stages. The subspherical- or spherical- shaped resting spores, which are about 3 μm in size and covered with spines, are released from decayed hosts. Each resting spore generates a primary zoospore with two flagella which can swim to the root hair and penetrate the cell wall of host plants (for review, see Kageyama and Asano 2009). During primary infection, *P. brassicae* attacks root hair and subsequently undergoes a series of nuclear divisions in epidermal cells and forms the zoosporangium. Secondary zoospores are produced in the zoosporangium (for review, see Kageyama and Asano 2009; Liu et al. 2020). The zoosporangium appears as small and pearl-shaped galls which first manifest on the lateral roots. A zoosporangium can release 4-16 secondary zoospores; they attack the young roots or older thickened roots which lead to the development of the secondary plasmodia within the attacked tissues. The secondary plasmodium undergoes a series of complicated proliferation and meiotic division and becomes multinucleate. The multinucleate plasmodia spread to the cortical cells, cambium and subsequently to the cortex and xylem, where the abnormal development of host cells occurs and results in the formation of characteristic galls full of resting spores in roots (for review, see Kageyama and Asano 2009 and Hasan et al. 2021a). The transportation of water through xylem tissue of the susceptible plants becomes severely interrupted due to clubroot infection, which causes above-ground symptoms like stunting and wilting of the plants, yellowing of the leaves and premature ripening and eventual death of the infected plants (for review, see Kageyama and Asano 2009; Hwang et al. 2012). Following the disintegration of the root galls, the resting spores of *P. brassicae* are

released back to the soil. The resting spores can remain pathogenic for a long period of time because they are well protected by a five-layer cell wall (Buczacki and Moxham 1983). According to Wallenhammar (1999), the half-life of the resting spores in the field is about 3.6 years, while the pathogenicity of the spores can remain up to 17 years. The zoospores germinated from the resting spores can be dispersed by water and can travel towards the root surface through the motion of the flagella (for review, see Dixon 2009b and Hwang et al. 2012). Of the different stages of the life cycle of *P. brassicae*, the zoospore stage is most vulnerable to environmental conditions for survival. During this time, it seeks hosts and is only equipped with a single cell wall; however, this condition is very short (for review, see Dixon 2009b).

The gemination of resting spores in soil can be affected by various biotic and abiotic factors. Among these, root exudates are critical for spore germination. The stimulus from root exudates can come either from the host or from non-host species (Macfarlane 1970; Friberg et al. 2005; Niwa et al. 2008; Feng et al. 2010), and ionic composition affects spore germination rate (Macfarlane 1970). Calcium is most likely a cofactor of serine protease *Pro1* which is highly expressed during germination and infection stages and can facilitate spore germination in the presence of root exudates (Feng et al. 2010). However, calcium can also increase the soil pH, which has been shown to have an inhibition effect on spore germination and root-hair infection (Niwa et al. 2008); the effectiveness of liming depends on the size of the lime particle, its chemical form and neutralizing value (for review, see Hasan et al. 2021a). Increasing level of nitrogen or nitrate ions can negatively affect the development of *P. brassicae* in the rhizosphere (for review, see Dixon 2009b). However, Laperche et al. (2017) showed that the level of host resistance under variable nitrogen conditions could vary depending on *P. brassicae* genotype and

resistance genes in the host. Soil moisture is another factor that can affect disease development (Dixon 2009b; Řičařová et al. 2016). Tap water has been found to encourage spore germination (Ayers 1944, cited by Dixon 2009b), and adequate soil moisture can facilitate the infection process by creating an aerobic condition for germination (Dixon 2009b; Řičařová et al. 2016). Microbes, such as some strains of *Lysobacter* and *Bacillus cereus*, can significantly inhibit the germination of resting spores of *P. brassicae* (Fu et al. 2018; Arif et al. 2021). Other factors such as spore load, soil boron, light intensity, and soil structure can also affect the development of *P. brassicae* (for review, see Dixon 2009b, Hwang et al. 2014 and Řičařová et al. 2016). In fact, infection by *P. brassicae* can be determined by a combined effect of various factors such as moisture, temperature, pH, soil texture and structure, spore biology, host plant genetics, and soil microfauna. The optimal conditions for clubroot infection are estimated to be 23-26 °C temperature and poorly drained acidic soil (for review, see Dixon 2009b; Gossen et al. 2012; Řičařová et al. 2016). Based on knowledge of the conditions required for resting spore germination and disease infection, common management practices have been investigated by different researchers for clubroot management (for review, see Hwang et al. 2014; Peng et al. 2014b). However, many of these, such as fungicide, bait crops and soil amendments, were found not to be economically viable for efficient clubroot management in the commercial production of canola (for review, see Hwang et al. 2014). Therefore, the development of resistant cultivars carrying diverse resistance genes and crop rotation has been identified to be the most effective strategy for controlling clubroot disease (for review, see Hwang et al. 2014 and Hasan et al. 2021a; Peng et al. 2014b). In addition, sanitation of the seed and farm equipment is also essential to impede the spread of the pathogen (for review, see Hwang et al. 2014).

## 1.7 Mechanisms of resistance to clubroot

To manage the disease through the development of resistant cultivars, an understanding of the interaction between the host and the pathogen is important to deploy the resistance genes in the best possible way. Two types of triggered host immunity are involved during plant-pathogen interaction: Pathogen-Associated Molecular Pattern (PAMP) triggered Immunity (PTI) and Effector-triggered Immunity (ETI) (Jones and Dangl 2006). PTI is initiated with the recognition of the conserved PAMP features of the pathogen by the pattern recognition receptors in the host plant cell membrane. PTI can result in a hypersensitive response which is considered to be non-specific to pathogen races (for review, see Neik et al. 2017 and Pérez-López et al. 2018). On the other hand, the effectors produced by avirulence genes in the pathogen are race-specific; they can be recognized by the receptors encoded by the R genes of the host plant and can stimulate ETI, and this stimulates a race-specific hypersensitive response (for review, see Jones and Dangl 2006 and Pérez-López et al. 2018). However, neither PTI nor ETI has yet been well studied in *P. brassicae* (for review, see Pérez-López et al. 2018); only a few effector genes of *P. brassicae* have so far been identified and characterized (for review, see Pérez-López et al. 2018 and Hasan et al. 2021a). The methyltransferase encoded by *PbBSMT* gene of *P. brassicae* can inhibit salicylic-acid related plant defense through methylation of the salicylic, benzoic and anthranilic acids (Ludwig-Müller et al. 2015; Ciaghi et al. 2019). Serine protease *Pro1* may play an important role in pathogenesis by inducing spore germination (Feng et al. 2010). Chitinase genes may also be involved in disease development and may act as a PAMP during host-*P. brassicae* interaction (Chen et al. 2018). Several *P. brassicae* genes have been reported to be upregulated during primary and secondary infection stages (Fei et al. 2016). For example, the expression of

the race-specific gene *Cr811* of *P. brassicae* pathotype 5 has been found to be upregulated during infection of canola plants (Zhang et al. 2015). Expression of other genes, such as *Y10, PbTPS, PbSTKL1, PbBrip9, and PbCC245* have also been reported to be associated with pathogenesis(Hwang et al. 2012). Several effectors, such as cysteine-rich proteins, nuclear localization domains, chitin-binding domains, protease inhibitors, plasmodium export element (Pexel) motif, and arginine-any amino acid-leucine arginine (RxLR) motifs have been identified in other plant pathogens, however, their role in the pathogenesis of *P. brassicae* has not been well understood (for reviw, see Pérez-López et al. 2018; Hasan et al. 2021a).

ETI is considered to be regulated by the host resistance gene. In plants, this is often mediated through the conserved motifs such as nucleotide-binding site (NBS), leucine-rich repeat (LRR), toll-interleukin-1 receptor domain (TIR), coiled-coil (CC) structure, leucine zipper (LZ) structure and protein kinase (PK) domain (Liu et al. 2007). The biggest group of plant-disease resistance genes is the NBS-LRR class (Glazebrook 2005), which is effective against biotrophic and hemi-biotrophic pathogens (for review, see Glazebrook 2005; Jones and Dangl 2006). Genes encoding TIR-NBS-LRR class proteins have been found in *B. rapa, B. oleracea* and *B. napus* (Yu et al. 2017; Dakouri et al. 2018; Hejna et al. 2019). The LRR domain may play an important role in resistant plants (Zhang et al. 2016). Several NBS-LRR genes can be found in the *Brassica* genome, which originated from a common ancestral genome through gene duplication and uneven crossovers; these genes subsequently may have evolved independently and developed unique resistance patterns (Yu et al. 2014; Hatakeyama et al. 2017). A genome-wide study identified 464, 202 and 146 putative functional NBS-encoding genes in *B. napus*, *B. rapa* and *B.*

*oleracea*, respectively; these genes are found to be distributed unevenly in a clustered pattern (Fu et al. 2019).

To date, many genes or QTL have been identified to be involved in resistance to *P. brassicae.* However, several resistance genes act in a race-specific manner; therefore, precise classification of the pathotypes is required to understand their roles. The races or pathotypes of *P. brassicae* are classified based on their capacity to infect different host plants. Historically, the Williams (1966, cited by Strelkov et al. 2018), Somé (Some et al. 1996), and the European Clubroot Differential (ECD) (Buczacki et al. 1975, cited by Strelkov et al. 2018) systems have been used by researchers; among these, the Williams differential set has been used most extensively by the Canadian researchers (Strelkov et al. 2018). However, the above-mentioned systems were not able to differentiate the newly evolved pathotypes from the old ones. Therefore, the Canadian Clubroot Differential (CCD) set with a greater capacity of differentiating the pathotypes has been developed by Strelkov et al. (2018). Nevertheless, all differential systems are based on screening of the isolates for virulence sets of various hosts rather than identification of the exact genotype of the pathogen races.

Before the release of the clubroot-resistant canola cultivars, the CCD pathotype H or 3H (Strelkov et al. 2018), which was previously designated as pathotype 3 (Williams, 1996), was found most commonly in canola fields in Alberta (for review, see Strelkov and Hwang 2014). The first incidence of overcoming this resistance by *P. brassicae* has been reported in 2013 by Strelkov et al. (2016), which was designated as pathotype X or 5X (Strelkov et al. 2018). More recently, the CCD pathotype A (or 3A), a variant of pathotype H (or 3H), was found to be capable of overcoming the resistance of the winter canola cv. 'Mendel' and the Canadian spring

canola cv. '45H29'; this pathotype has currently become most prevalent and virulent in Alberta. The CCD pathotype B (or 2B) can infect almost the same host spectrum as CCD pathotype A and, additionally, it is virulent on the *B. oleracea* host ECD 11. Similarly, CCD pathotype D (or 3D) shares the same host range with CCD pathotype A except it is avirulent on the cv. 'Mendel'. The breakdown of resistance has been found to occur as distinct clusters indicating that the new *P. brassicae* populations evolve independently in the fields (Strelkov et al. 2018). Therefore, the development of canola cultivars carrying resistance to multiple pathotypes has been considered the most important pillar of clubroot management, and this should be planned scientifically for maximizing the efficiency and longevity of the resistance.

### 1.8 Identification of *Brassica* germplasm for clubroot resistance

Screening of *Brassica* germplasm for resistance to CR has been carried out by researchers and many accessions carrying resistance have been identified (Table 1.1, Table 1.2 and Table 1.3). For example, Peng et al. (2014a) tested 955 *Brassica* accessions including a large number of *B. rapa* for resistance to pathotypes 2, 3, 5, 6 and 8 (Williams, 1966) and identified several *B. rapa* vegetables, such as pak choi cv. 'Flower Nabana', Chinese cabbage cvs.'Bejo 2833', 'Emiko', 'Jazz Napa Cabbage', and turnip cvs. 'Siloga', 'Taronda', 'Vedette' and 'Vollenda', carrying resistance to pathotype 3. On the contrary, most of the *B. napus* accessions showed almost no resistance to pathotype 3, while the rutabaga cvs. 'Wilhelmsburger', 'Askegarde' and the spring canola line 'SW 02763' showed moderate resistance to these pathotypes. Some accessions of *B. nigra* were completely resistant to all five pathotypes. Resistance to multiple pathotypes has also been found in cabbage (*B. oleracea*) cvs. 'Kilaherb' and 'Tekila'; however, none of the *B. juncea* and *B. carinata* accessions carried resistance. Similar results have also been reported by Hasan et

al. (2012) and Liu et al. (2018b). Nevertheless, Liu et al. (2018) reported one *B. juncea* accession to be resistant to pathotype 4. Hasan et al. (2012) found all five turnip accessions used in their study were highly resistant to pathotypes 2, 3, 5, 6, 8. Recently, Farid et al. (2020) investigated CR in *B. oleracea* and identified several accessions of var. *acephala* (kale) and few accessions of var. *gemmifera* (Brussels sprout) and var. *capitata* (cabbage) carrying resistance to the newly evolved pathotypes 3A and 5X (LG2) isolates. In contrast, no resistance was found in the vars. *italica* (broccoli), *alboglabra* (Chinese kale) and *sabauda* (savoy cabbage).

### 1.9 Clubroot resistance loci and introgression into *B. napus* canola

Several clubroot resistance loci, such as *CRa, CRb, CRc, CRd, CRk, Rcr1, Rcr2, Rcr4, Rcr8, Rcr9, Crr1, Crr2, Crr3, CrrA5, bBa3.3, QS_B3.1* and *PbBa8.1* (Table 1.1), have been identified in *B. rapa*, where the majority of the loci originated from European turnips and most of them show a large additive or dominance effect for resistance to *P. brassicae* pathotypes (Matsumoto et al. 1998; Suwabe et al. 2003; Hirai et al. 2004; Sakamoto et al. 2008; Piao et al. 2009; Chen et al. 2013; Pang et al. 2014, 2018; Huang et al. 2017; Yu et al. 2017; Laila et al. 2019; Choi et al. 2020). The chromosome A03 carries several CR loci hotspots, such as *PbBa3.1* located at 1.95 - 6.61 Mb region (Chen et al. 2013); *PbBa3.2, Crr3, CRd* and *CRk* at 13.54 - 16.37 Mb region (Chen et al. 2013; Pang et al. 2018); *PbBa3.3* at 18.43 - 22.10 Mb region (Chen et al. 2013); *Bra012688, CRa, CRb, CRbKato, QS_B3.1, Rcr 1, Rcr 2* and *Rcr 4* at 22.28 - 29.98 Mb region (Pang et al. 2014; Zhang et al. 2016; Yu et al. 2017; Hatakeyama et al. 2017; Fredua-Agyeman et al. 2020; Hasan et al. 2021b). Besides this chromosome, regions of A01 were found to carry *QS_B1.1, PbBa1.1* and *Crr2* (Chen et al. 2013; Pang et al. 2014); and regions of A08 carry *Crr1, CRs, PbBa8. 1, PbBrA08$^{Banglim}$, Rcr9* and *QS_B8.1* (Chen et al. 2013; Pang et al. 2014; Yu et al.

2017; Laila et al. 2019; Choi et al. 2020; Fredua-Agyeman et al. 2020). The genomic location of some of the loci was found to overlap; therefore, many of these could be the same locus but reported as different loci. For example, it has been reported that *QS_B3.1* contains both *CRa* and *CRb* loci, however, these two loci later have been confirmed to be one locus (Hatakeyama et al. 2017). Among the different CR loci of the A genome, functional characterization of only *Crr1a* (Hatakeyama et al. 2013) and *CRa* (Ueno et al. 2012) has been done through gain-of-function and loss-of-function methods, respectively. As mentioned above, a large number of CR loci of the A genome exhibit qualitative inheritance; however, quantitative genetic control of resistance has also been reported in *B. rapa* (Yu et al. 2017; Chen et al. 2013). Furthermore, epistatic interactions between the CR loci conferring resistance to this disease has also been reported (Sakamoto et al. 2008; Pang et al. 2014; Fredua-Agyeman et al. 2020).

To date, most of the efforts on mapping of clubroot resistance have been focused on the *Brassica* A genome. However, recently this has been shifted towards the C genome of *B. oleracea* (Lee et al. 2016; Dakouri et al. 2018; Peng et al. 2018; Farid et al. 2020) as well as *B. napus* (Li et al. 2016; Hejna et al. 2019; Wagner et al. 2019; Botero-Ramírez et al. 2020). A number of QTLs have been identified from all nine C genomes where some of the chromosomes were found to carry more than one QTL (Table 1.2 and Table 1.3). For example, at least four QTLs, such as *PbC7.1, PbC7.2, Rcr7, PbC7.3* and *BnC07_0238*, have been reported from C07 (Dakouri et al. 2018; Hejna et al. 2019; Farid et al. 2020). QTL mapping of clubroot resistance in *B. napus* also identified several QTL from the A and C genome chromosomes (Werner et al. 2008; Li et al. 2016; Hejna et al. 2019; Botero-Ramírez et al. 2020). Among these, the 21–27 Mb region of A03 (Li et al. 2016; Hejna et al. 2019) and 1–5 Mb region of C03 (Li et al. 2016; Botero-Ramírez et

al. 2020) were found to be the hotspots for the CR genes. Thus, it is evident that CR in the C genome of *B. oleracea* and *B. napus* is largely controlled by quantitative trait loci (Piao et al. 2009; Peng et al. 2018; Hejna et al. 2019; Farid et al. 2020). Moreover, epistatic interactions between the genomic regions have also been reported (Manzanares-Dauleux et al. 2000; Rocherieux et al. 2004).

Given the quantitative and qualitative nature of the CR genes and the feasibility of crossing different *Brassica* species, introgression of CR genes from allied species and exotic germplasm into canola is possible, and this has been achieved by different plant breeders and researchers. For example, Hasan and Rahman (2016) transferred a CR locus located on chromosome A08 of rutabaga (*B. napus* var. *napobrassica*) into spring *B. napus* canola; this gene showed resistance to multiple pathotypes including pathotype 3. Liu et al. (2018b) and Hasan et al. (2021b) transferred resistance to pathotype 4 and 3 from *B. rapa* subsp. *pekinensis* into *B. napus*, respectively. The introgression of multiple CR loci into canola is important for pyramiding clubroot-resistance genes into canola for resistance to multiple pathotypes, as has been demonstrated by Matsumoto et al. (2012) and Shaikh et al. (2021).

## 1.10 Research objectives

As reviewed above, clubroot is a devastating disease to canola production; therefore, the development of clubroot-resistant cultivars has been considered an indispensable tool for the successful management of this disease. Efforts have been made for the development of clubroot-resistant canola cultivars. However, most of the efforts have been focused on the use of the A-genome resistance. In contrast, the resistance genes from the C genome remain relatively less

exploited despite strong resistance to this disease has been reported in several accessions of *B. oleracea* (Lee et al. 2016; Li et al. 2016; Peng et al. 2018; Hejna et al. 2019; Wagner et al. 2019; Farid et al. 2020). To date, no research has been carried out to introgress clubroot-resistance genes of *B. oleracea* into *B. napus* through an interspecific cross between these species to enrich the resistance in the C genome of *B. napus* canola. The overall objective of this research project is to introgress clubroot-resistance genes from *B. oleracea* into *B. napus* canola and use this resistance in combination with the A genome resistance for the development of clubroot resistant canola cultivars. The tactical objectives of this M.Sc. thesis research are the followings:

1. Produce interspecific hybrids of *B. napus* canola and clubroot resistant *B. oleracea* var. *acephala* accessions and develop progeny generations of the interspecific hybrids.

2. Investigate the possibility of developing a canola quality clubroot resistant euploid *B. napus* line from a *B. napus* × *B. oleracea* interspecific cross.

Table 1.1 List of major clubroot resistance loci reported in *Brassica rapa*. The loci where TIR-NBS-LRR has been reported are marked by the tick ('√') sign

| Chromosome | Locus name | Resistance source | Pathotype/isolate | TIR-NBS-LRR | Reference |
|---|---|---|---|---|---|
| A01 | *Crr2* | European turnip cv. 'Siloga' | Wakayama-01 (Kuginuki et al. 1996) | | Suwabe et al. 2003, 2006 |
| A02 | *CRc* | European fodder turnip cv. 'Debra' | 2 | | Sakamoto et al. 2008 |
| A02 | *Rcr8* | European turnip cultivar cv. 'Pluto' | 5X (Strelkov et al. 2018) | √ | Yu et al. 2017 |
| A03 | *Bra012688* | Chinese cabbage cv. 'Bilko' | 3 | √ | Hasan et al. 2021b |
| A03 | *Crr3* | European turnip cv. 'Milan White' | ANo-01 (Kuginuki et al. 1999) | | Hirai et al. 2004; Saito et al. 2006 |
| A03 | *CRa* | Chinese cabbage, turnip ECD-02 cv. 'Gelria R', 'Debra' | 2, 5X, 5G (Strelkov et al. 2018) | √ | Matsumoto et al. 1998, 2012; Ueno et al. 2012; Zhang et al. 2016; Fredua-Agyeman et al. 2020 |
| A03 | *CRd* | Chinese cabbage | 4 | √ | Pang et al. 2018 |
| A03 | *CRb* | Chinese cabbage,<br>European fodder turnip cv. 'Gelria R' | 2, 4, 8 and group 3 (Hatakeyama et al. 2004) | √ | Piao et al. 2004; Kato et al. 2013; Zhang et al. 2014; Hatakeyama et al. 2017 |

| | | | | | |
|---|---|---|---|---|---|
| A03 | *CRk* | European fodder turnip cv. 'Debra' | Isolate M85 (Matsumoto et al. 2005)<br><br>Isolate K04 (Sakamoto el al. 2008) | | Sakamoto el al. 2008 |
| A03 | *PbBa3.3* | European fodder turnip ECD-04 | 7 | | Chen et al. 2013 |
| A03 | *QS_B3.1* | European fodder turnip | 4 | | Pang et al. 2014 |
| A03 | *Rcr1* | Pak choi (*B. rapa* ssp. chinensis) cv. 'Flower Nabana' | 2, 3, 5, 6 | √ | Chu et al. 2014; Yu et al. 2016 |
| A03 | *Rcr2* | Chinese cabbage cv. 'Jazz' | 2, 3, 5, 6, 8 | √ | Huang et al. 2017 |
| A03 | *Rcr4* | European turnip cv. 'Pluto' | 2, 3, 5, 6, 8 | √ | Yu et al. 2017 |
| A05 | *CrrA5* | Chinese cabbage | Unknown race | | Nguyen et al. 2018 |
| A08 | *Crr1* | European turnip cv. 'Siloga' | 2, 4, isolate 'Wakayama-01' and 'Ano-01'(Suwabe et al. 2003, 2006) | √ | Suwabe et al. 2003, 2006; Hatakeyama et al. 2013 |
| A08 | *CRs* | Turnip (SCNU-T2016) | 4, 5 | √ | Laila et al. 2019 |
| A08 | *PbBa8.1* | ECD-04 | 4 | | Chen et al. 2013 |
| A08 | *PbBrA08*$^{Bangli\ m}$ | ECD-04 | 2 | √ | Choi et al. 2020 |
| A08 | *Rcr9* | European turnip cv. 'Pluto' | 5X (Strelkov et al. 2018) | √ | Yu et al. 2017 |

Note: Otherwise stated, the pathotype/isolate names are based on Williams (1966) system.

Table 1.2 List of clubroot resistance loci reported in *B.rassica oleracea*. The gene loci where TIR-NBS-LRR has been reported are marked by the tick ('√') sign

| Chromosome | Locus name | Resistance source | Pathotype/isolate | TIR-NBS - LRR | Reference |
|---|---|---|---|---|---|
| C01 | *QTL- LG3* | Kale *B. oleracea* var. *alboglabra* cv. 'K269' | 1, 3, Isolates Kamogawa, Anno and Yuki | | Moriguchi et al. 1999; Nomura et al. 2005; Nagaoka et al. 2010 |
| C02 | *CRQTL-GN1* | Cabbage (*B. oleracea* L. var. *capitata*) inbred 'C1220' Cabbage cv. 'Anju' | 4, 9 | | Lee et al. 2016 Nagaoka et al. 2010 |
| | *Pb-Bo(Anju)1* | | | | |
| C03 | *CRQTL-GN2 (CRQTL-YC)* | Cabbage (*B. oleracea* L. var. *capitata*) inbred 'C1220' | 2, 4, 9 | | Lee et al. 2016 |
| | *Pb-Bo(Anju)3* | Cabbage cv. 'Anju' | | | Nagaoka et al. 2010 |
| LG3 | *pb-3* | Cabbage landrace *Bindsachsener* | ECD 16/3/30 (Buczacki et al. 1975) | | Voorrips et al. 1997 |
| C05 | *Pb-Bo(GC)1* *QTL- LG9* | Kale *B. oleracea* var. *alboglabra* cv. 'K269' | 1, 3, Isolates Kamogawa, Anno and Yuki | | Nomura et al. 2005; Nagaoka et al. 2010 |
| C06 | *NFR.I-5* *NFR.II-5* *PCR.1-1* *PCR.II-4* *PCR.II-5* | *B. oleracea* cv. 'GZ87' | 4 | | Peng et al. 2018 |
| C07 | *Rcr 7* | Cabbage cvs. 'Tekila' and 'Kilaherb' | 3,5X(Strelkov et al. 2018) | √ | Dakouri et al.2018 |
| C07 | *PbC7.2* | *B. oleracea* vars. *acephala, gemmifera, capitata* | 3A | | Farid et al. 2020 |

| | | | | |
|---|---|---|---|---|
| C07 | *PbC7.3* | *B. oleracea* vars. *acephala, gemmifera, capitata* | 5X, L-G2 | Farid et al. 2020 |
| C08 | *DIC.I-1 DIC.II-1* | *B. oleracea* cv. 'GZ87' | 4 | Peng et al. 2018 |
| | *NFR.II-8* | | | |
| | *PCR.II-6* | | | |
| C08 | *PbC8* | *B. oleracea* vars. *acephala, gemmifera, capitata* | 3A (Strelkov et al. 2018) | Farid et al. 2020 |
| C09 | *PbC9.2* | *B. oleracea* vars. *acephala, gemmifera, capitata* | 5X, L-G2 (Strelkov et al. 2018) | Farid et al. 2020 |
| LG1 | *CR2b* | Cabbage (*B. oleracea* L. ssp. *capitata* cv. 'Chiiteauguay') and rutabaga (*B. napus* L. ssp. *rapifera* (Metzg., Sinsk) cv. 'Wilhelmsburger') | 2 | Landry et al. 1992 |
| LG1 | *Locus 14a* | Broccoli *B. oleracea* var. *italica* | 7 | Figdore et al. 1993 |
| LG1 | *PbBo1* | French kale landrace *B. oleracea* var. *acephala* | 1, 2, 7 (Some´ et al. 1996) | Rocherieux et al. 2004 |
| LG1 | *pb-4* | Cabbage landrace *Bindsachsener* | ECD 16/3/30 (Buczacki et al. 1975) | Voorrips et al. 1997 |
| LG6 | *CR2a* | Cabbage (*B. oleracea* L. ssp. *capitata* cv. 'Chiiteauguay') and rutabaga (*B. napus* L. ssp. *rapifera* (Metzg., Sinsk) cv. 'Wilhelmsburger') | 2 | Landry et al. 1992 |

Note: Otherwise stated, the pathotype/isolate names are based on Williams (1966) system. LG stands for linkage group

Table 1.3 List of major clubroot resistance loci reported in *Brassica napus*. The gene loci where TIR-NBS-LRR has been reported are marked by the tick ('√') sign

| Chromosome | Locus name | Resistance source | Pathotype/isolate | TIR-NBS - LRR | Reference |
|---|---|---|---|---|---|
| A02 | *BnA02_0265* | *B. napus* accessions (2*n*=38) including winter oilseed rape, winter fodder, spring oilseed rape, swede, kale, semi-winter, and not assigned crop type | ECD 17/31/31 | | Hejna et al. 2019 |
| A02 | *PbBn_di_A02* | *B. napus* Winter oilseed rape cv. 'Aviso' and 'Montego' | P1(Some et al. 1996) | | Botero-Ramírez et al. 2020 |
| A03 | *BnA03_0263* | *B. napus* accessions (2*n*=38) including winter oilseed rape, winter fodder, spring oilseed rape, swede, kale, semi-winter, and not assigned crop type | ECD 17/31/31 | √ | Hejna et al. 2019 |
| A04 | *MCR-A4* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | | Li et al. 2016 |
| A10 | *SCR-A10a* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | | Li et al. 2016 |
| A10 | *SCR-A10b* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | | Li et al. 2016 |
| C03 | *MCR-C3* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | | Li et al. 2016 |
| C03 | *PbBn_di_C03* | *B. napus* Winter oilseed rape cv. 'Aviso' and 'Montego' | P1(Some et al. 1996) | √ | Botero-Ramírez et al. 2020 |
| C03 | *PbBn_rsp_C03* | *B. napus* Winter oilseed rape cv. 'Aviso' and 'Montego' | P1(Some et al. 1996) | √ | Botero-Ramírez et al. 2020 |
| C03 | *Region between PbBn_di_C03 and PbBn_rsp_C03* | *B. napus* Winter oilseed rape cv. 'Aviso' and 'Montego' | P1(Some et al. 1996) | √ | Botero-Ramírez et al. 2020 |

| | | | | | |
|---|---|---|---|---|---|
| C04 | *SCR-C4a* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | | Li et al. 2016 |
| C04 | *SCR-C4b* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | | Li et al. 2016 |
| C06 | *MCR-C6* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | √ | Li et al. 2016 |
| C09 | *MCR-C9* | *B. napus* accessions (2*n*=38) including winter oilseed rape, semi-winter oilseed rape, spring oilseed rape, spring fodder and winter fodder | 4 | √ | Li et al. 2016 |
| LG03 | *PbBn-k-2* | Resynthesized *B. napus* (*B. oleracea* cv. 'Böhmerwaldkohl' × *B. rapa* ECD-04) | Isolate 'k' | | Werner et al. 2008 |
| LG03 | *PbBn-01.07-1* | Resynthesized *B. napus* (*B. oleracea* cv. 'Bo¨hmerwaldkohl' × *B. rapa* ECD-04) | Isolate '01.07' | | Werner et al. 2008 |
| LG03 | *PbBn-1-1* | Resynthesized *B. napus* (*B. oleracea* cv. 'Bo¨hmerwaldkohl' × *B. rapa* ECD-04) | Isolate '1' | | Werner et al. 2008 |
| LG19 | *PbBn-e4x04-1* | Resynthesized *B. napus* (*B. oleracea* cv. 'Bo¨hmerwaldkohl' × *B. rapa* ECD-04) | Isolate 'e4x04' | | Werner et al. 2008 |

Note: Otherwise stated, the pathotype/isolate names are based on Williams (1966) system. LG stands for linkage group

# Chapter 2 *Brassica napus* × *B. oleracea* interspecific cross for introgression of clubroot resistance in the C genome of *B. napus* canola

## 2.1 Introduction

Canola (*Brassica napus*; AACC, 2*n* = 38) is an important source of vegetable oil in the world after palm, soybean and cottonseed oil (www.statista.com, retrieved on October 16, 2021). However, its production is often impeded by different abiotic and biotic stresses (Neik et al. 2017; Lohani et al. 2020). Plant breeders often search for genes conferring resistance to these stresses in allied *Brassica* species when they are not available in the crop germplasm (for review, see Rahman 2013). The genetic relationship between the six *Brassica* species, the well-known Triangle of U, allows crossing between these species to transfer genes from one species to the other through interspecific hybridization (for review, see Rahman 2013 and Katche et al. 2019). A number of agronomically important traits, such as silique shatter resistance (Prakash and Chopra 1988), yellow seed color (Rahman 2001), earliness of flowering (Rahman et al. 2011a), dwarf plant (Muangprom et al. 2006) and male sterility (Chamola et al. 2013) have been transferred from one *Brassica* species to the other. *B. oleracea* has been reported to carry clubroot resistance (Lee et al. 2016; Dakouri et al. 2018; Peng et al. 2018; Farid et al. 2020). However, to my knowledge, no effort has been made to introgress clubroot resistance from *B. oleracea* into *B. napus* through *B. napus* × *B. oleracea* interspecific cross. This is primarily due to the difficulty of producing interspecific hybrid of this cross, and high sterility in the hybrid progenies due to anomalies of the chromosomes in meiosis (for review, see Rahman 2013). Different cell and tissue culture techniques have been used for the efficient production of interspecific hybrids (Rahman 2004; Bennett et al. 2008; Gaebelein et al. 2019). For example,

Bennett et al. (2008) optimized the ovule culture technique for the production of $F_1$ plants of a *B. napus* × *B. oleracea* (CC, 2*n* = 18) interspecific cross. Despite high sterility occur in the progeny of *Brassica* interspecific hybrids, several researchers (e.g., Rahman et al. 2011b, 2015; Li et al. 2016) demonstrated the feasibility of recovering fertile euploid (2*n* = 38) *B. napus* plants from the progeny of this interspecific cross. The objectives of this research were to investigate the prospects of the production of interspecific hybrid plants from canola (*B. napus*) × kale (*B. oleracea*) interspecific cross, and develop recombinant *B. napus* lines from the progeny of this cross carrying the clubroot resistance of kale.

## 2.2 Materials and methods

### 2.2.1 Parent lines and interspecific crosses

Three *B. oleracea* var. *acephala* lines AM094, AM111 and AM114, developed from the Kale cvs. Dwarf Green Curled (AM094) and Kale Winterbor (AM111 and AM114) through self-pollination of single plants, and an elite *B. napus* canola (zero erucic acids in seed oil and low glucosinolate in seed meal) line A04-73NA were used as parents. The three *B. oleracea* lines were resistant to multiple *P. brassicae* pathotypes (Farid et al. 2020) and were self-incompatible, while the elite canola line is susceptible to this pathogen and self-compatible. All parental lines were obtained from the Canola Program of the University of Alberta. The *B. oleracea* parents required vernalization for flowering; therefore, they were initially grown in a greenhouse for four weeks and were vernalized (4 °C) for eight weeks in a plant growth room. After vernalization, they were transferred to a plant growth chamber set at 20 °C/15 °C (day/night) temperature and 16-hour photoperiod with photosynthetic photon flux density of 575 µmol/$m^2$/s. The *B. napus* parent A04-73NA was also grown in the same growth chamber; however, without vernalization.

The plants were fertilized every 2-3 weeks with 200 ppm 20-20-20 (N-P-K) fertilizer (Plant Products, Brampton, Ontario).

The interspecific cross between the *B. napus* and *B. oleracea* parents was performed using the *B. napus* line as female. For this, mature unopened flower buds (about a day before anthesis) of the female parent were emasculated with forceps and pollinated with fresh pollen from the male parent. The pollinated buds were bagged with water-proof envelopes to prevent any further cross-pollination. The envelopes were removed about a week after crossing and the developed siliques were used for ovule culture.

### 2.2.2 *In vitro* ovule culture

Following Bennett et al. (2008), the *in vitro* ovule culture technique was applied to rescue the interspecific hybrid embryos. For this, the developing siliques at the age of 14 to 21 days after pollination were collected and sterilized with 7% calcium hypochlorite solution for 10 min and were washed with distilled water two times. The siliques were cut along the replum with a sterilized surgical blade under a laminar flow hood, and the developed ovules were excised and placed on a sterile Petri dish. The ovules were cut into halves or left with an incision on the coat and were cultured in a 60 mm × 15 mm Petri dish filled with 5 ml liquid culture medium. The culture medium was prepared using NN medium (Nitsch and Nitsch 1967, cited by Bennett et al. 2008) mixed with 300 mg/L casein hydrolysate, 200 mg/L glutamine and 13% sucrose, and the pH was adjusted to 6.0; the medium was filtered to remove any microorganisms. The Petri-dishes were sealed with Parafilm tape and placed on a shaker at 60 rpm in dark under room temperature for 14 to 21 days. The developed embryos at torpedo stage were transferred to solid

B5 medium containing 0.1 mg/L gibberellic acid (GA3), 20 g/L sucrose and 8 g/L agar (Coventry et al. 1988, cited by Bennett et al. 2008) in Petri dish. The Petri-dishes were sealed and placed in an incubator at 4 °C and 8 h photoperiod for 2-4 days. After that, they were transferred to room temperature with a 16 h photoperiod until the tissue culture-derived plantlets had at least about 3 cm long roots.

### 2.2.3 Production of $F_2$, $BC_1$, $F_3$ and $BC_1F_2$ population

The tissue culture-derived interspecific $F_1$ plants were transplanted to soil in a greenhouse and were covered with transparent plastic cups for 1-2 weeks to maintain high humidity for the plants, and the plants were placed in a growth chamber set at 20 °C/15 °C (day/night) temperature and 16 h photoperiod with a flux density of 575 μmol/$m^2$/s. The plants were fertilized every 2-3 weeks with 200 ppm 20-20-20 (N-P-K) fertilizer (Plant Products, Brampton, Ontario).

The $F_1$ plants were manually self-pollinated for $F_2$ seeds and were also backcrossed to the *B. napus* parent A04-73NA for $BC_1$ seeds. To produce $F_2$ seeds, the unopened flower buds (about a day before anthesis) were opened at the tip with clean forceps, and 5% NaCl solution was added to the stigma by a cotton stick and left for 10-15 min; after that, they were pollinated with fresh pollen from the same plant. The pollinated buds were covered with pollination bags to avoid any cross-pollination. To produce $BC_1$ seeds, the unopened flower buds were emasculated and treated with 5% NaCl solution, as mentioned above, and were pollinated with pollen from the *B. napus* parent. The NaCl treatment was applied to break the self-incompatibility barriers (Fu et al. 1992).

The $F_2$ and $BC_1$ (≈$BC_1F_1$) plants were grown in a greenhouse to produce $F_3$ and $BC_1F_2$ seeds. To ensure the harvest of seeds from all plants, at least 30-40 flower buds were manually self-pollinated, as described above, and the plants were covered with self-pollination bags. The traits, including days to flower and seed yield (g) per plant, were recorded for this population.

### 2.2.4 Fatty acid analysis

Fatty acid profiles of the $F_3$ and $BC_1F_2$ seeds, harvested from the $F_2$ and $BC_1$ plants, were analyzed following Bennett et al. (2008). For this, 5-8 seeds from each plant were crushed in 5 ml hexane in a Fisherbrand™ Disposable Heavy-Wall Borosilicate glass tube. After centrifugation at 1500 rpm for 15 min, about 3 ml supernatant was transferred to a new tube and 1 ml Na+ methylation solution was added. The tube was covered with aluminum foil paper and incubated in dark for 30 min for maximum conversion of oil to methyl ester, and 1-2 ml of 20% NaCl solution was added to this for precipitation. About 0.5 ml hexane-methyl ester mixture from the upper layer of the tube was transferred to a gas chromatography vial and left overnight for evaporation. About 100 µl mixture was analyzed using a gas chromatograph (model 6890 N Hewlett-Packard) equipped with a DB-WAX column (polyethylene glycol stationary phase) for measurement of the fatty acids.

### 2.2.5 Oil, protein and glucosinolate analysis

Analysis of seed oil, protein and glucosinolate content was performed on bulk $F_3$ and $BC_1F_2$ seeds harvested from the $F_2$ and $BC_1$ plants using a Foss NIRsystem (FOSS, Hillerød, Demark) and following the near-infrared spectroscopy method. For this, depending on the availability of seeds, 1.7 to 4.0 g (for sample cup without insert) or 0.4 to 1.7 g (for sample cup with insert)

seeds were used. Oil and protein contents were reported as percent of the seeds and glucosinolate content reported as μmol/g seed at 8.5% moisture level.

### 2.2.6 Ploidy analysis

The $F_3$ and $BC_1F_2$ plants identified to be carrying resistance (as described in Chapter 3) to *P. brassicae* pathotype 3H at about 45 days after inoculation were analyzed for relative nuclear DNA content by a Partec ploidy analyzer (Partec GmbH, Münster, Germany). For this, approximately 2-3 $cm^2$ young leaf from each plant was chopped with a razor blade into fine pieces on a Petri dish containing 500 μl lysis and DNA extraction buffer. The content was filtered through a Partec CellTrics disposable filter and loaded in a glass tube where 1500 μl DNA staining buffer was added. After incubation for 2 minutes, the samples were analyzed by the flow cytometer. The probe of the flow cytometer was cleaned after running every 8-10 samples with 1% bleach solution (Sysmex cleaning and decontamination solution, Sysmex Partec GmBH, Germany) followed by washing twice with distilled water. Software settings were also re-calibrated each time, and relative nuclear DNA contents (Partec value) of the plants, including the *B. napus* and *B. oleracea* parents, were calculated.

### 2.2.7 Statistical analysis

Flowering date, seed weight as well as seed oil, protein, glucosinolate and erucic acid data were analyzed using one-way ANOVA in the software program R (v. 3.6.3) on Rstudio (v. 1.4.1106) to understand the extent of variation present in different families of the different generation populations. Comparison among the different families was performed by Tukey's HSD (honestly

significant difference) test with an alpha set to 0.05 using the cld and lsmeans function in R. The lm function was used to fit the linear model was as follows:

$$\text{trait.lm} = \text{lm}(\text{trait} \sim \text{family}, \text{data})$$

Chi-square test was performed in excel using the following formula:

$$\chi 2 = \sum (\text{O} - \text{E})^2 / \text{E}$$

where, O is the observed value and E is the expected value. The *p*-value was calculated using CHISQ.TES function in Excel.

Welch's *t*-test was performed to test for significant difference between the $F_2$ and $BC_1$ populations for flowering date, erucic acid, oil, protein and glucosinolate contents, and the t.test function in R was used for difference between the $F_3$ and $BC_1F_2$ populations for nuclear DNA content.

## 2.3 Results

### 2.3.1 Production of interspecific $F_1$ hybrids

A total of 145 developing siliques were excised from three interspecific crosses, which yielded 32 ovules and 12 $F_1$ plants; this translated into 0.22 ovule per silique and 0.07 $F_1$ plants per pollination. The number of $F_1$ plant/silique of the three crosses varied from 0.05 to 0.17 (Table 2.1), where the cross A04-73NA × AM111 gave the greatest number of $F_1$ plants per silique.

Table 2.1 Production of *Brassica napus* (♀) × *B. oleracea* (♂) interspecific $F_1$ plants through the application of *in vitro* ovule culture technique.

| Cross (♀×♂) | No. polli-nation | No. silique | No. embryo rescued | No. $F_1$ plant | No. ovule/ silique | No. $F_1$ plant/ silique |
|---|---|---|---|---|---|---|
| *B. nap* A04-73NA × *B. ole* AM094 | 38 | 20 | 6 | 2 | 0.30 | 0.10 |
| *B. nap* A04-73NA × *B. ole* AM111 | 32 | 29 | 5 | 5 | 0.17 | 0.17 |
| *B. nap* A04-73NA × *B. ole* AM114 | 105 | 96 | 21 | 5 | 0.22 | 0.05 |
| **Total** | **175** | **145** | **32** | **12** | **0.22** | **0.07** |

Note: *B. nap* = *B. napus*; *B. ole* = *B. oleracea*

### 2.3.2 Production of $F_2$ and $BC_1$ population and their fertility

A total of 498 $F_2$ seeds were obtained from 2,958 manual self-pollination of the $F_1$ plants of the three crosses; this translated to 0.168 seeds per self-pollination (Table 2.2). On the other hand, a total of 2,173 backcrossings of the $F_1$ plants to the *B. napus* parent yielded 0.064 seeds per pollination (Table 2.2). The $F_1$ plants of all three crosses had poor fertility (Supplementary Table 2.1); however, among the three crosses, the cross involving the *B. oleracea* line AM-114 developed from the kale cv. Winterbor (cross ID 1CA2525) showed higher fertility and produced a greater number (0.433 seeds/pollination) of self-pollinated seeds as compared with the other crosses. Wide variation was also found between the individual $F_1$ plants for seed set under self-pollination and backcrossing (Supplementary Table 2.1)

Out of all planted second-generation seeds, 375 $F_2$ and 81 $BC_1$ plants grew to the vegetative stage. Transferring the non-flowering plants to a growth chamber (20 °C/15 °C, temperature, 16-hour photoperiod) resulted in most of them flowering. Thus, 99% of the $F_2$ and 96% of the $BC_1$ plants flowered under either greenhouse or growth chamber conditions. On average, the $F_2$ population required about five-week longer time to flower as compared to the $BC_1$ population (t

= -6.49, p<0.001); wide variation was also found at the family level, where the earliest family 1CA2538.005-A6218 flowered 113.3 days earlier than the latest flowering family 1CA2525.005-A1228 (Supplementary Table 2.2). The $F_2$ population 1CA2526 of the *B. napus* × *B. oleracea* var. *acephala* cv. Dwarf Green Curled AM094 cross took on average 68.2±7.0 days to flower, while the 1CA2525 population took 116.2±2.1 days to flower. The earliest $F_2$ plant derived from *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM114 (1CA2525) flowered about 32 days after seeding (Table 2.3) when the *B. napus* parent A04-73NA flowered at about 45.0±0.63 days after seeding. A total of seven $F_2$ and three $BC_1$ plants flowered no later than the *B. napus* parent A04-73NA (data not shown). Of the flowering plants, about 90% of the $F_2$ and $BC_1$ plants produced at least one viable seed (Table 2.3). Seed set in the $F_2$ and $BC_1$ populations varied widely, from as low as 0.01 to as high as 8.62 g per plant; however, mean seed yield of these two populations was about 1.3 g per plant (Table 2.3) which was significantly lower than the *B. napus* parent (9.16 ± 0.13 g per plant) ($t$ = 2.365, $p$ =<0.001). No statistical difference in seed production was detected at $F_2$ and $BC_1$ family level or at the interspecific cross level.

Table 2.2 Production of $F_2$ seeds through manual self-pollination of the $F_1$ plants and $BC_1$ ($F_1$ × *Brassica napus*) seeds through crossing the $F_1$ plants to the *B. napus* parent of the *B. napus* × *B. oleracea* interspecific crosses.

| Cross | Cross ID | No. plants | No. poll. | No. silique | No. seeds | Silique / poll. | Seeds / silique | Seeds / poll. |
|---|---|---|---|---|---|---|---|---|
| $F_2$ by manual self-pollination: | | | | | | | | |
| *B. nap* × *B. ole* AM094 | 1CA2526 | 2 | 496 | 29 | 14 | 0.058 | 0.483 | 0.028 |
| *B. nap* × *B. ole* AM111 | 1CA2524 | 5 | 1522 | 107 | 77 | 0.070 | 0.720 | 0.051 |
| *B. nap* × *B. ole* AM114 | 1CA2525 | 5 | 940 | 82 | 407 | 0.087 | 4.963 | 0.433 |
| **Total** | | **12** | **2,958** | **218** | **498** | **0.074** | **2.284** | **0.168** |
| $BC_1$ ($F_1$ × *B. napus*) by backcrossing: | | | | | | | | |
| (*B. nap* × *B. ole* AM094) × *B. nap* | 1CA2540 | 2 | 166 | 11 | 4 | 0.066 | 0.364 | 0.024 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538 | 5 | 992 | 103 | 66 | 0.104 | 0.641 | 0.067 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539 | 5 | 1015 | 86 | 70 | 0.085 | 0.814 | 0.069 |
| **Total** | | **12** | **2,173** | **200** | **140** | **0.092** | **0.700** | **0.064** |

Note: *B. nap* = *B. napus*; *B. ole* = *B. oleracea*

Table 2.3 Growing of $F_2$ and $BC_1$ plants and production of $F_3$ and $BC_1F_2$ seeds of *Brassica napus* × *B. oleracea* interspecific crosses

| Cross | Cross ID | No. plants grew | No. plants flowered | % plants flowered | Days to flowering | | | No. fertile plants | % fertile plants[2] | Seeds (g) / plant | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Range | Mean ± S.E. [1] | | | | Range | Mean ± S.E. |
| $F_2$: | | | | | | | | | | | |
| *B. nap* × *B. ole* AM094 | 1CA2526 | 5 | 5 | 100% | 54-94 | 68.2±7.0 | ab | 4 | 80% | 1.15-2.38 | 1.77±0.22 |
| *B. nap* × *B. ole* AM111 | 1CA2524 | 43 | 42 | 98% | 39-169 | 107.9±4.7 | bc | 28 | 65% | 0.00-6.27 | 1.07±0.33 |
| *B. nap* × *B. ole* AM114 | 1CA2525 | 327 | 325 | 99% | 32-200 | 116.2±2.1 | c | 307 | 94% | 0.00-6.27 | 1.34±0.08 |
| **Total** | | **375** | **372** | **99%** | **32-200** | **114.6±1.9** | | **339** | **90%** | **0.01-7.63** | **1.32±0.08** |
| $BC_1$: | | | | | | | | | | | |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538 | 40 | 37 | 93% | 45-174 | 91.9±6.5 | ab | 34 | 85% | 0.00-4.06 | 1.23±0.2 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539 | 41 | 41 | 100% | 43-165 | 80.1±4.6 | a | 39 | 95% | 0.02-8.62 | 1.38±0.28 |
| **Total** | | **81** | **78** | **96%** | **43-174** | **85.7±4.0** | | **73** | **90%** | **0.00-8.62** | **1.31±0.17** |

Note: The spring canola parent A04-73NA flowered at 45.0 ± 0.63 days after seeding and all plants produced seeds on self-pollination; the amount of seed produced per plant was 9.16 ± 0.13 g.

[1] Comparison made between the different populations; mean ± S.E. following the same letter are not significantly different ( to 0.05).

[2] Plants having at least one viable seed was considered as fertile

### 2.3.3 Seed quality analysis

Self-pollinated seeds harvested from 203 $F_2$ and 51 $BC_1$ plants were used for the analysis of seed oil, protein and glucosinolate contents by the NIR machine, and seeds of 302 $F_2$ and 61 $BC_1$ plants were used for fatty acid analysis. Seed oil and protein contents of the *B. napus* parent A04-73NA were 48.4±0.9 % and 24.5±0.5 %, respectively. The average oil and protein content of the $F_2$ population was 38.6 ± 0.1% and 28.9 ± 0.1%, respectively; while for $BC_1$, it was 38.9 ± 0.5% and 29.1 ± 0.3%, respectively. Thus, the oil and protein content of the $F_2$ and $BC_1$ populations was similar ($t$ = -0.2102, $p$ = 0.8342), however, significantly ($t$ = -56.783, $p$ <0.001) lower in seed oil and higher ($t$ =39.047, $p$< 0.001) in seed protein than the *B. napus* parent. Of the three populations, the $F_2$ population derived from the cross involving AM111 had a relatively higher oil content (40.8±0.8%) as compared to the other populations (Figure. 2.1; Table 2.4). Wide variation for oil content was found in both populations where individual plants producing about 50% oil in seed could be found. A significant difference was found between the $F_2$ and $BC_1$ populations for seed glucosinolate (37.4 ± 0.7 vs. 30.3 ± 2.0 µmol/g seed; $t$ = 3.313, $p$ = 0.002) and erucic acid (13.4 ± 0.4 vs. 6.8 ± 0.7%; $t$ = 8.236, $p$ <0.001) contents, where the content of these two seed constituents was lower in the $BC_1$ population (Figure. 2.1; Table 2.4). Seeds of 1.5% $F_2$ and 19.6% $BC_1$ plants had glucosinolate lower than 15 µmol/g seed, and 5.0% $F_2$ and 19.7% $BC_1$ plants had erucic acid content less than 2% suggesting that canola quality lines can be extracted from these two populations (Table 2.4; Supplementary Table 2.3).

Figure 2.1 (A) Seed oil and protein, and (B) glucosinolate and erucic acid contents in $F_3$ and $BC_1F_2$ seeds harvested from $F_2$ and $BC_1$ plants of *Brassica napus* × *B. oleracea* interspecific crosses. Error bars showed for all populations. For a given trait, significant differences between the five populations (three $F_2$ and two $BC_1$) are indicated by letters above the error bars. Bars with same alphabet are not significantly different. B.nap = *B. napus* parent A04-73NA; *B. ole* AM094, AM111 and AM114 are the three *B. oleracea* var. *acephala* parents.

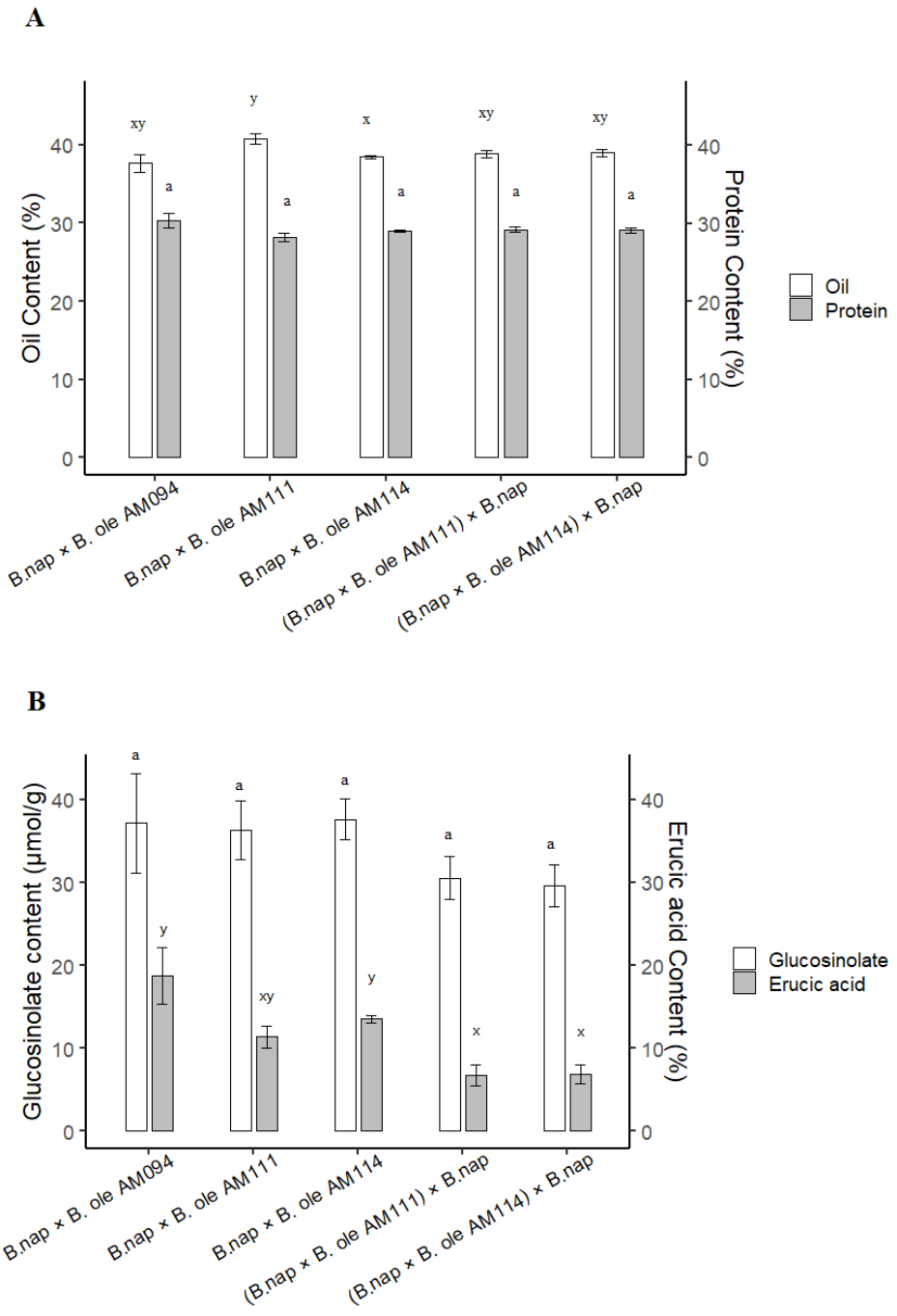


Table 2.4 Seed quality traits of the $F_3$ and $BC_1F_2$ seeds, produced, respectively on $F_2$ and $BC_1$ plants of *Brassica napus* × *B. oleracea* interspecific crosses. Near-infrared spectroscopy was used for estimation of oil, protein and glucosinolates, and gas chromatograph was used for estimation of erucic fatty acid in seed oil.

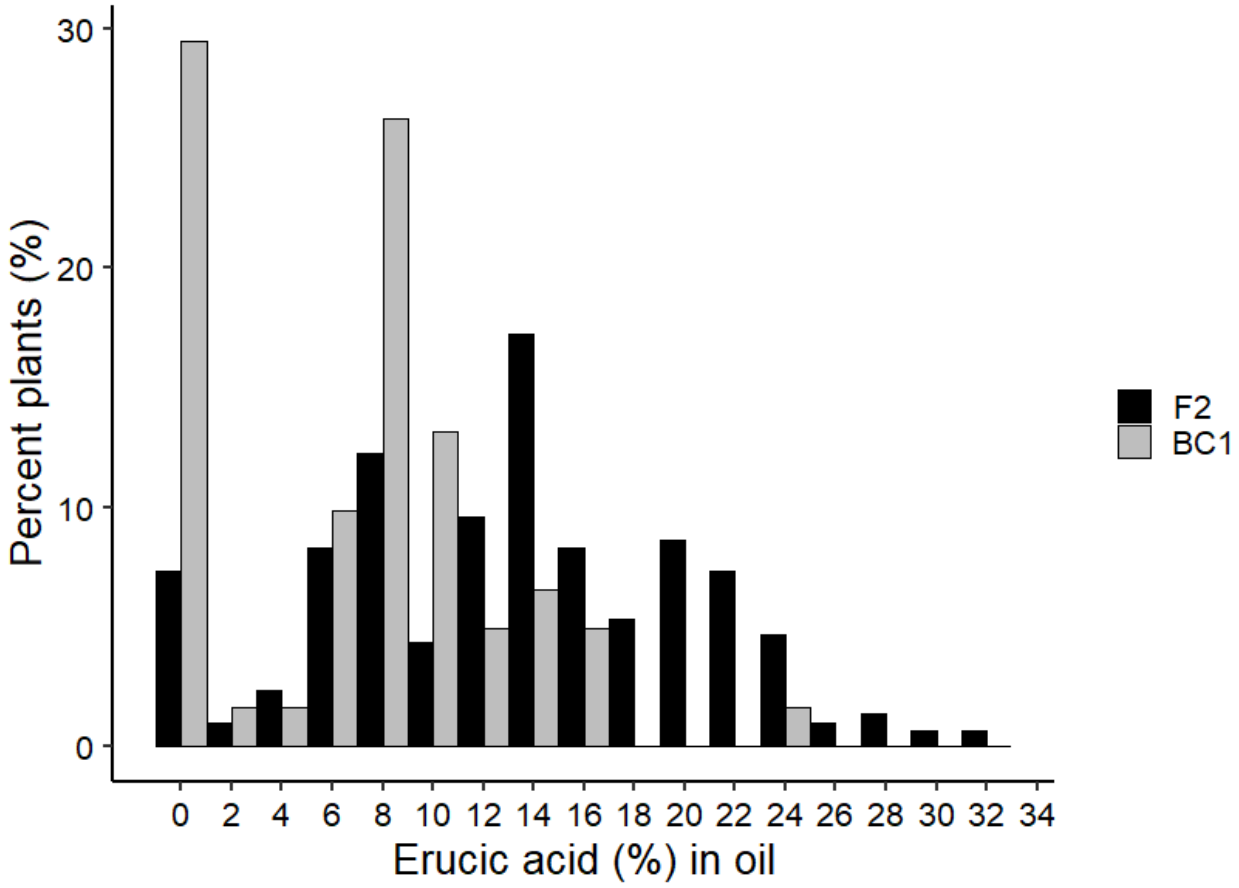
| Cross | Cross ID | No. plants for NIR | Oil (%) | | Protein (%) | | Glucosinolate (μmol/g seed) | | No. plants for GC | Erucic acid (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Range | Mean ±S.E. | Range | Mean ±S.E. | Range | Mean ±S.E. | | Range | Mean ±S.E. |
| $F_3$: | | | | | | | | | | | |
| *B. nap* × *B. ole* AM094 | 1CA2526 | 4 | 34.3-39.7 | 37.7±1.1 | 29.0-31.6 | 30.3±0.5 | 21.7-52.4 | 36.9±6.7 | 4 | 9.4-23.9 | 18.8±2.8 |
| *B. nap* × *B. ole* AM111 | 1CA2524 | 12 | 36.8-47.8 | 40.8±0.8 | 22.1-30.8 | 28.2±0.7 | 9.3-55.0 | 36.7±2.9 | 26 | 0.0-24.2 | 11.4±1.4 |
| *B. nap* × *B. ole* AM114 | 1CA2525 | 187 | 33.3-44.1 | 38.4±0.1 | 24.5-32.5 | 28.9±0.1 | 12.6-66.3 | 37.5±0.8 | 272 | 0.0-32.6 | 13.5±0.4 |
| **Total** | | **203** | **33.3-47.8** | **38.6±0.1** | **22.1-32.5** | **28.9±0.1** | **9.3-66.3** | **37.4 ±0.7** | **302** | **0.0-32.6** | **13.4±0.4** |
| $BC_1F_2$: | | | | | | | | | | | |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538 | 25 | 33.5-44.9 | 38.9±0.5 | 23.9-32.4 | 29.2±0.4 | 12.3-59.5 | 31±2.7 | 28 | 0.1-17.0 | 6.7±1 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539 | 26 | 32.2-53.6 | 39±0.8 | 18.6-32.9 | 29.1±0.5 | 10.9-55.7 | 29.6±3 | 33 | 0.1-23.3 | 6.9±1 |
| **Total** | | **51** | **32.2-53.6** | **38.9±0.5** | **18.6-32.9** | **29.1±0.3** | **10.9-59.5** | **30.3 ±2.0** | **61** | **0.1-23.3** | **6.8 ±0.7** |
| *B. nap* (A04-73NA) | | 2 | 47.2-49.6 | 48.4±0.9 | 23.6-25.3 | 24.5±0.5 | 7.1-8.6 | 7.6±0.5 | 2 | 0.1-0.2 | 0.1±0.0 |

Note: *B. nap* = *B. napus* parent A04-73NA; *B. ole* = *B. oleracea* parent

### 2.3.4 Inheritance of erucic acid

The two *B. oleracea* parents AM111 and AM114 had about 24% erucic acid in seed oil, while the content of this fatty acid in the seed oil of the *B. napus* parent A04-73NA was 0.1%. The frequency distribution of erucic acid content in the seeds of the $F_2$ and $BC_1$ populations is presented in Figure 2.2. The content of this fatty acid in seeds of the $F_2$ plants ranged from 0 to about 32%, while it was 0 to about 23% in seeds of the $BC_1$ plants. The $F_1$ plants showed high sterility, i.e. chromosomal anomalies occurred in these plants. Assuming that this chromosomal anomaly did not affect the erucic acid in $F_2$ and $BC_1$, and a single locus is involved in the control of this fatty acid in the seed oil of the *B. oleracea* parents, the segregation of low erucic acid (≤1%): high erucic acid (>1%) should follow a 1:3 ratio in $F_2$ and 1:1 ratio in $BC_1$. However, the $\chi^2$ tests based on 22 low erucic: 280 high erucic plants in $F_2$ and 18 low erucic: 43 high erucic plants in $BC_1$ showed a significant deviation from the expected segregation in both populations ($F_2$: $\chi^2$=50.5475, $p$<0.001; $BC_1$: $\chi^2$=10.2459, $p$<0.01 ).

Figure 2.2 Frequency distribution of the $F_2$ ($n$ = 302) and $BC_1$ ($n$ = 61) populations of *Brassica napus* × *B. oleracea* interspecific cross for erucic acid content in seed oil. Pooled data of the three crosses presented.



### 2.3.5 Ploidy of the $F_3$ and $BC_1F_2$ plants

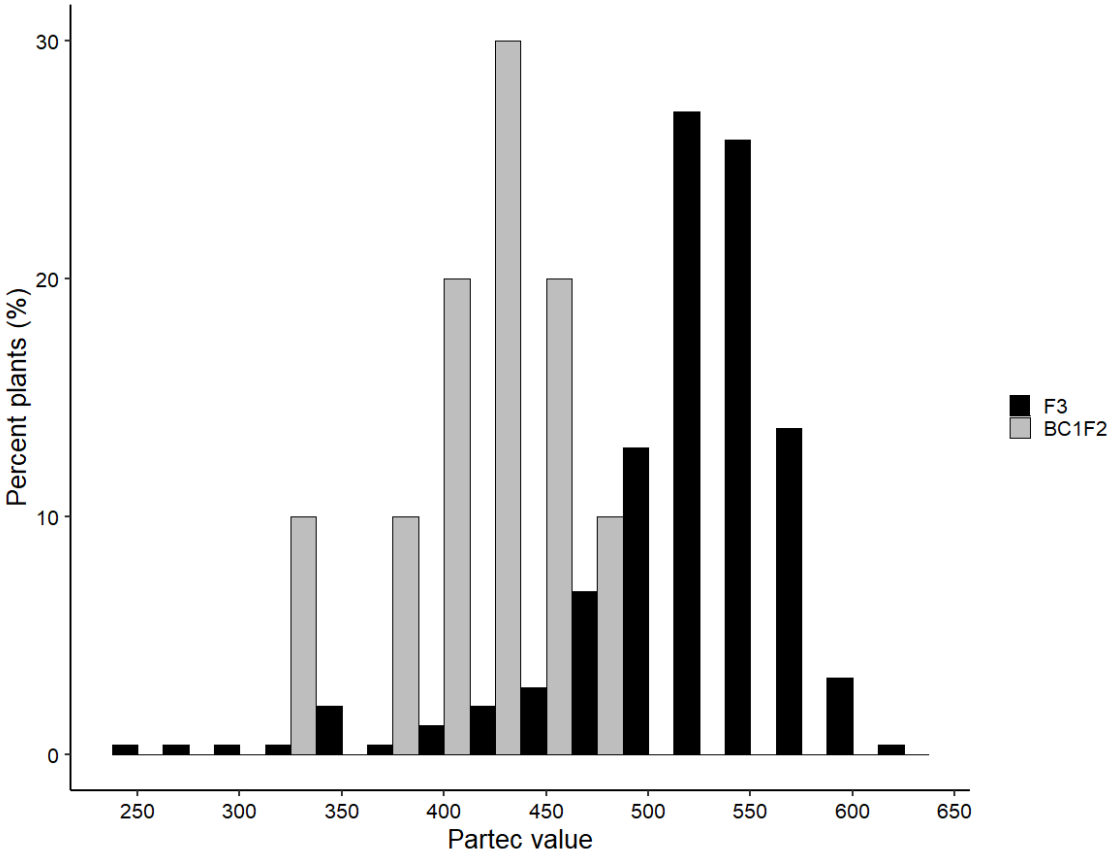
A total of 248 $F_3$ and 10 $BC_1F_2$ plants and their parents were analyzed by a flow cytometer for nuclear DNA contents (**Table 2.5**). The Partec value of the *B. oleracea* and *B. napus* parents were 199.7± 1.2 and 357. 2 ± 3.8, respectively (**Table 2.5**). Wide variation for this was found in these two populations, where the variation was wider in the $F_3$ population (Partec value 256 – 629) as compared to the $BC_1F_2$ population (Partec value 323 – 463). On average, the $F_3$ population had a significantly greater nuclear DNA content as compared to the $BC_1F_2$ population (521.2 ± 3.5 vs. 414.0 ± 12.7; $t = -7.751$; $p < 0.001$) as well as compared to the *B. napus* parent A04-73NA (521.2 ± 3.5 vs. 357. 2 ± 3.8; $t = -30.877$; $p < 0.001$). In contrast, the difference between the $BC_1F_2$ and the *B. napus* parent was smaller, however, it was still statistically significant (414.0 ± 12.7 vs. 357. 2 ± 3.8; $t = -4.076$; $p = 0.002$). None of the interspecific progeny had Partec value similar to the *B. oleracea* parent.

Table 2.5 Relative nuclear DNA content (Partec value) of the selected $F_3$ and $BC_1F_2$ plants of *Brassica napus* × *B. oleracea* interspecific crosses

| Cross | No. plants | Partec value | |
|---|---|---|---|
| | | Range | Mean ± S.E. |
| $F_3$: | | | |
| *B. nap* × *B. ole* AM094 | 6 | 337-572 | 442.2 ± 41.5 |
| *B. nap* × *B. ole* AM111 | 7 | 370-601 | 489.1 ± 31.3 |
| *B. nap* × *B. ole* AM114 | 235 | 256-629 | 524.2 ± 3.3 |
| **Total** | **248** | **256-629** | **521.2 ± 3.5** |
| $BC_1F_2$: | | | |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 8 | 379-463 | 404.0 ± 13.7 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 2 | 450-458 | 454.0 ± 2.8 |
| **Total** | **10** | **323-463** | **414.0 ± 12.7** |
| Parents: | | | |
| *B. napus* A04-73NA | 8 | 337-400 | 357. 2 ± 3.8 |
| *B. oleracea* | 8 | 190-208 | 199.7± 1.2 |

Note: The *B. oleracea* parents were AM094, AM111 and AM114

Figure 2.3 Frequency distribution of the $F_3$ ($n = 247$) and $BC_1F_2$ ($n = 10$) plants of *Brassica napus* × *B. oleracea* interspecific crosses for relative nuclear DNA content (Partec value)



## 2.4 Discussion

Interspecific cross in the genus *Brassica* has been carried out by different researchers with varying success in the production of $F_1$ plants (Bennett et al. 2008; Attri and Rahman 2018; Iftikhar et al. 2018; Kamiński et al. 2020; Nikzad et al. 2020). Among the different crosses, the cross between *B. napus* × *B. oleracea* is the most difficult ones (Quazi 1988; Fitzjohn et al. 2007; Bennett et al. 2008; Iftikhar et al. 2018). Quazi (1988), Bennett et al. (2008) and Iftikhar et al. (2018) reported 0.189, 0.041 and 0.042 $F_1$ per pollination respectively, while using *B. napus* as the female parent in the cross. In case of the reciprocal cross, Quazi (1988) and Bennett et al. (2008) reported 0.90 and 0.78 $F_1$ per pollination. In this study, while using a single *B. napus* line as female and three kale accessions as male, 0.068 $F_1$ (12/175) per pollination was obtained. This is similar to the results reported by Bennett et al. (2008) and Iftikhar et al. (2018); however, the success was much lower than the finding by Quazi (1988). Wide variation for the efficiency of the production of *B. napus* (♀) × *B. oleracea* (♂) interspecific hybrids can be seen while using different var. *acephala* accessions. For example, using a single *B. napus* line as female and multiple *B. oleracea* as male, Quazi (1988) reported 0.077 to 0.306 $F_1$ per pollination, while Iftikhar et al. (2018) reported 0.19 to 0.96 $F_1$ per pollination. Thus, the genotype constitution of the *B. oleracea* parent might affect the efficiency of the production of the *B. napus* (♀) × *B. oleracea* (♂) interspecific $F_1$ hybrid, and this might be one of the reasons for the low success rate in this study.

The $F_1$ hybrid plants showed low fertility and produced, on average, less than one seed/pollination, which is consistent with the results reported by Li et al. (2014c) and Iftikhar et al. (2018). However, wide variation between the $F_1$ plants for seed set under self-pollination and

backcrossing to the *B. napus* parent was found. High sterility in the early generation population is a common phenomenon in different interspecific crosses, such as *B. napus* × *B. oleracea* (Rahman et al. 2011a; Bennett et al. 2012; Kamiński et al. 2020), *B. napus* × *B. rapa* (Attri and Rahman 2018) and *B. napus* × *B. juncea* (Prakash and Chopra 1988; Rashid et al. 2018). This mainly results from chromosomal anomalies in meiosis of the interspecific hybrid progenies. Fertility in *Brassica* interspecific hybrid progeny also depends on other factors, such as genome composition, cytoplasmic effect, and the extent of homoeologous chromosome pairing in meiosis including auto- and allo-syndesis (Leflon et al. 2006; Mason et al. 2010; Cui et al. 2012; Mwathi et al. 2019) Compared to $F_1$ (ACC genome), greater fertility was found in $F_2$ and $BC_1$ populations, which was apparently due to less meiotic anomalies in these populations; this is consistent with the results reported by Li et al. (2014c) and Iftikhar et al. (2018).

The *B. oleracea* var. *acephala* parents used in this study were self-incompatible, and plants of this species are generally heterozygous. The self-incompatiblity (SI) in *B. oleracea* is regulated by the sporophytic SI mechanism, which is controlled by multi-allelic single S locus(Thompson and Taylor 1971). Rahman (2005) has demonstrated that the SI allele of the C genome of *B. oleracea* can exhibit Mendelian segregation in AACC genome background. Thus, in the present study, the inclusion of S alleles in the *B. napus* × *B. oleracea* var. *acephala* interspecific cross might have also affected seed set in the $F_1$ and later generation populations.

The genome composition of the *B. napus* × *B. oleracea* interspecific $F_1$ hybrids was ACC. In this composition, it was expected that the C genome chromosomes will form normal bivalents and evenly segregate to two poles in meiosis, while the haploid set of the A genome chromosomes will randomly segregate to the two poles. Based on this, it was expected that the chromosomal

number in the progeny of this interspecific cross will vary from $2n = 18$ (CC) to $2n = 38$ (AACC). However, flow cytometric analysis revealed that none of the $F_3$ and $BC_1F_2$ plants carried chromosome numbers similar to the *B. oleracea* parent, and most of them were closer to the *B. napus* parent. However, a large number of the plants had chromosome numbers greater than the *B. napus* parent as inferred from the Partec values. In a study with a synthetic digenomic allotriploid (ACC) and an allotetraploid (AACC), Cui et al. (2012) demonstrated that meiotic anomalies can occur in interspecific hybrids and this can result in unreduced gametes, which therefore, can generate progeny with a greater number of chromosomes than *B. napus*. The occurrence of hexaploid or near-hexaploid plants in the $F_1$, $F_2$ and $BC_1F_1$ populations of a *B. napus* × *B. oleracea* interspecific cross has been reported by Li et al. (2014b) and Kamiński et al. (2020). However, several researchers (Bennett et al. 2012; Rahman et al. 2015; Attri and Rahman 2018; Iftikhar et al. 2018) demonstrated that the advanced generation populations of *B. napus* × *B. oleracea* and *B. napus* × *B. rapa* interspecific cross tend to stabilize into *B. napus* type (AACC, $2n = 38$). This was also evident in this study from flow cytometric analysis of the $F_2$ and $BC_1$ derived plants. Of the two populations ($F_3$ and $BC_1F_2$), the genomic composition of the $BC_1$ derived plants were theoretically expected to be closer to the *B. napus* parent, and this has been confirmed by the Partec values. Thus, based on flow cytometry analysis, it was expected that self-pollinated progenies of the $F_3$ and $BC_1F_2$ populations will stabilize into *B. napus* type; euploid, and clubroot resistant canola plants can be achieved from this interspecific cross.

As expected, segregations for different traits such as days to flowering, seed oil and erucic acid contents were found in the population derived from *B. napus* × Kale interspecific cross. Days to

flowering in *Brassica* is a quantitative trait controlled by multiple loci (Raman et al. 2013; Rahman et al. 2017, 2018; Li et al. 2018; Jian et al. 2019). In the present study, wide variation for this trait was found at both the family and interspecific cross level in the $F_2$ and $BC_1$ population, where one of the $F_2$ plants flowered 13 days earlier than the *B. napus* canola parent. This suggests that alleles of *B. oleracea* can be used to improve the earliness in *B. napus* as has been suggested by Rahman et al. (2011a). The erucic acid content in *B. napus* seed oil is controlled by two major loci located on A8 and C3 chromosomes with additive effects of the loci (Rahman et al. 2008; Li et al. 2014a). The *B. napus* parent used in the *B. napus* × *B. oleracea* interspecific cross was a canola quality type; therefore, segregation for this trait in the seeds of $F_2$ and $BC_1$ plants were expected from segregation of the C genome erucic acid alleles only. Based on this, the segregation of plants lacking erucic acid and plants containing erucic acid should follow a 1:3 and 1:1 ratio in $F_2$ and $BC_1$ populations, respectively. However, $\chi^2$ tests showed a significant deviation from this expected segregation in both populations. Similar distorted segregations for erucic acid content have also been reported by Bennett et al. (2008) and Rahman et al. (2015). Seed glucosinolate (Howell et al. 2003; Li et al. 2014a; He et al. 2018; Rahman and Kebede 2021) and oil (Chao et al. 2017; Tang et al. 2018; Rahman and Kebede 2021) and protein (Chao et al. 2017; Tang et al. 2018; Rahman and Kebede 2021) contents are quantitative traits controlled by multiple loci located on both A and C genomes, where glucosinolate content is controlled by a relatively fewer number of loci (Rücker and Röbbelen 1994; Toroser et al. 1995; Uzunova et al. 1995; Rahman et al. 2001; Howell et al. 2003). Therefore, segregation for these traits was also expected in the $F_2$- and $BC_1$- derived populations of the *B. napus* × *B. oleracea* interspecific cross, and plants with low erucic acid and low glucosinolate could be identified in these populations. As expected, both glucosinolate and erucic acid contents were

lower in the $BC_1$ population as compared with the $F_2$ population, which should have resulted from a greater contribution of the canola quality *B. napus* genome in the $BC_1$ population (75%) as compared with the $F_2$ (50%) population. Thus, the results from this study demonstrated that canola quality spring growth habit plants could be achieved from Canola × Kale interspecific cross, and this paved the path for introgression of clubroot resistance from kale into canola.

# Chapter 3 Introgression of Clubroot Resistance from *Brassica oleracea* into *B. napus*

## 3.1 Introduction

Among the different diseases affecting canola (*Brassica napus* L.) production in Canada, clubroot disease, caused by *Plasmodiophora brassicae* Woronin is one of the most devastating (for review. See Rahman et al. 2014). The crop species *B. napus* evolved from interspecific hybridization between *B. rapa* (AA, 2*n* = 20) and *B. oleracea* (CC, 2*n* = 18). Resistance to this disease is generally not available in canola (Neik et al. 2017); however, resistance can be found in its parental species *B. rapa* and *B. oleracea* (Hasan et al. 2012; Ning et al. 2018). Among them, resistance in the A genome of *B. rapa* is often controlled by Mendelian genes (for review, see Piao et al. 2009 and Hasan et al. 2021a); this might be one of the reasons for the frequent use of this resistance in the breeding of clubroot resistant canola cultivars (for review, see Rahman et al. 2014). However, the ineffectiveness of the qualitative resistance controlled by a major gene has been reported in many countries due to the evolution of new pathotypes (Strelkov et al. 2016, 2018; Pang et al. 2020). In contrast, clubroot resistance in the C genome of *B. oleracea* has been reported to be under quantitative genetic control (for review, see Piao et al. 2009 and Hasan et al. 2021a); some of the *B. oleracea*, especially the var. *acephala*, carry excellent resistance to several *P. brassicae* pathotypes (Farid et al. 2020). In this regard, the use of C genome resistance in the breeding of *B. napus* canola is expected to increase the pool of clubroot resistance genes in this crop for durable resistance to this disease. A few researchers have made *B. napus* × *B. oleracea* interspecific cross for introgression of agronomic traits or to broaden the genetic base of *B. napus* canola (Rahman et al. 2011a; Iftikhar et al. 2018). However, clubroot resistance in *B. napus* has mostly been introgressed from *B. napus* (rutabaga and winter canola) and *B. rapa*

(Nomura et al. 2005; Liu et al. 2018; for review, see Rahman et al. 2014); recently, an attempt has also been made to introgress resistance from *Raphanus sativus* (Zhan et al. 2017). The objective of this thesis research was to investigate the prospects of introgression of clubroot resistance from *B. oleracea* into *B. napus* canola through interspecific hybridization.

## 3.2 Materials and methods

### 3.2.1 Plant materials

The $F_3$ and $BC_1F_1$ populations derived from interspecific crosses involving the *B. napus* canola line A04-73NA (low erucic acid and low glucosinolate contents) and three *B. oleracea* var. *acephalla* lines AM094, AM111 and AM114, as described in Chapter 2, has been used for this research. The line A04-73NA is susceptible to clubroot, while the three *B. oleracea* lines derived from cvs. Dwarf Green Curled (AM-094) and Winterbor (AM-111 and AM-114) were resistant to this disease (Hasan et al. 2012; Farid et al. 2020).

In addition to the above-mentioned $F_3$ and $BC_1F_1$ populations, eight $F_3$, $F_4$, $F_5$ and $BC_1F_2$ populations derived from four interspecific crosses involving the *B. napus* canola line A04-73NA and four clubroot resistant non-canola quality accessions, viz. *B. oleracea* var. *gemmifera* cv. Diablo, *B. oleracea* var. *capitata* cv. Badger Shipper, *B. oleracea* var. *capitata* cv. Bindsachsener and *B. oleracea* var. *capitata* cv. Balbro (Hasan et al. 2012), were used in this study to identify clubroot-resistant plants. These populations were obtained from the Canola Program of the University of Alberta; the details of the development of these populations can be found in Iftikhar et al. (2018) and Nikzad et al. (2020). Plants of these populations were self-pollinated with selection for clubroot resistance to develop a homozygous resistant line.

All populations were grown in a greenhouse of the Faculty of Agricultural, Life and Environmental Sciences of the University of Alberta. The temperature in the greenhouse was about 20-22/15°C day/night and the photoperiod was 16 h. Seeding was performed either in 72-cell or 32-cell trays filled with Sunshine Professional growing mix (Sun Gro Horticulture Canada Ltd, Seba Beach, Canada). The canola cv. Hi-Q was included as the susceptible check. A clubroot resistant canola line carrying resistance of the *B. napus* cv. Mendel was also included as a check to ensure that inoculation was being carried out with *P. brassicae* pathotype 3H.

### 3.2.2 Inoculum preparation and inoculation

Single-spore isolates of *P. brassicae* pathotype 3 (Williams 1966), which are designated as pathotype 3H and 3A based on Williams and Canadian Clubroot Differential Set (Strelkov et al. 2018), respectively, were used in this study. Both pathotypes were obtained from Professor Stephen Strelkov, University of Alberta. These isolates were multiplied by infecting the susceptible *B. napus* canola cv. Hi-Q and the galls were collected and stored in a -20 ℃ freezer. Resting spores were extracted following the method described by Strelkov et al. (2007). For this, 36-gram frozen clubroot galls were finely ground in a blender with 1000 ml distilled water for 3-min. The suspension was filtered through two layers of cheesecloth to filter out all the solid residues, and spore concentration in the inoculum was adjusted to about $1\times10^7$ spores per milliliter using distilled water.

Seedlings at the age of 12 days after seeding were inoculated following the pipette method of inoculation as described by Voorrips and Visser (1993). For this, 1 ml of inoculum was pipetted to the base of each seedling. To ensure successful inoculation, seedlings were also inoculated for

the second time the next day. After inoculation, the trays were kept saturated with water for a week to ensure successful infection. After that, watering was done once a day to maintain good soil moisture. N-P-K fertilizer 15-30-15 was applied twice a week with the water.

### 3.2.3 Evaluation for clubroot resistance

The disease severity of the plants was evaluated visually at 45 days after inoculation (DAI) and was scored following the scale described by Kuginuki et al. (1999). For this, the plants were uprooted carefully and washed in water, and scored on a 0 to 3 scale, where 0 = no disease incidence, 1 = few small galls on lateral roots, 2 = moderate galling on the main root, and 3 = large galls on the major and lateral roots. The resistant plants with scores of 0 or 1 were transferred to a 32-cell tray and manually self-pollinated to harvest progeny seeds. In addition to this, some susceptible plants with disease score of 2 and 3 were also taken to the following generation to develop homozygous susceptible lines. The plants were also scored for disease resistance at harvest or both stages.

The disease severity of each family was estimated based on the disease severity index (DSI), which was calculated as follows (Strelkov et al. 2006):

$$\text{DSI } \% = \sum \left[(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3) / N \times 3\right] \times 100$$

where, 0, 1, 2 and 3 are disease symptom classes, $N_0$, $N_1$, $N_2$ and $N_3$ are the number of plants in these disease classes, and N is the total number of plants.

### 3.2.4 Statistical analysis

One-way analysis of variance (ANOVA) was performed using anova function in the program R (v.3.6.3) on Rstudio (v.1.4.1106) to test for statistical significance of the fixed effects (different generations) for resistance to pathotypes 3H or 3A. Two-way ANOVA was performed to assess the fixed effects (pedigree/family and pathotypes) of the different generation families of the interspecific crosses and two pathotypes (3H and 3A). For this, the lm function was used to fit the linear model as follows: $\mathrm{DI.lm} = \mathrm{lm}(\mathrm{DI} \sim \mathrm{Pedigree} + \mathrm{Pathotype} + \mathrm{Pedigree{:}Pathotype}, \mathrm{scroring.dat})$

To test the disease score or DSI at 45 days after inoculation (DAI) and at harvest for significant difference, one-tailed paired *t*-test was carried out using the t.test function in R. Tukey's HSD (honestly significant difference) test or Dunn–*Šidák* significant difference test with alpha equal to 0.05 was also carried out in R for pairwise comparison of the mean values in all other cases. Pearson correlation analysis was performed using the cor.test function in R and the *p*-values were also obtained from this analysis.

Chi-square ($\chi^2$) values were calculated as $\chi 2 = \sum(\mathrm{O} - \mathrm{E})^2/\mathrm{E}$, where O is the observed value and E is the expected value. The *p*-value was calculated using CHISQ.TES function in Excel.

### 3.2.5 Molecular marker analysis

Young leaf samples of the resistant $F_3$ and $BC_1F_2$ plants of *B. napus* A04-73NA × *B. oleracea* var. *acephala*, and $F_8$ plants of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener were collected in 1.5 ml Eppendorf tubes for DNA extraction. The samples were placed in liquid

nitrogen for 1-2 min and ground into fine powder, and 400 µl nuclei lysis buffer (Invitrogen, Carlsbad, CA, USA) was added to each sample. The mixture was incubated in a water bath at 65 ℃ for 15 min and 400 µl chloroform was added. The samples were centrifuged at 12,000 rpm for 5 min at room temperature and 300 µl supernatant was transferred to a new tube where 300 µl isopropanol was added and the samples were mixed by inverting gently. The samples were kept on ice for 20-30 min and were centrifuged at 12,000 rpm for 3 min. The supernatant was discarded and 500 µl of 70% ethanol was added; the samples were mixed by inverting gently and were centrifuged again at 12,000 rpm for 2 min, and the supernatant was discarded. After that, the lids of the sample tubes were kept open to dry the DNA which precipitated at the bottom of the tubes. 200 µl milliQ water was added to each tube to dissolve the DNA, and the concentration and quality of the DNA were measured with a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The samples were diluted to 10-20 ng/µl with Invitrogen UltraPure DNase/RNase-free distilled water for use.

The SSR markers collected from different sources, such as Agriculture and AgriFood Canada (AAFC), Biotechnology and Biological Sciences Research Council (BBSRC), and other research groups, and available in the University of Alberta Canola program, were used in this study. Polymerase chain reaction (PCR) of the DNA samples was carried out in a total volume of 12 µl reaction mixture comprising 50 ng genomic DNA (25 ng/µl × 2 µl), 1.25 µl 10× PCR buffer, 0.125 µl of 20 mM dNTPs, 1.25 µl of 50 mM $MgCl_2$, 0.5 µl of each forward and reverse primer, 6.225 µl of nuclease-free water, and 0.15 µl (0.75U) of GoTaq DNA polymerase enzyme. Electrophoretic separation of the PCR products was carried out on 3% agarose gel (Invitrogen, Carlsbad, CA, USA). The loaded gels were run in TAE buffer (ThermoFisher Scientific,

Waltham, MA, USA) at 180V for about 2 hand scanned using a Typhoon FLA 9500 biomolecular imager (GE Healthcare Bio-Sciences, Piscataway, NJ, USA).

## 3.3 Results

### 3.3.1 Selection for clubroot resistance in the progeny of *B. napus*× *B. oleracea* var. *acephala* interspecific cross

A total of 2,264 $F_3$ plants belonging to 313 families and 468 $BC_1F_2$ plants belonging to 66 families of *B. napus* (A04-73NA) × *B. oleracea* var. *acephala* (lines AM094, AM111 and AM114) interspecific crosses were evaluated for resistance to *P. brassicae* pathotype 3H at 45 DAI. About 11.8% (268/2,264) $F_3$ plants and 3.2% (15/468) $BC_1F_2$ plants showed resistance at this stage (Table 3.1). The resistant $F_3$ and $BC_1F_2$ plants along with susceptible plants from the same family were transferred to larger pots (12.7cm×12.7cm×16.5cm) after screening at 45 DAI; these plants were kept until harvest and scored again, which allows a comparison of resistance at 45 DAI and at the harvest stage (Table 3.2). In the case of the $F_3$, a total of 1,042 plants from 141 families were evaluated at the harvest stage. These 1,042 plants included the above-mentioned 268 resistant (disease score 0 and 1) plants and 774 susceptible plants (disease score 2 and 3). At harvest, only 13 plants (1.2%) of the *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor line AM-114 cross were resistant (Table 3.2). In the case of the $BC_1F_2$ population, a total of 76 plants, including the above-mentioned 15 plants found to be resistant at 45 DAI, were evaluated for resistance at the harvest stage; however, none showed resistance. A significant difference was found for DSI at 45 DAI and at harvest for all populations except *B. napus* × *B. oleracea* var. *acephala* cv. Dwarf Green Curled AM-094, which was apparently due to the small population size (Table 3.2).

A total of 245 $F_4$ plants descendent of the above-mentioned 13 resistant $F_3$ plants, and 296 $F_4$ plants descendent of 29 susceptible $F_3$ plants were evaluated for resistance to pathotype 3H at harvest (Table 3.3). The mean DSI of the $F_4$ progeny of the resistant $F_3$ plants was slightly lower than the DSI of the $F_4$ progeny of the susceptible $F_3$ plants (87.5 ± 4.3% vs. 90.3 ± 2.8%). Of the 245 $F_4$ plants of the progeny of the resistant $F_3$ plants, 20.0% (49/245) plants were resistant. On the other hand, 12.5% (37/296) of the $F_4$ plants derived from the susceptible $F_3$ plants were also found to be resistant at the harvest stage.

### 3.3.2 Molecular marker analysis of the resistant $F_3$ plants of *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-114

A total of 56 SSR markers from nine C-genome chromosomes were tested for polymorphism between the *B. napus* parent A04-73NA and *B. oleracea* var. *acephala* cv. Winterbor. Among these, 15 markers were found to be polymorphic and were used to genotype the clubroot resistant and susceptible $F_3$ plants (Table 3.4). Nine markers from chromosomes 1, 5, 6, 7 and 8 detected *B. oleracea* alleles in this $F_3$ population; however, no association of resistance to pathotype 3H and *B. oleracea* SSR marker allele, i.e., presence of the allele in all resistant plants but lacking in the susceptible plants, could be established (Table 3.5).

Table 3.1 Evaluation of the $F_3$ and $BC_1F_2$ populations derived from *Brassica napus* × *B. oleracea* var. *acephala* interspecific crosses for resistance to *Plasmodiophora brassicae* pathotype 3H at 45 days after inoculation (DAI)

| Cross | Generation | No. Family | Total plants | No. resistant | No. susceptible | Disease severity index DSI (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Range | Mean±SE[1] | |
| *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-111 | $F_3$ | 26 | 182 | 20 | 162 | 33.3-100.0 | 87.7±3.3 | a |
| *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-114 | $F_3$ | 285 | 2073 | 242 | 1831 | 16.7-100.0 | 86.0±0.8 | a |
| *B. napus* × *B. oleracea* var. *acephala* cv. *Dwarf Green Curled* AM-094 | $F_3$ | 2 | 9 | 6 | 3 | 44.4-55.6 | 50.0±4.0 | b |
| **Total** | **$F_3$** | **313** | **2264** | **268** | **1996** | 16.7-100.0 | **85.9±0.8** | |
| *(B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-111) × *B. napus* | $BC_1F_2$ | 32 | 226 | 10 | 216 | 66.7-100.0 | 93.1±1.8 | a |
| (*B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-114) × *B. napus* | $BC_1F_2$ | 34 | 242 | 5 | 237 | 50.0-100.0 | 89.3±2.2 | a |
| **Total** | **$BC_1F_2$** | **66** | **468** | **15** | **453** | **50.0-100.0** | **91.2±1.4** | |
| *B. napus* A04-73NA | | 1 | 8 | 0 | 8 | 100 | | |
| *B. oleracea* var. *acephala* cv. Winterbor | | 1 | 7 | 6 | 1 | 19 | | |
| *B. oleracea* var. *acephala* cv. Dwarf Green Curled | | 1 | 8 | 6 | 2 | 33.3 | | |

Note: Evaluation of the parents *B. napus* A04-73NA and *B. oleracea* var. *acephala* cvs. Winterbor and Dwarf Green Curled for clubroot resistance performed at harvest stage.

[1] Comparison made between the three $F_3$ and two $BC_1F_2$ populations separately using Dunn–*Šidák* significant difference test with alpha equal to 0.05; the populations with the same letter are not significantly different.

Table 3.2 Resistance to *Plasmodiophora brassicae* pathotype 3H in $F_3$ and $BC_1F_2$ populations of *Brassica napus* × *B. oleracea* var. *acephala* interspecific crosses at 45 days after inoculation (DAI) and at harvest.

| Cross and generation | No. family | No. plants | Disease scoring at 45 DAI | | | Disease scoring at harvest | | | *p*-value[1] |
|---|---|---|---|---|---|---|---|---|---|
| | | | No. resistant plants | No. susceptible plants | Mean DSI±SE[2] | No. resistant plants | No. susceptible plants | Mean DSI±SE[2] | |
| $F_3$: | | | | | | | | | |
| *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-111 | 9 | 65 | 20 | 45 | 71.8±6.4 | 0 | 65 | 86.6±3.1 | 0.003 |
| *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-114 | 130 | 968 | 242 | 726 | 75.6±1.1 | 13 | 955 | 83.8±0.7 | <0.001 |
| *B. napus* × *B. oleracea* var. *acephala* cv. Dwarf Green Curled AM-094 | 2 | 9 | 6 | 3 | 50.0±4.0 | 0 | 9 | 69.5±1.9 | 0.129 |
| **Pooled $F_3$** | **141** | **1042** | **268** | **774** | **75.0±1.1** | **13** | **1029** | **83.8±0.7** | **<0.001** |
| $BC_1F_2$: | | | | | | | | | |
| *(B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-111*)* × *B. napus* | 7 | 50 | 10 | 40 | 77.2±3.0 | 0 | 50 | 83.9±2.3 | <0.001 |
| (*B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-114) × *B. napus* | 4 | 26 | 5 | 21 | 67.7±7.3 | 0 | 26 | 83.4±5.7 | 0.047 |
| **Pooled $BC_1F_2$** | **11** | **76** | **15** | **61** | **73.8±3.6** | **0** | **76** | **83.7±2.6** | **0.002** |
| **Total ($F_3$ and $BC_1F_2$)** | **152** | **1118** | **283** | **835** | **74.9±1.1** | **13** | **1105** | **83.8±0.7** | **<0.001** |

Note: Disease scores 0 and 1 were considered as resistant, and scores 2 and 3 were considered as susceptible.

[1] Paried *t*-test was performed for DSI at 45 DAI vs. DSI at harvest stage.

[2] Mean disease severity index (DSI) ± standard error

Table 3.3 Evaluation of the $F_4$ populations derived from resistant and susceptible $F_3$ plants of *Brassica napus* × *B. oleracea* var. *acephala* interspecific cross for resistance to *Plasmodiophora brassicae* pathotype 3H at harvest stage.

| Cross | Generation | No. Family | Total plants | No. resistant plants | No. susceptible plants | Disease severity index DSI (%) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Range | Mean±SE[1] |
| *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-114 | $F_4$ (resistant $F_3$) | 13 | 245 | 49 | 196 | 50.0-100.0 | 87.5±4.3 |
| *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor AM-114 | $F_4$ (susceptible $F_3$) | 29 | 296 | 37 | 259 | 33.3-100.0 | 90.3±2.8 |
| **Total** | **$F_4$** | **42** | **541** | **86** | **455** | **33.3-100.0** | **89.4±2.4** |

[1] No significant difference was found between the $F_4$ population derived from the resistant $F_3$ plants and the $F_4$ population derived from the susceptible $F_3$ plants for disease severity index.

Table 3.4 List of polymorphic SSR markers used to genotype the clubroot resistant and susceptible $F_3$ plants of *Brassica napus* × *B. oleracea* var. *acephala* interspecific cross

| Primer no. | Linkage group | Name | Source | Forward Primer 5`-3` | Reverse Primer 5`-3` | size |
|---|---|---|---|---|---|---|
| 2279 | C1 | sN3734 | AAFC | CACGACGTTGTAAAACCCCCTTCCGGTTAAACAAAT | AAAACAGACTTTGCCCGTTG | 273 |
| 2115 | C4 | sN3817 | AAFC | CACGACGTTGTAAAACGACCCTGCCGTAACGTTCTTGTT | ATCTTCGAAGCAATCTCGGA | 169 |
| 4114 | C5 | PbC5.1 | Parkin et al. (2014) | CACGACGTTGTAAAACGACAGAGAGAGAGAGGGAGGGAGG | CACCCTACAGGCGGGATATAA | 459 |
| 2374 | C6 | sN11862 | AAFC | CACGACGTTGTAAAACAGGGACAACGAGCATACCAC | AGGCGCCTTCAATCCTATTT | 290 |
| 2379 | C6 | sN12743J | AAFC | CACGACGTTGTAAAACGACCTAGCCACCATGAAAGGAGC | AAACCAAGCAAACCCATCAG | 370 |
| 2380 | C6 | sN9539 | AAFC | CACGACGTTGTAAAACGACCTCGTAAACTGGCAAGCCTC | AAGTTTTGGGCTGACCATGA | 168 |
| 3476 | C6 | PbC4.1 | Parkin et al. (2014) | GGAAATGTCAGCCGGTTCAT | GAGACCCTTTTCCACCTGACA | 810 |
| 4237 | C6 | PbC6.2 | Parkin et al. (2014) | CCTAATCCTAAGTCGGCCAAG | AACCAAAACCAGAAGAGAAGGC | 241 |
| 2410 | C7 | sN1975 | AAFC | CACGACGTTGTAAAACGACTCCCTTGCCTTCTCTTCTTG | TCGGCCAAGCATCTCTAACT | 140 |
| 3519 | C7 | PbC7.2 | Parkin et al. (2014) | GATAACAATGCCAAGCCGACC | TCAATGCGTATTGTCGTCGT | 806 |
| 4350 | C7 | C1M0166 | Farid et al. (2020) | CTAAATTCCCAACACTAACCA | ACGAGGAAATTACGACGATA | 167 |
| 4351 | C7 | C1M0166 | Farid et al. (2020) | GAAGAGGGGAGAGAGTAGTGA | GGTGGTGATAAGTATGACTAAGG | 153 |
| 4243 | C8 | PbC8 | Parkin et al. (2014) | GGCACAAAAGAAGATGGAAAAC | TAGGGTTTGGGTTTAAGGTTCA | 188 |
| 4244 | C8 | PbC8 | Parkin et al. (2014) | ATCCATACCCTAAACCCCAATC | CCTAAACCCAAACCTTAAACCC | 254 |
| 4341 | C8 | C1M0255 | Farid et al. (2020) | TTCTATGAAGCAGAGCGTTAG | CGGTCTTTTTAATCTGACCAT | 166 |

Note: AAFC = Agriculture and Agri-Food Canada

Table 3.5 Occurrence of simple sequence repeat (SSR) marker alleles of *Brassica oleracea* (Ole) in 10 clubroot resistant and 10 susceptible $F_3$ plants of *Brassica napus* × *B. oleracea* var. *acephala* interspecific cross

| SSR marker[1] | Linkage group | No. plants detected SSR marker allele | Ole. allele in % resistant plants[2] | Ole. allele in % susceptible plants[2] |
|---|---|---|---|---|
| 2279 | C1 | 20 | 90.0 | 70.0 |
| 2115 | C4 | 9 | 0.0 | 0.0 |
| 4114 | C5 | 17 | 37.5 | 88.9 |
| 2374 | C6 | 18 | 44.4 | 55.6 |
| 2379 | C6 | 20 | 0.0 | 0.0 |
| 2380 | C6 | 20 | 0.0 | 0.0 |
| 3476 | C6 | 20 | 30.0 | 30.0 |
| 4237 | C6 | 19 | 100.0 | 90.0 |
| 2410 | C7 | 20 | 0.0 | 0.0 |
| 3519 | C7 | 20 | 50.0 | 30.0 |
| 4350 | C7 | 18 | 0.0 | 44.4 |
| 4351 | C7 | 17 | 75.0 | 88.9 |
| 4243 | C8 | 20 | 0.0 | 0.0 |
| 4244 | C8 | 20 | 50.0 | 100.0 |
| 4341 | C8 | 20 | 50.0 | 100.0 |

[1] SSR markers from the chromosomes carrying clubroot resistance in the C genome (Hasan et al. 2021) were used.
[2] Proportion of the total number of plants carrying *B. oleracea* allele.

### 3.3.3 Selection for clubroot resistance in the progeny of *B. napus*× *B. oleracea* vars. *gemmifera* and *capitata* interspecific crosses

All *B. oleracea* parents, viz. *B. oleracea* cvs. Badger Shipper, Bindsachsener, Diablo and Balbro showed a certain level of resistance to *P. brassicae* pathotypes 3H and 3A (Table 3.6). Therefore, it was expected that the populations derived from *B. napus* × *B. oleracea* interspecific crosses involving these *B. oleracea* parents will carry resistance to these pathotypes. A total of 1,119 $F_3$, $F_4$, $F_5$ and $BC_1F_2$ plants belonging to 223 families and 1,323 plants belonging to 220 families of the four interspecific crosses were tested for resistance to pathotype 3H and 3A, respectively (Table 3.6). No significant difference for resistance to pathotype 3H and 3A was found between the different interspecific crosses, and pooled data of these crosses also did not show a significant difference for resistance to these two pathotypes ($t = 0.0626$, $p = 0.9501$). However, one of the $F_5$ families of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener cross showed DSI less than 80% (Table 3.6). Single plant selection for resistance was performed on $F_5$ families. Three plants, including two plants (5CA1363.290-A1258 and 5CA1363.266-A1258) showing resistance to pathotype 3H and one plant (5CA1392.453-A1258) showing resistance to pathotype 3A were selected and self-pollinated. Several $F_6$ plants derived from 5CA1392.453-A1258 through self-pollination were found to be resistant to pathotype 3H at 45 DAI; however, they became totally susceptible at harvest. On the other hand, two $F_6$ populations, derived from two resistant (pathotype 3H) $F_5$ plants 5CA1363.266-A1258 and 5CA1363.290-A1258, were tested for resistance to pathotype 3A (Table 3.7); the resistant $F_6$ plants at harvest were selected to produce $F_7$ populations, which were tested with pathotype 3H again. Resistant plants were observed in both $F_7$ populations at 45 DAI, but only one $F_7$ plant (5CA1363.323-A1279)

remained to be resistant at harvest, which resulted the $F_8$ family (5CA1363.325-A1280) with resistance to pathotype 3H (48% DSI) at harvest stage (Table 3.7).

A total of 64 $F_8$ plants were evaluated for resistance to pathotype 3H at 45 DAI and harvest stage. At harvest stage, one plant (1.6%) had disease score of 0, 34 (53.1%) had disease score of 1, 28 (43.8%) had disease score of 2, and one (1.6%) had a disease score of 3. Correlation between disease score of the resistant $F_8$ plants at 45 DAI and harvest was $r = 0.34$ ($p = 0.008$). Moreover, a significant ($t$ =-7.078, $p< 0.001$) increase in average disease score was also observed in the selected $F_8$ population at 45 DAI (mean disease score 0.89) as compared at harvest stage (mean disease score 1.39). A total of 54 $F_9$ families derived from one $F_8$ plant with disease score of 0, 29 families with $F_8$ disease score of 1, 23 families with $F_8$ disease score of 2, and one $F_9$ family with $F_8$ disease score of 3 were evaluated for resistance to pathotypes 3 at 45 days after inoculation. The average DSI of the 54 $F_9$ families was 20.5% at 45 days after inoculation. The $F_9$ families derived from the $F_8$ plants with disease score 0 and 1 had significantly ($t$ = -2.916, $p$ = 0.003) lower average DSI (17.6±1.6%) as compared to the families derived from the plants with disease score of 2 and 3 (average 23.8±1.4% DSI). The coefficient of correlation between $F_8$ (disease score of 54 plants) and $F_9$ (average score of the 54 families) generations disease score was 0.41 ($p$ = 0.002). Thus, partially resistant plants predominantly occurred in this population; *B. napus* families partially resistant to pathotype 3H could be developed from this interspecific cross.

Table 3.6 Evaluation of different generation populations of *B. napus* × *B. oleracea* vars. *gemmifera* and *capitata* interspecific crosses for resistance to *Plasmodiophora brassicae* pathotypes 3H and 3A at harvest stage.

| Cross[1] | Genera-tion | Pathotype 3H | | | | Pathotype 3A | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. family | No. plant | DSI (%) Range | Mean DSI±S.E.[2] | No. family | No. plant | DSI[3] (%) Range | Mean DSI±S.E.[2] |
| *B. nap.* × *B. ole.* var. *gemmifera* cv. Diablo | $BC_1F_2$ | 9 | 112 | 96-100 | 99.3±0.5 | 9 | 100 | 93-100 | 98.8±0.7 |
| *B. nap.* × *B. ole.* var. *capitata* cv. Badger Shipper | $F_5$ | 49 | 503 | 89-100 | 99.1±0.3 | 49 | 624 | 92-100 | 99.2±0.3 |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | $F_4$ | 14 | 133 | 97-100 | 99.5±0.3 | 12 | 262 | 100 | 100±0.0 |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | $F_5$ | 59 | 522 | 78-100 | 99.4±0.4 | 58 | 739 | 89-100 | 99.7±0.2 |
| *B. nap.* × *B. ole.* var. *capitata.* cv. Balbro | $BC_1F_2$ | 5 | 53 | 98-100 | 99.5±0.4 | 5 | 112 | 99-100 | 99.7±0.2 |
| *B. nap.* × *B. ole.* var. *capitata.* cv. Balbro | $F_5$ | 87 | 1119 | 89-100 | 99.0±0.3 | 87 | 1323 | 92-100 | 98.8±0.2 |
| *B. napus* A04-73NA | | 1 | 19 | | 100 | 1 | 17 | | 100 |
| *B. ole.* var. *gemmifera* cv. Diablo | | 1 | 14 | | 86 | 1 | 16 | | 2 |
| *B. ole.* var. *capitata* cv. Badger Shipper | | 1 | 17 | | 41 | 1 | 16 | | 4 |
| *B. ole.* var. *capitata* cv. Bindsachsener | | 1 | 17 | | 29 | 1 | 13 | | 10 |
| *B. ole.* var. *capitata.* cv. Balbro | | 1 | 12 | | 67 | 1 | 8 | | 100 |

[1] *B. nap.* = *B. napus* line A04-73NA, *B. ole* = *B. oleracea*

[2] The differences between resistance to pathotype 3H and 3A were not statistically significant ($p<0.05$)

[3] Disease severity index

Table 3.7 Selection for resistance to *Plasmodiophora brassicae* pathotypes 3H and 3A in $F_5$ and in subsequent generations of *B. napus* ×*B. oleracea* var. *capitata* interspecific cross

| Cross | Parent generation | Progeny generation[1] | Progeny generation | Patho-type | No. family | No. plant | No. resistant plants | No. suscepti-ble plants | DSI[2] (%) | Mean disease score ± S.E.[3] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.039 -A1242 | 5CA1363.266-A1258 | $F_5$ | 3H | 1 | 15 | 1 | 14 | 95.6 | 2.9±1.3 | a |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.051 -A1242 | 5CA1363.290-A1258 | $F_5$ | 3H | 1 | 3 | 1 | 2 | 77.8 | 2.3±0.5 | a |
| *B. nap.* × *B. ole.* var. *capitata*. cv. Balbro | 5CA1392.040 -A1242 | 5CA1392.453-A1258 | $F_5$ | 3A | 1 | 16 | 1 | 15 | 95.8 | 2.9±0.1 | a |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.266 -A1258 | 5CA1363.321-A1269 | $F_6$ | 3A | 1 | 49 | 1 | 48 | 98.6 | 3.0±0.0 | a |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.290 -A1258 | 5CA1363.322-A1269 | $F_6$ | 3A | 1 | 7 | 0 | 7 | 95.2 | 2.9±0.1 | a |
| *B. nap.* × *B. ole.* var. *capitata*. cv. Balbro | 5CA1392.453 -A1258 | 5CA1392.455-A1269 | $F_6$ | 3H | 1 | 392 | 0 | 392 | 100 | 3.0±0.0 | a |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.321 -A1269 | 5CA1363.323-A1279 | $F_7$ | 3H | 1 | 280 | 1 | 279 | 98.1 | 3.0±0.0 | a |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.322 -A1269 | 5CA1363.324-A1279 | $F_7$ | 3H | 1 | 87 | 0 | 87 | 100 | 3.0±0.0 | a |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.323 -A1279 | 5CA1363.325-A1280 | $F_8$ | 3H | 1 | 64 | 35 | 29 | 48.4 | 1.5±0.1 | b |
| *B. nap.* × *B. ole.* var. *capitata* cv. Bindsachsener | 5CA1363.325 -A1280 | 5CA1363.326-380-A1290 | $F_9$ | 3H | 54 | 903 | 903 | 0 | 20.5 | 0.6±0.0 | c |

[1] The 5CA1363.326-380-A1290 families were scored for resistance at 45 DAI only, while the resistant plants of all other families were presented with scores at harvest.

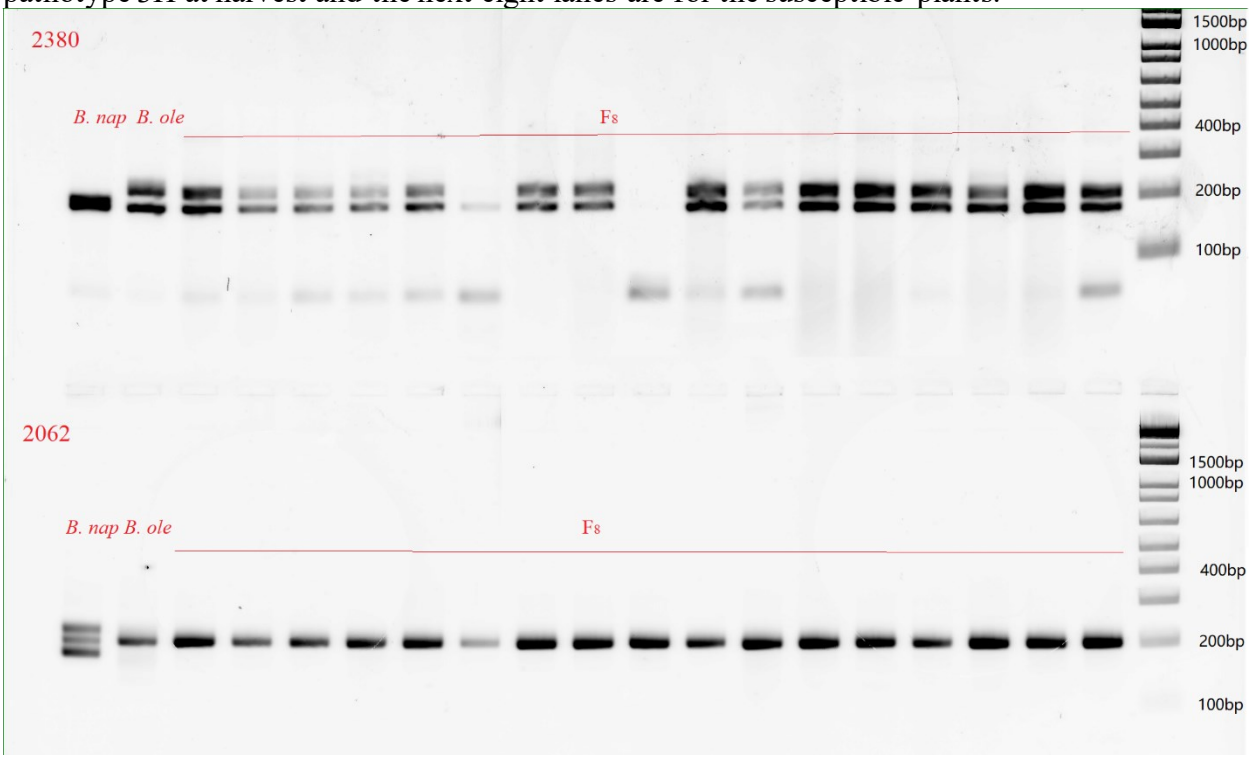
[2] Disease severity index

[3] Comparison made between the different generation populations; mean ± S.E. following the same letters are not significantly different.

### 3.3.4 Molecular marker analysis of the partially resistant $F_8$ plants of *B. napus*× *B. oleracea* var. *capitata* cv. Bindsachsener

A total of 119 SSR markers from nine C genome chromosomes were tested for polymorphism between the parents *B. napus* A04-73NA and *B. oleracea* var. *capitata* cv. Bindsachsener where 71 markers found to be polymorphic (Supplement Table 3.1). These 71 markers were used to genotype the bulk of pathotype 3H resistant and susceptible plants of the $F_8$ family 5CA1363.325-A1280. Of the 71 markers, 69 detected only the *B. napus* allele in both the resistant and susceptible bulks, while two markers, 2062 and 2380, detected the *B. oleracea* allele, however, in both the resistant and susceptible bulks. The marker 2062 is known to amplify the genomic regions of C2 and C3 chromosomes (Supplement Table 3.1), while marker 2380 amplifies the genomic region of C6. These two markers were tested on nine resistant and eight susceptible $F_8$ plants. In the case of SSR marker 2062, 15 (88.2%) plants carried the *B. oleracea* allele (Figure 3.1) while for 2380, all 17 plants carried the *B. oleracea* allele. The occurrence of *B. oleracea*-specific alleles in the $F_8$ population demonstrated that the resistance in the $F_8$ line derived from the *B. oleracea* parent Bindsachsener; however, none of the markers showed linkage association with this resistance.

Figure 3.1 Genotypic results of the *Brassica* C-genome specific simple sequence repeats (SSR) markers 2380 and 2062 from an $F_8$ population of *B. napus* derived from *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener interspecific cross. The *B. napus* parent A04-73NA and the *B. oleracea* parent var. *capitata* cv. Bindsachsener is annotated as *B. nap* and *B. ole*, respectively. After these two lanes, the first nine lanes are for the plants resistant to *Plasmodiophora brassicae* pathotype 3H at harvest and the next eight lanes are for the susceptible plants.



## 3.4 Discussion

Clubroot resistance in *B. napus* have been introduced by several researchers from different sources (e.g. Rahman et al. 2011b; Hasan and Rahman 2016; Zhan et al. 2017; Liu et al. 2018; Shaikh et al. 2021; Hasan et al. 2021); however, resistance was mostly introduced into the A genome of this crop. Attempts have been made to introduce clubroot resistance in *B. napus* from *B. oleracea* through resynthesis of this species using clubroot resistant *B. rapa* and *B. oleracea* (Diederichsen and Sacristan 1996). However, no effort has been made to introgress clubroot resistance from *B. oleracea* into *B. napus* canola through *B. napus* × *B. oleracea* interspecific cross. This is apparently due to the difficulty of producing interspecific hybrids through this cross (Fitzjohn et al. 2007; Bennett et al. 2008; Iftikhar et al. 2018) and the quantitative nature of clubroot resistance in *B. oleracea* (Peng et al. 2018; Farid et al. 2020). The results from this study demonstrated the prospects of developing a clubroot resistant euploid ($2n = 38$) *B. napus* line from this interspecific cross.

*Brassica oleracea* var. *acephala* has been reported to carry resistance to several *P. brassicae* pathotypes, including 3H and 3A (Laurens and Thomas 1993; Rocherieux et al. 2004; Farid et al. 2020). In the present study, more than 2,500 $F_3$ and $BC_1F_2$ plants were evaluated for resistance to pathotype 3H, where only 0.6% $F_3$ plants of *B. oleracea* var. *acephala* cv. Winterbor (AM114) found to be resistant. This low frequency of resistant plants in the $F_3$, as well as the occurrence of only 20% resistant plants in the progeny ($F_4$) of the resistant $F_3$ plants, suggests that clubroot resistance in this population is under quantitative genetic control. Quantitative genetic control of resistance in the C genome of *B. oleracea* has also been reported by Rocherieux et al. (2004) and Farid et al. (2020). On the other hand, the occurrence of resistant $F_4$ plants, in the progeny of the

susceptible $F_3$ plants, indicates that recessive genes might also be involved in the control of clubroot resistance in this population (Table 3.3). This contradicts the results reported by Laurens and Thomas (1993) that resistance in *B. oleracea* var. *acephala* is under dominant gene control. Chromosomal anomalies affecting the resistance phenotype in the early generation population of this interspecific cross can also not be denied; however, further research will be needed to prove this. Nevertheless, the occurrence of an increased proportion of resistant plants in $F_4$ as compared with the $F_3$ demonstrates the possibility of developing a clubroot resistant line from this interspecific cross.

In the case of the interspecific crosses involving the *B. oleracea* parents var. *gemmifera* cv. Diablo and var. *capitata* cvs. Badger Shipper and Bindsachsener, all these *B. oleracea* parents were found to carry resistance to pathotype 3H and, to some extent, resistance to pathotype 3A (Table 3.6). This is in accordance with the results from the previous studies (Hasan et al. 2012; Farid et al. 2020) that *B. oleracea* vars. *gemmifera* and *capitata* carries clubroot resistance, and this was the basis for evaluation of the $F_4$, $F_5$ and $BC_1F_2$ populations for resistance to this disease. Of the 2,442 $F_4$, $F_5$ and $BC_1F_2$ plants belonging to 223 families tested, only 2 $F_5$ families were found to carry resistance to pathotype 3H. The occurrence of such a low number of families with partial resistance (mean disease score = 2.6; mean DSI = 86.7%) to pathotype 3H in $F_5$ might be for the reason that these families have never been tested for clubroot resistance and multiple gene loci might be involved in the control of resistance in this population. Results from this study agree with the results reported by other researchers (Rocherieux et al. 2004; Diederichsen et al. 2009; Farid et al. 2020) that clubroot resistance in *B. oleracea* is under control of multiple gene loci. The recessive genetic control of clubroot resistance in *B. oleracea*

(Chiang and Crete 1976; Voorrips et al. 1997; for review, see Piao et al. 2009) might also be a reason for the occurrence of resistant plants at a low frequency and for taking several generations to recover a resistant line. Further study with the $F_9$ lines of a *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener interspecific cross will be needed to confirm the genetic control of resistance that has been introgressed into *B. napus* from the cabbage cv. Bindsachsener.

Genotyping of the clubroot resistant plants of the $F_8$ family of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener by SSR markers identified *B. oleracea*-specific alleles in this family. This provided evidence of the origin of this family from this interspecific cross. The utility of the SSR markers for the identification of the alleles of the allied species in *B. napus* has been demonstrated by several researchers (Bennett et al. 2012; Attri and Rahman 2018; Liu et al. 2018b). Based on SSR marker alleles, it was apparent that high homozygosity was attained in the clubroot resistant $F_8$ families of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener. For the markers tested on the $F_8$ plants, the occurrence of alleles mostly from the *B. napus* parent as compared with the occurrence of the *B. oleracea* alleles could have resulted from the effect of selection for some of the *B. napus* traits, such as low erucic acid and glucosinolates and the earliness of flowering, in the previous generations (Iftikhar et al. 2018; Nikzad et al. 2020).

A difference for resistance at 45 DAI and at harvest was found in the progeny of the interspecific crosses involving *B. oleracea* var. *capitata* cv. Bindsachsener and var. *acephala*. For example, about a 39.7% decrease in the number of resistant plants was found at harvest as compared to the number of resistant plants at 45 DAI in the $F_8$ population of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener cross, and this decline was about 95% in the $F_3$ population of *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor cross. This indicates that a different genetic control might

be involved in resistance to this disease at growth and development stage and at maturity stage. To my knowledge, no study has so far been conducted to study the genetic basis of clubroot resistance at different growth stages. However, Laurens and Thomas (1993) also observed a greater level of disease symptom in adult plants as compared to young plants screened at 47 DAI, while using the same plant material and inoculum. Several researchers (Summanwar et al. 2019; Wagner et al. 2019) found a similar level of primary infection in both resistant and susceptible plants at early infection stage; however, the level of infection significantly reduces at the secondary infection stage in the resistant plants. Mei et al. (2019) observed a similar trend of root hair infection in both resistant and susceptible plants up to 15 days after inoculation, while the cortical infection was reduced at this stage in the resistant plants. In contrast, Zhang et al. (2015) and Fei et al. (2016) reported a similar or even greater level of primary and secondary infection in the resistant plants; this might be due to the interaction of the resistance gene in the host and avirulence genes in the pathogen. The resistance gene in interaction with different pathotypes or different resistance genes in interaction with a single pathotype may result in different disease symptoms; this was the basis of the development of the clubroot differential sets (Strelkov et al. 2018).

Clubroot resistance in *B. oleracea* is primarily controlled by multiple gene loci; this type of quantitative trait is generally influenced by the environment to a greater extent as compared to the resistance controlled by a Mendelian gene. In this regard, the longer growing time and some other factors, such as the temperature (Gossen et al. 2012), the level of nitrogen in the soil (Laperche et al. 2017), and molecular metabolism in the plants such as ethanol fermentation in cells and expression of the pyruvate decarboxylase genes in pathogen (Gravot et al. 2016), as

well as epigenetic factors such as DNA methylation (Liégard et al. 2019) have been reported to influence clubroot resistance phenotype, especially in case of the quantitative genetic control of resistance. Nevertheless, results from this study indicate that a greater complexity of resistance mechanisms is involved in the control of *B. oleracea*-resistance in canola at the maturity stage as compared with the resistance at 45 DAI; further study will be needed to understand this.

## Chapter 4 General Discussion

Clubroot disease, caused by *Plasmodiophora brassicae*, is one of the major threats to canola production worldwide. Rapid spread of this disease has been reported in many countries including Canada (Dixon 2009; Strelkov and Hwang 2014; Řičařová et al. 2016). This disease causes at least 30% yield loss in canola (Tewari et al. 2005; Hwang et al. 2012). Currently, there is no effective method for eradicating this pathogen from soil. Several cultural practices such as increasing soil pH, crop rotation, changing seeding date, and sanitation of equipment have been investigated; however, none of the practices were found to be economic and feasible for use in canola. Growing clubroot-resistant cultivars with appropriate management practices has been suggested to be the best strategy for the management of this disease (Hwang et al. 2014; Peng et al. 2014b). However, due to the evolution of new *P. brassicae* pathotypes, many of the available sources of resistance became ineffective in Canada (Strelkov et al. 2018). This highlights the need of increasing diversity for clubroot resistance genes in canola for sustainable management of this disease. Several efforts have been made in the last decade for introgression of resistance from the diploid parental species *Brassica rapa* into *B. napus* canola and mapping of the resistance genes (Bradshaw et al. 1997; Chen et al. 2013; Hirani et al. 2016; Hasan and Rahman 2016; Liu et al. 2018b; Pang et al. 2018; Laila et al. 2019; Hasan et al. 2021b). *Brassica oleracea*, the other parental species of *B. napus*, carries excellent resistance to clubroot disease(Lee et al. 2016; Dakouri et al. 2018; Peng et al. 2018; Farid et al. 2020); however, almost no effort has been made to introgress this resistance into canola. This thesis research was carried out to investigate the prospects of introgression of clubroot resistance from *B. olearcea* into *B. napus* canola.

According to Farid et al. (2020), *B. oleracea* var. *acephala* (kale) carries excellent resistance to multiple *P. brassicae* pathotypes including the recently evolved ones. Therefore, this *B. oleracea* was crossed to a clubroot susceptible *B. napus* line to develop a clubroot resistant canola line carrying resistance in its C genome. Several researchers (Quazi 1988; Bennett et al. 2008; Iftikhar et al. 2018) reported that production of $F_1$ hybrids of a *B. napus* × *B. oleracea* interspecific cross is extremely difficult following the conventional approach of harvesting the viable $F_1$ seeds; therefore, application of embryo rescue technique (Rahman 2004) is essential for successful production of $F_1$ hybrids of this interspecific cross. In this study, the *in vitro* ovule culture technique was applied, and 0.07 $F_1$ hybrid per pollination were obtained from the *B. napus* × *B. oleracea* var. *acephala* interspecific crosses. Backcrossing of the $F_1$'s to the *B. napus* parent produced 0.064 $BC_1$ seeds per pollination.

The *B. oleracea* var. *acephala* parents used in this study require vernalization for flowering, and their seed oil contains a high content of erucic fatty acid and seed meal contain a high content of glucosinolates. Therefore, segregation for days to flowering, as well as seed quality traits such as oil, protein, and glucosinolate and erucic acid contents were expected in the $F_2$ and $BC_1$ populations. On average, these populations flowered later and had lower seed oil and higher erucic acid and glucosinolate contents than the *B. napus* parent; however, plants flowering earlier than the *B. napus* parent could be found in these populations. Development of canola quality type is needed for use of the clubroot resistant *B. napus* lines developed from this interspecific cross. Indeed, seeds of several plants contained zero erucic acid in oil and low glucosinolate in seed meal. This is apparently due to a simpler genetic control of these two canola quality traits (Howell et al. 2003; Rahman et al. 2008; Li et al. 2014a; He et al. 2018; Rahman and Kebede

2021) as compared with the genetic control of seed oil content (Chao et al. 2017; Tang et al. 2018; Rahman and Kebede 2021). Nevertheless, based on agronomic and seed quality data, it is apparent that spring growth habit plants with acceptable seed quality traits can be achieved from this population. This agrees with the results reported by other researchers (Rahman et al. 2011a, 2015; Iftikhar et al. 2018) that canola quality *B. napus* plants can be achieved from the *B. napus* × *B. oleracea* interspecific crosses.

High sterility in regards to silique and seed set is commonly seen in early generation populations of *Brassica* interspecific crosses (Rahman et al. 2011a; Bennett et al. 2012; Li et al. 2014b; Kamiński et al. 2020), as was found in the present study. Sterility occurs mainly due to chromosomal anomalies in meiosis. This chromosomal anomaly can also result in unreduced male and female gametes, which after fertilization is expected to generate progeny with chromosome numbers greater than the parental species (Cui et al. 2012; Kamiński et al. 2020), as has been found in the present study. The flow cytometry analysis data from this thesis research also supported this. Nuclear DNA content of some of the $F_3$ plants was about 1.5-times greater than that of the *B. napus* parent; however, the majority of the plants had nuclear DNA content similar to the *B. napus* parent. Theoretically, the $BC_1F_2$ population was expected to have experienced less meiotic anomalies and be closer to the *B. napus* parent. Indeed, the mean nuclear DNA content of this population was significantly less than that of the $F_3$ and was closer to the *B. napus* parent. This indicates that progeny plants with a *B. napus* chromosome number ($2n = 38$, AACC genome) can be achieved from both $F_3$ and $BC_1F_2$ populations. Rahman et al. (2015) and Attri and Rahman (2018) also demonstrated that the interspecific progenies derived from an amphidiploid × diploid cross stabilize into an amphidiploid type.

Several researchers (Laurens and Thomas 1993; Hasan et al. 2012; Ning et al. 2018; Farid et al. 2020) reported that *B. oleracea* vars. *acephala* (Kale), *capitata* (Cabbage) and *gemmifera* (Brussels sprout) carry resistance to clubroot disease. Therefore, progenies derived from *B. napus* × *B. oleracea* interspecific crosses involving these varieties of *B. oleracea* was expected to generate clubroot resistant *B. napus* plants. Of the total 2,264 $F_3$, 468 $BC_1F_2$ and 541 $F_4$ plants of three *B. napus* × *B. oleracea* var. *acephala* interspecific crosses evaluated for resistance to pathotype 3H, only 13 $F_3$ and 86 $F_4$ plants of *B. oleracea* var. *acephala* cv. Winterbor (AM114) were resistant at the harvest stage. The occurrence of such low proportion of resistant plants in the segregation populations suggested that multiple gene loci might be involved in the control of clubroot resistance in *B. oleracea*. Quantitative genetic control of clubroot resistance in *B. oleracea* has also been reported by other researchers, such as Rocherieux et al. (2004) and Farid et al. (2020). In addition to this, the involvement of recessive genes in the control of resistance (Voorrips and Visser 1993) may be another reason for the occurrence of such a low number of resistant plants. This was even more evident in the progenies of *B. napus* × *B. oleracea* interspecific crosses involving the var. *gemmifera* cv. Diablo and var. *capitata* cvs. Badger shipper, Bindsachsener and Balbro. In this case, a total of 4,168 plants belonging to 14 $BC_1F_2$, 14 $F_4$, 195 $F_5$, 1 $F_6$, 2 $F_7$, 1 $F_8$ and 54 $F_9$ families were screened for resistance to pathotype 3H; and 3,216 plants belonging to 14 $BC_1F_2$, 12 $F_4$, 194 $F_5$ and 2 $F_6$ families were screened for resistance to pathotype 3A. In this case, only one $F_8$ family of *B. napus* × *B.oleracea* var. *capitata* cv. Bindsachsener cross was found to carry resistance (48.4% DSI at harvest) to pathotype 3H, and $F_9$ progeny of the selected plants of this family exhibited 20.5% DSI. In contrast, no plants exhibiting resistance to pathotype 3A could be selected from this population, which might be due to a complex genetic control of resistance to this pathotype as compared to the genetic control of

resistance to pathotype 3H (Shaikh et al. 2021). Thus, the quantitative genetic control with possible involvement of recessive genes in the control of clubroot resistance in the C genome of *B. oleracea* might be the reason for the need of repeated selection for several generations to achieve a homozygous clubroot resistant line from a *B. napus* × *B. oleracea* interspecific cross.

Researchers commonly score the plants at 6-8 weeks after inoculation to assess clubroot disease severity (Rocherieux et al. 2004; Chen et al. 2013; Strelkov et al. 2018). In this study, in addition to scoring the plants at 45 days after inoculation, scoring was also done at harvest time. A significant increase in disease severity was observed at harvest stage in $F_3$ and $BC_1F_2$ populations of *B. napus* × *B. oleracea* var. *acephala* and in the $F_8$ population of *B. napus* × *B. oleracea* cv. Bindsachsener crosses. A greater level of disease symptoms in adult plants as compared to young plants has also been reported by Laurens and Thomas (1993). Several researchers (Zhang et al. 2015; Fei et al. 2016; Mei et al. 2019) also found a variety of changes in resistance patterns in resistant and susceptible plants at different growth stages. The effect of longer growing time including the effect of environment (Gossen et al. 2012; Laperche et al. 2017), and gene expression(Gravot et al. 2016; Zhang et al. 2019) and epigenetic (Liégard et al. 2019) control of clubroot resistance may also have contributed to the occurrence of greater disease severity at harvest stage as compared with 45 days after inoculation.

In this study, SSR markers were used to investigate the origin of resistance in the $F_3$ plants of *B. napus* × *B. oleracea* var. *acephala* cv. Winterbor and in $F_8$ plants of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener crosses. Marker analysis could confirm the occurrence of *B. oleracea* alleles in the resistant plants; however, no association between marker and clubroot resistance could be established. Nevertheless, the results from this study have contributed to the knowledge

of the prospects of developing a clubroot-resistant *B. napus* line from *B. napus* × *B. oleracea* cross including mapping of the *B. oleracea*-resistance introgressed into *B. napus*.

## 4.1 Conclusion

The following conclusions can be drawn from the present study:

- Viable hybrids of *B. napus* × *B. oleracea* interspecific cross can be achieved through the application of embryo rescue techniques, such as the application of *in vitro* ovule culture technique.
- Clubroot resistant spring growth habit plants with *B. napus* chromosome number and canola quality traits (zero erucic acid in oil and low glucosinolate in seed meal) can be developed from canola × kale interspecific crosses.
- Clubroot resistance from other *B. oleracea*, such as cabbage, can also be introgressed into *B. napus* through *B. napus* × *B. oleracea* interspecific cross.
- The clubroot resistance of *B. oleracea* can broaden the genetic base of *B. napus* canola for clubroot resistance genes in its C genome.

## 4.2 Future research

- Selection needs to be carried out in the progeny of the $F_4$ population of *B. napus* × *B. oleracea* var. *acephala* interspecific cross that I developed in this study to achieve a homozygous clubroot resistant euploid *B. napus* line.

- Study with the clubroot resistant lines developed from the crosses involving *B. oleracea* vars. *acephala* and *capitata* need to be carried out to understand the genetic control of this resistance.
- Mapping populations need to be developed using the clubroot resistant lines carrying the resistance of var. *acephala* and var. *capitata*, and mapping of these resistances need to be carried out using molecular markers and to develop markers associated with resistance for use in breeding.
- Gene expression and metabolomics study need to be carried out with the resistant lines to understand the molecular basis of the resistance as well as to understand the changes occurring in the plants for increased disease severity at the adult plant stage

## Bibliography

Abbaoui B, Lucas CR, Riedl KM, et al (2018) Cruciferous vegetables, isothiocyanates, and bladder cancer prevention. Molecular Nutrition and Food Research 62 (18):e1800079 https://doi.org/https://doi.org/10.1002/mnfr.201800079

Aghaalikhani M, Yaghoobi SR (2008) Critical period of weed control in winter canola in a semi-arid region. Pakistan Journal of Biological Sciences 11:773–777. https://doi.org/10.3923/pjbs.2008.773.777.

Ahmar S, Liaqat N, Hussain M, et al (2019) Effect of abiotic stresses on *Brassica* species and role of transgenic breeding for adaptation. Asian Journal of Research in Crop Science: 1–10. https://doi.org/10.9734/ajrcs/2019/v3i130037

Al-shehbaz IA (2011) Brassicaceae (mustard family). In eLS, (Ed). https://doi.org/10.1002/9780470015902.a0003690.pub2

Arif S, Liaquat F, Yang S, et al (2021) Exogenous inoculation of endophytic bacterium *Bacillus cereus* suppresses clubroot (*Plasmodiophora brassicae*) occurrence in pak choi (*Brassica campestris* sp. *chinensis* L.). Planta 253(2):25. https://doi.org/10.1007/s00425-020-03546-4

Attri R, 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 and Pasture Science 69:94–106. https://doi.org/10.1071/CP17193

Bennett RA, Séguin-Swartz G, Rahman H (2012) Broadening genetic diversity in canola using the C-genome species *Brassica oleracea* L. Crop Science 52:2030–2039. https://doi.org/10.2135/cropsci2011.11.0580

Bennett RA, Thiagarajah MR, King JR, Rahman H (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 164:593–601. https://doi.org/10.1007/s10681-008-9788-0

Botero-Ramírez A, Laperche A, Guichard S, et al (2020) Clubroot symptoms and resting spore production in a doubled haploid population of oilseed rape (*Brassica napus*) are controlled by four main QTLs. Frontiers in Plant Science 11: 604527. https://doi.org/10.3389/fpls.2020.604527

Bradshaw JE, Gemmell DJ, Wilson RN (1997) Transfer of resistance to clubroot (*Plasmodiophora brassicae*) to swedes (*Brassica napus* L. var. *napobrassica Peterm*) from *B. rapa*. Annals of Applied Biology 130:337–348. https://doi.org/10.1111/j.1744-7348.1997.tb06837.x

Buczacki ST, Moxham SE (1983) Structure of the resting spore wall of *Plasmodiophora brassicae* revealed by electron microscopy and chemical digestion. Transactions of the British Mycological Society 81:221–231. https://doi.org/10.1016/s0007-1536(83)80073-4

Bus A, Körber N, Snowdon RJ, Stich B (2011) Patterns of molecular variation in a species-wide germplasm set of *Brassica napus*. Theoretical and Applied Genetics 123:1413–1423. https://doi.org/10.1007/s00122-011-1676-7

Chamola R, Balyan HS, Bhat SR (2013) Transfer of cytoplasmic male sterility from alloplasmic *Brassica juncea* and *B. napus* to cauliflower (*B. oleracea* var. *botrytis*) through interspecific hybridization and embryo culture. Indian Journal of Genetics and Plant Breeding 73:203–210. https://doi.org/10.5958/j.0975-6906.73.2.030

Champolivier L, Merrien A (1996) Effects of water stress applied at different growth stages to *Brassica napus* L. var. *oleifera* on yield, yield components and seed quality. European Journal of Agronomy 5:153–160. https://doi.org/10.1016/S1161-0301(96)02004-7

Chao H, Wang H, Wang X, et al (2017) Genetic dissection of seed oil and protein content and identification of networks associated with oil content in *Brassica napus*. Nature Publishing Group 1–16. https://doi.org/10.1038/srep46295

Chen J, Jing J, Zhan Z, et al (2013) Identification of novel QTLs for isolate-specific partial resistance to *Plasmodiophora brassicae* in *Brassica rapa*. PLoS ONE 8:e85307. https://doi.org/10.1371/journal.pone.0085307

Chen J, Piao Y, Liu Y, et al (2018) Genome-wide identification and expression analysis of chitinase gene family in *Brassica rapa* reveals its role in clubroot resistance. Plant Science 270:257–267. https://doi.org/10.1016/j.plantsci.2018.02.017

Chen M, Shelton AM, Hallett RH, et al (2011a) Swede Midge (Diptera: Cecidomyiidae), ten years of invasion of crucifer crops in north America. Journal of Economic Entomology 104:709–716. https://doi.org/10.1603/EC10397

Chen S, Nelson MN, Chèvre A-M, et al (2011b) Trigenomic bridges for *Brassica* improvement. Critical Reviews in Plant Sciences 30:524–547. https://doi.org/https://doi.org/10.1080/07352689.2011.615700

Cheng X, Xu J, Xia S, et al (2009) Development and genetic mapping of microsatellite markers from genome survey sequences in *Brassica napus*. Theoretical and Applied Genetics 118:1121–1131. https://doi.org/10.1007/s00122-009-0967-8

Chiang MS, Crete R (1976) Diallel analysis of the inheritance of resistance to race 6 of *Plasmodiophora brassicae* in cabbage. Canadian Journal of Plant Science 56:865–868. https://doi.org/10.4141/cjps76-140

Choi SR, Oh SH, Chhapekar SS, et al (2020) Quantitative trait locus mapping of clubroot resistance and *Plasmodiophora brassicae* pathotype banglim-specific marker development in *Brassica rapa*. International Journal of Molecular Sciences 21:1–15. https://doi.org/10.3390/ijms21114157

Ciaghi S, Schwelm A, Neuhauser S (2019) Transcriptomic response in symptomless roots of clubroot infected kohlrabi (*Brassica oleracea* var. *gongylodes*) mirrors resistant plants. BMC Plant Biology 19:1–14. https://doi.org/10.1186/s12870-019-1902-z

Cui C, Ge X, Gautam M, et al (2012) Cytoplasmic and genomic effects on meiotic pairing in *Brassica* hybrids and allotetraploids from pair crosses of three cultivated diploids. Genetics 191:725–738. https://doi.org/10.1534/genetics.112.140780

Dakouri A, Zhang X, Peng G, et al (2018) Analysis of genome-wide variants through bulked segregant RNA sequencing reveals a major gene for resistance to *Plasmodiophora brassicae* in *Brassica oleracea*. Scientific Reports 8:1–10. https://doi.org/10.1038/s41598-018-36187-5

de Silva Weeraddana C, Evenden ML (2018) Canola nutrition and variety affect oviposition and offspring performance in the generalist herbivore, mamestra configurata (Lepidoptera: Noctuidae). Journal of Economic Entomology 111:1702–1710. https://doi.org/10.1093/jee/toy158

Diederichsen E, Frauen M, Linders EGA, et al (2009) Status and perspectives of clubroot resistance breeding in crucifer crops. Journal of Plant Growth Regulation 28:265–281. https://doi.org/10.1007/s00344-009-9100-0

Dixon GR (2009a) The occurrence and economic impact of *Plasmodiophora brassicae* and clubroot disease. Journal of Plant Growth Regulation 28:194–202. https://doi.org/10.1007/s00344-009-9090-y

Dixon GR (2009b) *Plasmodiophora brassicae* in its environment. Journal of Plant Growth Regulation 28:212–228. https://doi.org/10.1007/s00344-009-9098-3

Dunker S, Keunecke H, Steinbach P, Von Tiedemann A (2008) Impact of *Verticillium longisporum* on yield and morphology of winter oilseed rape (*Brassica napus*) in relation to systemic spread in the plant. Journal of Phytopathology 156:698–707. https://doi.org/10.1111/j.1439-0434.2008.01429.x

Elferjani R, Soolanayakanahally R (2018) Canola responses to drought, heat, and combined stress: shared and specific effects on carbon assimilation, seed yield, and oil composition. Frontiers in Plant Science 9:1–17. https://doi.org/10.3389/fpls.2018.01224

Evenden ML, Gries R (2010) Assessment of commercially available pheromone lures for monitoring diamondback moth (Lepidoptera: Plutellidae) in canola. Journal of Economic Entomology 103:654–661. https://doi.org/10.1603/EC09339

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

Fei W, Feng J, Rong S, et al (2016) Infection and gene expression of the clubroot pathogen *Plasmodiophora brassicae* in resistant and susceptible canola cultivars. Plant Disease 100:824–828. https://doi.org/10.1094/PDIS-11-15-1255-RE

Feng J, Hwang RU, Hwang SF, et al (2010) Molecular characterization of a serine protease Pro1 from *Plasmodiophora brassicae* that stimulates resting spore germination. Molecular Plant Pathology 11:503–512. https://doi.org/10.1111/j.1364-3703.2010.00623.x

Fitzjohn RG, Armstrong TT, Newstrom-Lloyd LE, et al (2007) Hybridisation within *Brassica* and allied genera: Evaluation of potential for transgene escape. Euphytica 158:209–230. https://doi.org/10.1007/s10681-007-9444-0

Fredua-Agyeman R, Jiang J, Hwang SF, Strelkov SE (2020) QTL mapping and inheritance of clubroot resistance genes derived from *Brassica rapa* subsp. *rapifera* (ECD 02) reveals resistance loci and distorted segregation ratios in two $F_2$ populations of different crosses. Frontiers in Plant Science 11:. https://doi.org/10.3389/fpls.2020.00899

Friberg H, Lagerlöf J, Rämert B (2005) Germination of *Plasmodiophora brassicae* resting spores stimulated by a non-host plant. European Journal of Plant Pathology 113:275–281. https://doi.org/10.1007/s10658-005-2797-0

Fu L, Li H, Wei L, et al (2018) Antifungal and biocontrol evaluation of four *Lysobacter* strains against clubroot disease. Indian Journal of Microbiology 58:353–359. https://doi.org/10.1007/s12088-018-0716-2

Fu TD, Si P, Yang XN, Yang GS (1992) Overcoming self-incompatibility of *Brassica napus* by salt (NaCl) spray. Plant Breeding 109:255–258

Fu Y, Zhang Y, Mason AS, et al (2019) NBS-encoding genes in *Brassica napus* evolved rapidly after allopolyploidization and co-localize with known disease resistance loci. Frontiers in Plant Science 10:1–18. https://doi.org/10.3389/fpls.2019.00026

Gaebelein R, Alnajar D, Koopmann B, Mason AS (2019) Hybrids between *Brassica napus* and *B. nigra* show frequent pairing between the B and A/C genomes and resistance to blackleg. Chromosome Research 27:221–236. https://doi.org/10.1007/s10577-019-09612-2

Ge JC, Yoon SK, Choi NJ (2017) Using canola oil biodiesel as an alternative fuel in diesel engines: A review. Applied Sciences 7 (9): 881. https://doi.org/10.3390/app7090881

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review of Phytopathology 43:205–227. https://doi.org/10.1146/annurev.phyto.43.040204.135923

Gossen BD, Adhikari KKC, Mcdonald MR (2012) Effects of temperature on infection and subsequent development of clubroot under controlled conditions. Plant Pathology 61:593–599. https://doi.org/10.1111/j.1365-3059.2011.02536.x

Gravot A, Richard G, Lime T, et al (2016) Hypoxia response in *Arabidopsis* roots infected by *Plasmodiophora brassicae* supports the development of clubroot. BMC Plant Biology 16:. https://doi.org/10.1186/s12870-016-0941-y

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

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

Hasan MJ, Shaikh R, Basu U, Rahman H (2021b) Mapping clubroot resistance of *Brassica rapa* introgressed into *Brassica napus* and development of molecular markers for the resistance. Crop Science. https://doi.org/10.1002/csc2.20626

Hasan MJ, Strelkov SE, Howard RJ, Rahman H (2012) Screening of *Brassica* germplasm for resistance to *Plasmodiophora brassicae* pathotypes prevalent in Canada for broadening diversity in clubroot resistance. Canadian Journal of Plant Science 92:501–515. https://doi.org/10.4141/CJPS2010-006

Hatakeyama K, Fujimura M, Suzuki T (2004) New classification method for *Plasmodiophora brassicae* field isolates in Japan based on resistance of $F_1$ cultivars of Chinese cabbage (*Brassica rapa* L.) to clubroot. Breeding Science 54:197–201. https://doi.org/https://doi.org/10.1270/jsbbs.54.197

Hatakeyama K, Niwa T, Kato T, et al (2017) The tandem repeated organization of NB-LRR genes in the clubroot-resistant CRb locus in *Brassica rapa* L. Molecular Genetics and Genomics 292:397–405. https://doi.org/10.1007/s00438-016-1281-1

Hatakeyama K, Suwabe K, Tomita RN, et al (2013) Identification and characterization of Crr1a, a gene for resistance to clubroot disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. PLoS ONE 8:1–10. https://doi.org/10.1371/journal.pone.0054745

He Y, Fu Y, Hu D, et al (2018) QTL Mapping of seed glucosinolate content responsible for environment in *Brassica napus*. Frontiers in Plant Science 9:1–8. https://doi.org/10.3389/fpls.2018.00891

Hejna O, Havlickova L, He Z, et al (2019) Analysing the genetic architecture of clubroot resistance variation in *Brassica napus* by associative transcriptomics. Molecular Breeding 39. https://doi.org/10.1007/s11032-019-1021-4

Hirai M, Harada T, Kubo N, et al (2004) A novel locus for clubroot resistance in *Brassica rapa* and its linkage markers. Theoretical and Applied Genetics 108:639–643. https://doi.org/10.1007/s00122-003-1475-x

Hirani AH, Gao F, Liu J, et al (2016) Transferring clubroot resistance from Chinese cabbage (*Brassica rapa*) to canola (*B. napus*). Canadian Journal of Plant Pathology 38:82–90. https://doi.org/10.1080/07060661.2016.1141799

Howell PM, Sharpe AG, Lydiate DJ (2003) Homoeologous loci control the accumulation of seed glucosinolates in oilseed rape (*Brassica napus*). Genome 46:454–460. https://doi.org/10.1139/g03-028

Huang Z, Peng G, Liu X, et al (2017) Fine mapping of a clubroot resistance gene in Chinese cabbage using SNP markers identified from bulked segregant RNA sequencing. Frontiers in Plant Science 8:1–9. https://doi.org/10.3389/fpls.2017.01448

Hwang SF, Howard RJ, Strelkov SE, et al (2014) Special Issue: Management of clubroot (*Plasmodiophora brassicae*) on canola (*Brassica napus*) in western Canada. Canadian Journal of Plant Pathology 36:49–65. https://doi.org/https://doi.org/10.1080/07060661.2013.863806

Hwang SF, Strelkov SE, Feng J, et al (2012) *Plasmodiophora brassicae*: A review of an emerging pathogen of the Canadian canola (*Brassica napus*) crop. Molecular Plant Pathology 13:105–113. https://doi.org/https://doi.org/10.1111/j.1364-3703.2011.00729.x

Hwang SF, Strelkov SE, Peng G, et al (2016) Blackleg (*Leptosphaeria maculans*) severity and yield loss in canola in Alberta, Canada. Plants 5:10–27. https://doi.org/10.3390/plants5030031

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

Jian H, Zhang A, Ma J, et al (2019) Joint QTL mapping and transcriptome sequencing analysis reveal candidate flowering time genes in *Brassica napus* L. BMC Genomics 20: 21. https://doi.org/10.1186/s12864-018-5356-8

Jiang D, Wang X, Liu S, Guo H (2011) Rapeseed oil monoester of ethylene glycol monomethyl ether as a new biodiesel. Journal of Biomedicine and Biotechnology 2011. https://doi.org/10.1155/2011/293161

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329. https://doi.org/https://doi.org/10.1038/nature05286

Kageyama K, Asano T (2009) Life cycle of *Plasmodiophora brassicae*. Journal of Plant Growth Regulation 28:203–211. https://doi.org/10.1007/s00344-009-9101-z

Kamiński P, Marasek-Ciolakowska A, Podwyszyńska M, et al (2020) Development and characteristics of interspecific hybrids between *Brassica oleracea* L. and *B. napus* L. Agronomy 10(9): 1339. https://doi.org/10.3390/agronomy10091339

Katche E, Quezada-martinez D, Katche EI (2019) Interspecific hybridization for *Brassica* crop improvement. Crop Breeding, Genetics and Genomics 1–32. https://doi.org/10.20900/cbgg20190007

Knodel JJ, Olson DL, Hanson BK, Henson RA (2008) Impact of planting dates and insecticide strategies for managing crucifer flea beetles (Coleoptera: Chrysomelidae) in spring-planted canola. Journal of Economic Entomology 101:810–821. https://doi.org/https://doi.org/10.1093/jee/101.3.810

Knop Wright M, Brandt SL, Coudron TA, et al (2006) Characterization of digestive proteolytic activity in *Lygus hesperus* Knight (Hemiptera: Miridae). Journal of Insect Physiology 52:717–728. https://doi.org/10.1016/j.jinsphys.2006.03.012

Laila R, Park JI, Robin AHK, et al (2019) Mapping of a novel clubroot resistance QTL using ddRAD-seq in Chinese cabbage (*Brassica rapa* L.). BMC Plant Biology 19:. https://doi.org/10.1186/s12870-018-1615-8

Laperche A, Aigu Y, Jubault M, et al (2017) Clubroot resistance QTL are modulated by nitrogen input in *Brassica napus*. Theoretical and Applied Genetics 130:669–684. https://doi.org/10.1007/s00122-016-2842-8

Laurens F, Thomas G (1993) Inheritance of resistance to clubroot (*Plasmodiophora brassicae* Wor.) in kale (*Brassica oleracea* ssp. *acephala*). Hereditas 119:253–262. https://doi.org/10.1111/j.1601-5223.1993.00253.x

Lee J, Izzah NK, Choi BS, et al (2016) Genotyping-by-sequencing map permits identification of clubroot resistance QTLs and revision of the reference genome assembly in cabbage (*Brassica oleracea* L.). DNA Research 23:29–41. https://doi.org/10.1093/dnares/dsv034

Leflon M, Eber F, Letanneur JC, et al (2006) Pairing and recombination at meiosis of *Brassica rapa* (AA) × *Brassica napus* (AACC) hybrids. Theoretical and Applied Genetics 113:1467–1480. https://doi.org/10.1007/s00122-006-0393-0

Li B, Zhao W, Li D, et al (2018) Genetic dissection of the mechanism of flowering time based on an environmentally stable and specific QTL in *Brassica napus*. Plant Science 277:296–310. https://doi.org/10.1016/j.plantsci.2018.10.005

Li F, Chen B, Xu K, et al (2014a) Genome-wide association study dissects the genetic architecture of seed weight and seed quality in rapeseed (*Brassica napus* L.). DNA Research 21:355–367. https://doi.org/10.1093/dnares/dsu002

Li H, Chen X, Yang Y, et al (2011) Development and genetic mapping of microsatellite markers from whole genome shotgun sequences in *Brassica oleracea*. Molecular Breeding 28:585–596. https://doi.org/10.1007/s11032-010-9509-y

Li L, Luo Y, Chen B, et al (2016) A genome-wide association study reveals new loci for resistance to clubroot disease in *Brassica napus*. Frontiers in Plant Science 7:1–12. https://doi.org/10.3389/fpls.2016.01483

Li Q, Zhou Q, Mei J, et al (2014b) Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B. oleracea*. Molecular Breeding 34:1955–1963. https://doi.org/10.1007/s11032-014-0153-9

Liégard B, Baillet V, Etcheverry M, et al (2019) Quantitative resistance to clubroot infection mediated by transgenerational epigenetic variation in *Arabidopsis*. New Phytologist 222:468–479. https://doi.org/10.1111/nph.15579

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

Liu L, Qin L, Zhou Z, et al (2020) Refining the life cycle of *Plasmodiophora brassicae*. Phytopathology 110:1704–1712. https://doi.org/10.1094/PHYTO-02-20-0029-R

Liu Y, Oliveira MSF, Stein HH (2018a) Canola meal produced from high-protein or conventional varieties of canola seeds may substitute soybean meal in diets for gestating and lactating sows without compromising sow or litter productivity. Journal of Animal Science 96:5179–5187. https://doi.org/10.1093/jas/sky356

Liu Y, Xu A, Liang F, et al (2018b) Screening of clubroot-resistant varieties and transfer of clubroot resistance genes to *Brassica napus* using distant hybridization. Breeding Science 68:258–267. https://doi.org/10.1270/jsbbs.17125

Lohani N, Jain D, Singh MB, Bhalla PL (2020) Engineering multiple abiotic stress tolerance in canola, *Brassica napus*. Frontiers in Plant Science 11:1–26. https://doi.org/10.3389/fpls.2020.00003

Ludwig-Müller J, Jülke S, Geiß K, et al (2015) A novel methyltransferase from the intracellular pathogen *Plasmodiophora brassicae* methylates salicylic acid. Molecular Plant Pathology 16:349–364. https://doi.org/10.1111/mpp.12185

Macfarlane I (1970) Germination of resting spores of *Plasmodiophora brassicae*. Transactions of the British Mycological Society 55:97–112. https://doi.org/10.1016/s0007-1536(70)80100-0

Madden MK, Widick IV, Blubaugh CK (2021) Weeds impose unique outcomes for pests, natural enemies, and yield in two vegetable crops. Environmental Entomology 50:330–336. https://doi.org/10.1093/ee/nvaa168

Manzanares-Dauleux MJ, Delourme R, Baron F, Thomas G (2000) Mapping of one major gene and of QTLs involved in resistance to clubroot in *Brassica napus*. Theoretical and Applied Genetics 101:885–891. https://doi.org/10.1007/s001220051557

Mason AS, Huteau V, Eber F, et al (2010) Genome structure affects the rate of autosyndesis and allosyndesis in AABC, BBAC and CCAB *Brassica* interspecific hybrids. Chromosome Research 18:655–666. https://doi.org/10.1007/s10577-010-9140-0

Matsumoto E, Yasui C, Ohi M, Tsukada M (1998) Linkage analysis of RFLP markers for clubroot resistance and pigmentation in Chinese cabbage (*Brassica rapa ssp. pekinensis*) Euphytica 104: 79. https://doi.org/10.1023/A:1018370418201

Megha S, Basu U, Joshi RK, Kav NNV (2018) Physiological studies and genome-wide microRNA profiling of cold-stressed *Brassica napus*. Plant Physiology and Biochemistry 132:1–17. https://doi.org/10.1016/j.plaphy.2018.08.027

Mei J, Guo Z, Wang J, et al (2019) Understanding the resistance mechanism in *Brassica napus* to clubroot caused by *Plasmodiophora brassicae*. Phytopathology 109:810–818. https://doi.org/10.1094/PHYTO-06-18-0213-R

Mei J, Qian L, Disi JO, et al (2011) Identification of resistant sources against *Sclerotinia sclerotiorum* in *Brassica* species with emphasis on *B. oleracea*. Euphytica 177:393–399. https://doi.org/10.1007/s10681-010-0274-0

Mejicanos G, Sanjayan N, Kim IH, Nyachoti CM (2016) Recent advances in canola meal utilization in swine nutrition. Journal of Animal Science and Technology 58. https://doi.org/10.1186/s40781-016-0085-5

Moriguchi K, Chiak K, Kinji I, Kanzunari N (1999) A genetic map based on RAPD, RFLP, isozyme, morphological markers and QTL analysis for clubroot resistance in *Brassica oleracea*. Breeding Science 49:257–265. https://doi.org/https://doi.org/10.1270/jsbbs.49.257

Muangprom A, Mauriera I, Osborn TC (2006) Transfer of a dwarf gene from *Brassica rapa* to oilseed *B. napus*, effects on agronomic traits, and development of a "perfect" marker for selection. Molecular Breeding 17:101–110. https://doi.org/10.1007/s11032-005-3734-9

Mwathi MW, Schiessl S v., Batley J, Mason AS (2019) "Doubled-haploid" allohexaploid *Brassica* lines lose fertility and viability and accumulate genetic variation due to genomic instability. Chromosoma 128:521–532. https://doi.org/10.1007/s00412-019-00720-w

Nagaoka T, Doullah MAU, Matsumoto S, et al (2010) Identification of QTLs that control clubroot resistance in *Brassica oleracea* and comparative analysis of clubroot resistance genes between *B. rapa* and *B. oleracea*. Theoretical and Applied Genetics 120:1335–1346. https://doi.org/10.1007/s00122-010-1259-z

Neik TX, Barbetti MJ, Batley J (2017) Current status and challenges in identifying disease resistance genes in *Brassica napus*. Frontiers in Plant Science 8: 1788.https://doi.org/ 10.3389/fpls.2017.01788. eCollection 2017.

Nikzad A, Kebede B, Pinzon J, et al (2020) Potential of the C genome of the different variants of *Brassica oleracea* for heterosis in spring *B. napus* canola. Frontiers in Plant Science 10:. https://doi.org/10.3389/fpls.2019.01691

Ning Y, Wang Y, Fang Z, et al (2018) Identification and characterization of resistance for *Plasmodiophora brassicae* race 4 in cabbage (*Brassica oleracea* var. *capitata*). Australasian Plant Pathology 47:531–541. https://doi.org/10.1007/s13313-018-0590-8

Niwa R, Nomura Y, Osaki M, Ezawa T (2008) Suppression of clubroot disease under neutral pH caused by inhibition of spore germination of *Plasmodiophora brassicae* in the rhizosphere. Plant Pathology 57:445–452. https://doi.org/10.1111/j.1365-3059.2007.01817.x

Nomura K, Minegishi Y, Kimizuka-Takagi C, et al (2005) Evaluation of $F_2$ and $F_3$ plants introgressed with QTLs for clubroot resistance in cabbage developed by using SCAR markers. Plant Breeding 124:371–375. https://doi.org/10.1111/j.1439-0523.2005.01105.x

Pang W, Fu P, Li X, et al (2018) Identification and mapping of the clubroot resistance gene CRd in chinese cabbage (*Brassica rapa* ssp. *pekinensis*). Frontiers in Plant Science 9:1–9. https://doi.org/10.3389/fpls.2018.00653

Pang W, Liang S, Li X, et al (2014) Genetic detection of clubroot resistance loci in a new population of *Brassica rapa*. Horticulture Environment and Biotechnology 55:540–547. https://doi.org/10.1007/s13580-014-0079-5

Pang W, Liang Y, Zhan Z, et al (2020) Development of a sinitic clubroot differential set for the pathotype classification of *Plasmodiophora brassicae*. Frontiers in Plant Science 11:. https://doi.org/10.3389/fpls.2020.568771

Paterson AH, Lan TH, Amasino R, et al (2001) *Brassica* genomics: A complement to, and early beneficiary of, the *Arabidopsis* sequence. Genome Biology 2:1–4. https://doi.org/10.1186/gb-2001-2-3-reviews1011

Peng G, Falk KC, Gugel RK, et al (2014a) Sources of resistance to *Plasmodiophora brassicae* (clubroot) pathotypes virulent on canola. Canadian Journal of Plant Pathology 36:89–99. https://doi.org/10.1080/07060661.2013.863805

Peng G, Lahlali R, Hwang SF, et al (2014b) Special Issue: Crop rotation, cultivar resistance, and fungicides/ biofungicides for managing clubroot (*Plasmodiophora brassicae*) on canola. Canadian Journal of Plant Pathology 36:99–112. https://doi.org/10.1080/07060661.2013.860398

Peng L, Zhou L, Li Q, et al (2018) Identification of quantitative trait loci for clubroot resistance in *Brassica oleracea* with the use of brassica SNP microarray. Frontiers in Plant Science 9:1–8. https://doi.org/10.3389/fpls.2018.00822

Pérez-López E, Waldner M, Hossain M, et al (2018) Identification of *Plasmodiophora brassicae* effectors — a challenging goal. Virulence 9:1344–1353. https://doi.org/10.1080/21505594.2018.1504560

Piao Z, Ramchiary N, Lim YP (2009) Genetics of clubroot resistance in brassica species. Journal of Plant Growth Regulation 28:252–264. https://doi.org/10.1007/s00344-009-9093-8

Piquemal J, Cinquin E, Couton F, et al (2005) Construction of an oilseed rape (*Brassica napus* L.) genetic map with SSR markers. Theoretical and Applied Genetics 111:1514–1523. https://doi.org/10.1007/s00122-005-0080-6

Prakash S, Chopra VL (1988) Introgression of resistance to shattering in *Brassica napus* from *Brassica juncea* through non-homologous recombination. Plant Breeding 101:167–168.

https://doi.org/10.1111/j.1439-0523.1988.tb00283.x

Quazi MH (1988) Interspecific hybrids between *Brassica napus* L. and *B. oleracea* L. developed by embryo culture. Theoretical and Applied Genetics 75:309–318. https://doi.org/10.1007/BF00303970

Rahman H (2013) Review: Breeding spring canola (*Brassica napus* L.) by the use of exotic germplasm. Canadian Journal of Plant Science 93:363–373. https://doi.org/10.4141/CJPS2012-074

Rahman H (2004) Optimum age of siliques for rescue of hybrid embryos from crosses between *Brassica oleracea*, *B. rapa* and *B. carinata*. Canadian Journal of Plant Science 84:965–969. https://doi.org/10.4141/P04-003

Rahman H, Bennett RA, Kebede B (2017) Mapping of days to flower and seed yield in spring oilseed *Brassica napus* carrying genome content introgressed from *Brassica oleracea*. Molecular Breeding 37:. https://doi.org/10.1007/s11032-016-0608-2

Rahman H, Bennett RA, Kebede B (2018) Molecular mapping of QTL alleles of *Brassica oleracea* affecting days to flowering and photosensitivity in spring *Brassica napus*. PLoS ONE 13:1–17. https://doi.org/10.1371/journal.pone.0189723

Rahman H, Bennett RA, 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. Canadian Journal of Plant Science 95:29–41. https://doi.org/10.4141/CJPS-2014-017

Rahman H, Bennett RA, Yang RC, et al (2011a) Exploitation of the late flowering species *Brassica oleracea* L. for the improvement of earliness in *B. napus* L.: An untraditional approach. Euphytica 177:365–374. https://doi.org/10.1007/s10681-010-0253-5

Rahman H, 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. The Plant Genome 1–18. https://doi.org/10.1002/tpg2.20078

Rahman H, Shakir A, Jakir HM (2011b) 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? Canadian Journal of Plant Science 91:447–458. https://doi.org/10.4141/cjps10073

Rahman M, Sun Z, McVetty PBE, Li G (2008) High throughput genome-specific and gene-specific molecular markers for erucic acid genes in *Brassica napus* (L.) for marker-assisted selection in plant breeding. Theoretical and Applied Genetics 117:895–904. https://doi.org/10.1007/s00122-008-0829-9

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

Rahman H (2001) Production of yellow-seeded *Brassica napus* through interspecific crosses. Plant Breeding 120:463–472. https://doi.org/10.1046/j.1439-0523.2001.00640.x

Rahman H, M. Joersbo, M.H. Poulsen (2001) Development of yellow-seeded *Brassica napus* of double low quality. Plant Breeding 120:473–478. https://doi.org/doi:10.1046/j.1439-0523.2001.00639.x

Rakow G (2004) Species origin and economic importance of *Brassica*. Biotechnology in Agriculture and Forestry 54:3–11. https://doi.org/10.1007/978-3-662-06164-0_1

Raman H, Raman R, Eckermann P, Coombes N (2013) Genetic and physical mapping of flowering time loci in canola (*Brassica napus* L.). Theoretical and Applied Genetics 126: 119–132. https://doi.org/10.1007/s00122-012-1966-8

Rashid MH, Hausner G, Fernando WGD (2018) Molecular and phenotypic identification of B-genome introgression linked to *Leptosphaeria maculans* resistant gene Rlm6 in *Brassica napus* × *B. juncea* interspecific hybrids. Euphytica 214:1–11. https://doi.org/10.1007/s10681-018-2287-z

Řičařová V, Kazda J, Singh K, Ryšánek P (2016) Clubroot caused by *Plasmodiophora brassicae* Wor.: A review of emerging serious disease of oilseed rape in the Czech Republic. Plant Protection Science 52:71–86. https://doi.org/10.17221/87/2015-PPS

Rocherieux J, Glory P, Giboulot A, et al (2004) Isolate-specific and broad-spectrum QTLs are involved in the control of clubroot in *Brassica oleracea*. Theoretical and Applied Genetics 108:1555–1563. https://doi.org/10.1007/s00122-003-1580-x

Rücker B, Röbbelen G (1994) Inheritance of total and individual glucosinolate contents in seeds of winter oilseed rape (*Brassica napus* L.). Plant Breeding 113:206–216. https://doi.org/doi:10.1111/j.1439-0523.1994.tb00724.x

Sakamoto K, Saito A, Hayashida N, et al (2008) Mapping of isolate-specific QTLs for clubroot resistance in Chinese cabbage (*Brassica rapa* L. *ssp. pekinensis*). Theoretical and Applied Genetics 117:759–767. https://doi.org/10.1007/s00122-008-0817-0

Salehi B, Quispe C, Butnariu M, et al (2021) Phytotherapy and food applications from *Brassica* genus. Phytotherapy Research 35:3590–3609. https://doi.org/10.1002/ptr.7048

Šamec D, Salopek-Sondi B (2019) Cruciferous (Brassicaceae) vegetables. Nonvitamin and Nonmineral Nutritional Supplements 195–202. https://doi.org/10.1016/B978-0-12-812491-8.00027-8

Šamec D, Urlić B, Salopek-Sondi B (2019) Kale (*Brassica oleracea* var. *acephala*) as a superfood: Review of the scientific evidence behind the statement. Critical Reviews in Food Science and Nutrition 59:2411–2422. https://doi.org/10.1080/10408398.2018.1454400

Shaikh R, Farid M, Rahman H (2021) Inheritance of resistance to the newly identified *Plasmodiophora brassicae* pathotypes in *Brassica napus* L. Canadian Journal of Plant Pathology 43:256–266. https://doi.org/10.1080/07060661.2020.1823483

Sharma RV, Somidi AKR, Dalai AK (2015) Preparation and properties evaluation of biolubricants derived from canola oil and canola biodiesel. Journal of Agricultural and Food Chemistry 63:3235–3242. https://doi.org/10.1021/jf505825k

Some A, Manzanares MJ, Laurens F, et al (1996) Variation for virulence on *Brassica napus* L. amongst *Plasmodiophora brassicae* collections from France and derived single-spore isolates. Plant Pathology 45:432–439. https://doi.org/10.1046/j.1365-3059.1996.d01-155.x

Strelkov SE, Hwang S, Manolii VP, et al (2018) Virulence and pathotype classification of *Plasmodiophora brassicae* populations collected from clubroot resistant canola (*Brassica napus*) in Canada Soilborne pathogens. Canadian Journal of Plant Pathology 40:284–298. https://doi.org/10.1080/07060661.2018.1459851

Strelkov SE, Hwang SF (2014) Special Issue: Clubroot in the Canadian canola crop: 10 years into the outbreak. Canadian Journal of Plant Pathology 36:27–36. https://doi.org/10.1080/07060661.2013.863807

Strelkov SE, Hwang SF, Manolii VP, et al (2016) Emergence of new virulence phenotypes of *Plasmodiophora brassicae* on canola (*Brassica napus*) in Alberta, Canada. European Journal of Plant Pathology 145:517–529. https://doi.org/10.1007/s10658-016-0888-8

Strelkov SE, Manolii VP, Cao T, et al (2007) Pathotype classification of *Plasmodiophora brassicae* and its occurrence in *Brassica napus* in Alberta, Canada. Journal of Phytopathology 155:706–712. https://doi.org/10.1111/j.1439-0434.2007.01303.x

Strelkov SE, Tewari JP, Smith-Degenhardt E (2006) Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. Canadian Journal of Plant Pathology 28:467–474. https://doi.org/10.1080/07060660609507321

Summanwar A, Basu U, Rahman H, 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. Molecular Plant-Microbe Interactions 32:1360–1377. https://doi.org/10.1094/MPMI-12-18-0341-R

Suwabe K, Tsukazaki H, Iketani H, et al (2003) Identification of two loci for resistance to clubroot (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* L. Theoretical and Applied Genetics 107:997–1002. https://doi.org/10.1007/s00122-003-1309-x

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

Tang M, Zhang Y, Liu Y, et al (2018) Mapping loci controlling fatty acid profiles, oil and protein content by genome-wide association study in *Brassica napus*. The Crop Journal 7:217–226. https://doi.org/10.1016/j.cj.2018.10.007

Tewari JP, Strelkov SE, Orchard D, et al (2005) Identification of clubroot of crucifers on canola (*Brassica napus*) in Alberta. Canadian Journal of Plant Pathology 27:143–144. https://doi.org/10.1080/07060660509507206

Thompson KF, Taylor JP (1971) Self-compatibility in kale. Heredity 27:459–471. https://doi.org/https://doi.org/10.1038/hdy.1971.108

Toroser D, Thormann CE, Osborn TC, Mithen R (1995) RFLP mapping of quantitative trait loci controlling seed aliphatic-glucosinolate content in oilseed rape (*Brassica napus* L.). Theoretical and Applied Genetics 91:802–808. https://doi.org/10.1007/BF00220963

Truco MJ, Hu J, Sadowski J, Quiros CF (1996) Inter- and intra-genomic homology of the *Brassica* genomes: implications for their origin and evolution. Theoretical and Applied Genetics 93:1225–1233. https://doi.org/10.1007/s001220050360

Ueno H, Matsumoto E, Aruga D, et al (2012) Molecular characterization of the CRa gene conferring clubroot resistance in *Brassica rapa*. Plant Molecular Biology 80:621–629. https://doi.org/10.1007/s11103-012-9971-5

Uzunova M, Ecke W, Weissleder K, Röbbelen G (1995) Mapping the genome of rapeseed (*Brassica napus* L.). I. Construction of an RFLP linkage map and localization of QTLs for seed glucosinolate content. Theoretical and Applied Genetics 90:194–204. https://doi.org/10.1007/BF00222202

Voorrips RE, Jongerius MC, Kanne HJ (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. Theoretical and Applied Genetics 94:75–82. https://doi.org/10.1007/s001220050384

Voorrips RE, Visser DL (1993) Examination of resistance to clubroot in accessions of *Brassica oleracea* using a glasshouse seedling test. Netherlands Journal of Plant Pathology 99:269–276. https://doi.org/10.1007/BF01974308

Wagner G, Laperche A, Lariagon C, et al (2019) Resolution of quantitative resistance to clubroot into QTL-specific metabolic modules. Journal of Experimental Botany 70:5375–5390. https://doi.org/10.1093/jxb/erz265

Werner S, Diederichsen E, Frauen M, et al (2008) Genetic mapping of clubroot resistance genes in oilseed rape. Theoretical and Applied Genetics 116:363–372. https://doi.org/10.1007/s00122-007-0674-2

Woods DL, Turkington TK, McLaren D, Davidson JGN (2000) Breeding summer turnip rape for resistance to brown girdling root rot. Canadian Journal of Plant Science 80:199–202. https://doi.org/10.4141/P99-023

Yu F, Zhang X, Peng G, et al (2017) Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*. Scientific Reports 7:1–11. https://doi.org/10.1038/s41598-017-04903-2

Yu J, Tehrim S, Zhang F, et al (2014) Genome-wide comparative analysis of NBS-encoding genes between *Brassica* species and *Arabidopsis thaliana*. BMC Genomics 15:1–18. https://doi.org/10.1186/1471-2164-15-3

Zhan Z, Nwafor CC, Hou Z, et al (2017) Cytological and morphological analysis of hybrids between *Brassicoraphanus*, and *Brassica napus* for introgression of clubroot resistant trait into *Brassica napus* L. PLoS ONE 12:1–17. https://doi.org/10.1371/journal.pone.0177470

Zhang H, Feng J, Hwang SF, et al (2016) Mapping of clubroot (*Plasmodiophora brassicae*) resistance in canola (*Brassica napus*). Plant Pathology 65:435–440. https://doi.org/10.1111/ppa.12422

Zhang H, Feng J, Manolii VP, et al (2015) Characterization of a gene identified in pathotype 5 of the clubroot pathogen *Plasmodiophora brassicae*. Phytopathology 105:764–770. https://doi.org/10.1094/PHYTO-10-14-0270-R

Zhang W, Wang S, Yu F, et al (2019) 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. https://doi.org/10.3390/genes10010071

Zhong R, Adeola O (2019) Energy values of solvent-extracted canola meal and expeller-derived canola meal for broiler chickens and growing pigs determined using the regression method. Journal of Animal Science 97:3415–3425. https://doi.org/10.1093/jas/skz215

## Appendices

Supplementary Table 2.1 Production of $F_2$ and $BC_1$ ($F_1$ × *Brassica napus*) seeds, respectively, through manual self-pollination and crossing of the $F_1$ plants of *B. napus* × *B. oleracea* interspecific crosses

| Cross | $F_2$ and $BC_1$ family | No. poll. | No. silique | No. seeds | No. silique / poll. | No. seed / silique | No. seed / poll. |
|---|---|---|---|---|---|---|---|
| $F_2$: | | | | | | | |
| *B. nap* × *B. ole* AM094 | 1CA2526.001-A1228 | 316 | 19 | 11 | 0.06 | 0.58 | 0.03 |
| *B. nap* × *B. ole* AM094 | 1CA2526.002-A1228 | 180 | 10 | 3 | 0.06 | 0.30 | 0.02 |
| | **Total** | **496** | **29** | **14** | 0.06 | 0.48 | 0.03 |
| *B. nap* × *B. ole* AM111 | 1CA2524.001-A1228 | 238 | 11 | 5 | 0.05 | 0.45 | 0.02 |
| *B. nap* × *B. ole* AM111 | 1CA2524.002-A1228 | 347 | 34 | 25 | 0.10 | 0.74 | 0.07 |
| *B. nap* × *B. ole* AM111 | 1CA2524.003-A1228 | 354 | 23 | 17 | 0.06 | 0.74 | 0.05 |
| *B. nap* × *B. ole* AM111 | 1CA2524.004-A1228 | 274 | 14 | 10 | 0.05 | 0.71 | 0.04 |
| *B. nap* × *B. ole* AM111 | 1CA2524.005-A1228 | 309 | 25 | 20 | 0.08 | 0.80 | 0.06 |
| | **Total** | **1522** | **107** | **77** | 0.07 | 0.72 | 0.05 |
| *B. nap* × *B. ole* AM114 | 1CA2525.001-A1228 | 222 | 13 | 10 | 0.06 | 0.77 | 0.05 |
| *B. nap* × *B. ole* AM114 | 1CA2525.002-A1228 | 248 | 8 | 6 | 0.03 | 0.75 | 0.02 |
| *B. nap* × *B. ole* AM114 | 1CA2525.003-A1228 | 96 | 28 | 370 | 0.29 | 13.21 | 3.85 |
| *B. nap* × *B. ole* AM114 | 1CA2525.004-A1228 | 215 | 6 | 1 | 0.03 | 0.17 | 0.00 |
| *B. nap* × *B. ole* AM114 | 1CA2525.005-A1228 | 159 | 27 | 20 | 0.17 | 0.74 | 0.13 |
| | **Total** | **940** | **82** | **407** | 0.09 | 4.96 | 0.43 |
| $BC_1$ ($F_1$ × *B. napus*): | | | | | | | |
| (*B. nap* × *B. ole* AM094) × *B. nap* | 1CA2540.001-A6218 | 126 | 7 | 4 | 0.06 | 0.57 | 0.03 |
| (*B. nap* × *B. ole* AM094) × *B. nap* | NA | 40 | 4 | 0 | 0.10 | 0.00 | 0.00 |
| | **Total** | **166** | **11** | **4** | 0.07 | 0.36 | 0.02 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.001-A6218 | 305 | 13 | 5 | 0.04 | 0.38 | 0.02 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.002-A6218 | 137 | 28 | 19 | 0.20 | 0.68 | 0.14 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.003-A6218 | 143 | 21 | 14 | 0.15 | 0.67 | 0.10 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.004-A6218 | 211 | 28 | 20 | 0.13 | 0.71 | 0.09 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.005-A6218 | 196 | 13 | 8 | 0.07 | 0.62 | 0.04 |
| | **Total** | **992** | **103** | **66** | 0.10 | 0.64 | 0.07 |
| (*B. nap* × *B. ole* AM114) × B.*nap* | 1CA2539.001-A6218 | 152 | 23 | 15 | 0.15 | 0.65 | 0.10 |
| (*B. nap* × *B. ole* AM114) × B.*nap* | 1CA2539.002-A6218 | 207 | 29 | 17 | 0.14 | 0.59 | 0.08 |
| (*B. nap* × *B. ole* AM114) × B.*nap* | 1CA2539.003-A6218 | 20 | 4 | 20 | 0.20 | 5.00 | 1.00 |
| (*B. nap* × *B. ole* AM114) × B.*nap* | 1CA2539.004-A6218 | 569 | 20 | 9 | 0.04 | 0.45 | 0.02 |
| (*B. nap* × *B. ole* AM114) × B.*nap* | 1CA2539.005-A6218 | 67 | 10 | 9 | 0.15 | 0.90 | 0.13 |
| | **Total** | **1015** | **86** | **70** | 0.08 | 0.81 | 0.07 |

Note: *B. nap* = *B. napus* parent A04-73NA; *B. ole* = *B. oleracea* parent

Supplementary Table 2.2 Days to flower and seed weight of $F_2$ and $BC_1$ ($F_1$ × *Brassica napus*) plants

| Cross | $F_2$ and $BC_1$ family | No. plants grew | No. plants flowered | days to flower | | | No. fertile plants | Seed weight/ plant | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Range | Mean ± S.E. [1] | | | Range | Mean ± S.E. |
| $F_2$: | | | | | | | | | |
| *B. nap* × *B. ole* AM111 | 1CA2524.001-A1228 | 7 | 7 | 69-139 | 110.9±9.3 | abcd | 3 | 0-0.96 | 0.62±0.25 |
| *B. nap* × *B. ole* AM111 | 1CA2524.002-A1228 | 12 | 12 | 39-136 | 95.8±10.2 | abc | 11 | 0.1-5.62 | 1.02±0.48 |
| *B. nap* × *B. ole* AM111 | 1CA2524.003-A1228 | 8 | 8 | 62-140 | 104.4±9.1 | abc | 5 | 0.12-7.63 | 1.88±1.3 |
| *B. nap* × *B. ole* AM111 | 1CA2524.004-A1228 | 2 | 2 | 89-117 | 103±9.9 | abcd | 2 | 0.05-1.92 | 0.99±0.66 |
| *B. nap* × *B. ole* AM111 | 1CA2524.005-A1228 | 14 | 13 | 55-169 | 120.4±7.7 | abcd | 7 | 0-3.17 | 0.78±0.41 |
| | **Total** | **43** | **42** | **39-169** | **107.9±4.7** | | **28** | **0.00-6.27** | **1.07±1.99** |
| *B. nap* × *B. ole* AM114 | 1CA2525.001-A1228 | 8 | 8 | 79-149 | 116.1±8.4 | abcd | 5 | 0-0.91 | 0.3±0.17 |
| *B. nap* × *B. ole* AM114 | 1CA2525.002-A1228 | 4 | 4 | 137-161 | 148.3±4.5 | cd | 1 | 0.09 | 0.09 |
| *B. nap* × *B. ole* AM114 | 1CA2525.003-A1228 | 303 | 302 | 32-199 | 114±2.1 | bc | 291 | 0-6.27 | 1.39±0.09 |
| *B. nap* × *B. ole* AM114 | 1CA2525.005-A1228 | 12 | 11 | 114-200 | 163.6±6.8 | d | 10 | 0-3.45 | 0.45±0.32 |
| | **Total** | **327** | **325** | **32-200** | **116.2±2.1** | | **307** | **0.00-6.27** | **1.34±0.08** |
| *B. nap* × *B. ole* AM094 | 1CA2526.001-A1228 | 4 | 4 | 54-94 | 71.8±7.8 | abc | 3 | 1.77-2.38 | 1.98±0.16 |
| *B. nap* × *B. ole* AM094 | 1CA2526.002-A1228 | 1 | 1 | 54 | 54 | abcd | 1 | 1.15 | 1.15 |
| | **Total** | **5** | **5** | **54-94** | **68.2±7.0** | | **4** | **1.15-2.38** | **1.77±0.22** |
| $BC_1$ ($F_1$ × *B. napus*): | | | | | | | | | |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.001-A6218 | 4 | 4 | 45-71 | 54.5±5 | ab | 4 | 0.44-2.09 | 0.98±0.34 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.002-A6218 | 7 | 6 | 65-161 | 108.7±15.9 | abcd | 6 | 0-1.26 | 0.49±0.2 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.003-A6218 | 12 | 11 | 57-174 | 119.4±12.2 | abcd | 9 | 0.02-3.69 | 1.37±0.45 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.004-A6218 | 13 | 13 | 49-135 | 81.9±7.0 | abc | 12 | 0.02-4.06 | 1.69±0.35 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.005-A6218 | 4 | 3 | 45-57 | 50.3±2.9 | ab | 3 | 0.02-1.39 | 0.75±0.33 |
| | **Total** | **40** | **37** | **45-174** | **91.9±6.5** | | **34** | **0.00-4.06** | **1.23±0.20** |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.001-A6218 | 14 | 14 | 43-160 | 83.5±8.2 | abc | 13 | 0.07-4.29 | 1.46±0.39 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.002-A6218 | 12 | 12 | 47-165 | 90.5±9.3 | abc | 12 | 0-8.62 | 1.16±0.67 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.003-A6218 | 4 | 4 | 49-116 | 71.3±13.4 | abc | 4 | 0.02-2.28 | 0.89±0.43 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.004-A6218 | 6 | 6 | 46-82 | 60.3±5.1 | a | 6 | 0.14-4.27 | 1.88±0.55 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.005-A6218 | 5 | 5 | 54-110 | 76.8±9.1 | abc | 4 | 0-4.00 | 1.5±0.8 |
| | **Total** | **41** | **41** | **43-165** | **80.1±4.6** | | **39** | **0.02-8.62** | **1.38±0.28** |

Note: *B. nap* = *B. napus* parent A04-73NA; *B. ole* = *B. oleracea* paren

[1] Comparison made between the different populations; same letter mean±S.E. are not significantly different ( to 0.05).

Supplementary Table 2.3 Seed quality traits of the $F_3$ and $BC_1F_2$ seeds produced, respectively on $F_2$ and $BC_1$ plants of *Brassica napus* × *B. oleracea* interspecific cross

| Cross | $F_2$ and $BC_1$ family | No. plants for NIR | Oil (%) | | Protein (%) | | Glucosinolate (μmol/g seed) | | No. plants for FA | Erucic acid (%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Range | Mean ±S.E. | Range | Mean ±S.E. | Range | Mean ±S.E. | | Range | Mean ±S.E. |
| $F_3$ seeds from $F_2$ plants: | | | | | | | | | | | |
| *B. nap* × *B. ole* AM111 | 1CA2524.001-A1228 | 2 | 39.8-40.9 | 40.3±0.4 | 27.1-28.1 | 27.6±0.4 | 36.6-39.2 | 37.9±0.9 | 2 | 7.5-22.9 | 15.2±5.4 |
| *B. nap* × *B. ole* AM111 | 1CA2524.002-A1228 | 5 | 38.4-41.3 | 40.0±0.5 | 26.7-30.8 | 29.2±0.6 | 35.5-55.0 | 43.0±3.1 | 11 | 0.2-21.4 | 10.3±1.9 |
| *B. nap* × *B. ole* AM111 | 1CA2524.003-A1228 | 2 | 40.1-47.8 | 44.0±2.7 | 22.1-29.7 | 25.9±2.7 | 9.3-31.9 | 20.6±8.0 | 5 | 6.6-16.7 | 12.1±3.5 |
| *B. nap* × *B. ole* AM111 | 1CA2524.004-A1228 | 1 | | 36.8 | | 30.7 | | 32.5 | 2 | 11.2-24.2 | 17.7±4.6 |
| *B. nap* × *B. ole* AM111 | 1CA2524.005-A1228 | 2 | 38.8-45.6 | 42.2±2.4 | 25.0-29.6 | 27.3±1.6 | 37.3-38.9 | 38.1±0.6 | 6 | 0.1-21.1 | 9.6±2.7 |
| *B. nap* × *B. ole* AM114 | 1CA2525.001-A1228 | 2 | 38.8-39.1 | 39.0±0.1 | 27.5-29.4 | 28.5±0.7 | 31.3-35.7 | 33.5±1.6 | 2 | 8.3-17.2 | 12.8±3.1 |
| *B. nap* × *B. ole* AM114 | 1CA2525.002-A1228 | 0 | | | | | | | 1 | | 4.4 |
| *B. nap* × *B. ole* AM114 | 1CA2525.003-A1228 | 183 | 33.3-44.1 | 38.5±0.1 | 24.5-32.5 | 28.9±0.1 | 12.6-66.3 | 37.5±0.8 | 262 | 0-32.6 | 13.6±0.4 |
| *B. nap* × *B. ole* AM114 | 1CA2525.005-A1228 | 2 | 34.7-38.7 | 36.7±1.4 | 27.7-29.3 | 28.5±0.6 | 40.2-46.1 | 43.2±2.1 | 7 | 0.5-22.7 | 13.5±2.7 |
| *B. nap* × *B. ole* AM094 | 1CA2526.001-A1228 | 3 | 34.3-39.7 | 37.0±1.3 | 30.1-31.6 | 30.7±0.4 | 21.7-52.4 | 33.2±7.9 | 3 | 9.4-23.9 | 18.5±3.7 |
| *B. nap* × *B. ole* AM094 | 1CA2526.002-A1228 | 1 | | 39.8 | | 29 | | 48.1 | 1 | | 19.5 |
| $BC_1F_2$ seeds from $BC_1$ plants: | | | | | | | | | | | |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.001-A6218 | 4 | 38.1-42.1 | 39.8±0.8 | 27.7-30.2 | 29.1±0.5 | 18.1-40.6 | 30.5±4.5 | 4 | 8.3-17.0 | 11.8±1.6 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.002-A6218 | 3 | 39.1-43.6 | 40.6±1.2 | 25.3-32.4 | 28.5±1.7 | 12.3-23.9 | 18.2±2.7 | 4 | 0.1-9.4 | 6.3±1.8 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.003-A6218 | 6 | 35.3-40.3 | 37.2±0.8 | 28.6-31.5 | 30.0±0.5 | 13.6-45.9 | 28.3±5.2 | 7 | 0.1-13.6 | 4.7±2.0 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.004-A6218 | 10 | 37.3-42.8 | 38.9±0.5 | 24.6-32.0 | 29.2±0.7 | 13.7-59.5 | 36.1±4.7 | 11 | 0.1-15.3 | 6.8±1.5 |
| (*B. nap* × *B. ole* AM111) × *B. nap* | 1CA2538.005-A6218 | 2 | 33.6-44.9 | 39.2±4.0 | 23.9-31.3 | 27.6±2.6 | 26.9-40.4 | 33.7±4.8 | 2 | 0.2-7.4 | 3.8±2.5 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.001-A6218 | 10 | 36.8-42.5 | 38.6±0.6 | 27.4-31.4 | 30.0±0.4 | 10.9-52.9 | 29.2±5.0 | 12 | 0.1-23.3 | 8.5±1.8 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.002-A6218 | 6 | 35.0-53.6 | 39.3±2.7 | 18.6-31.4 | 27.6±1.8 | 15.7-53.5 | 34.3±5.7 | 9 | 0.1-14.1 | 6.0±1.6 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.003-A6218 | 3 | 32.2-40.7 | 36.2±2.0 | 28.6-32.9 | 30.4±1.1 | 27.8-32.0 | 30.6±1.1 | 3 | 0.1-15.7 | 9.0±3.8 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.004-A6218 | 5 | 38.6-42.6 | 40.7±0.7 | 27.1-30.5 | 28.3±0.6 | 10.9-55.7 | 22.3±7.6 | 6 | 0.1-9.6 | 5.2±1.5 |
| (*B. nap* × *B. ole* AM114) × *B. nap* | 1CA2539.005-A6218 | 2 | 37.9-41.8 | 39.9±1.4 | 29.0-29.2 | 29.1±0.1 | 20.2-17.8 | 34.0±9.8 | 3 | 0.3-7.0 | 4.6±1.7 |
| *B. nap* (A04-73NA) | | 2 | 47.2-49.6 | 48.4±0.9 | 23.6-25.3 | 24.5 ±0.5 | 7.1-8.6 | 7.6 ±0.5 | 2 | 0.1-0.2 | 0.1 ±0.0 |

Note: *B. nap* = *B. napus* parent A04-73NA; *B. ole* = *B. oleracea* parent

Supplementary Table 3.1 List of 71 SSR markers amplifying *Brassica napus* A04-73NA and *B. oleracea* var. *capitata* cv. Bindsachsener alleles in $F_8$ plants of *B. napus* × *B. oleracea* var. *capitata* cv. Bindsachsener interspecific cross

| Primer No. | Name | Linkage group | Allele in $F_8$ plants | Primer Source | Forward primer | Reverse primer | Expected fragment size |
|---|---|---|---|---|---|---|---|
| 2278 | sN2087 | C1 | Nap | AAFC | CACGACGTTGTAAAACGAACC TCGAAAACGGTTGAA | CTCCCCCGATCTATACCCAT | 475 |
| 2279 | sN3734 | C1 | Nap | AAFC | CACGACGTTGTAAAACCCCCT TCCGGTTAAACAAAT | AAAACAGACTTTGCCCGTTG | 273 |
| 2286 | sN0691 | C1 | Nap | AAFC | CACGACGTTGTAAAACGCAA ATCTTGTTTTTGTGAGTACA | GTCTTGGAAGCAGCCTAACG | 375 |
| 2300 | sNRF$_9$4 | C1 | Nap | AAFC | CACGACGTTGTAAAACGATGA CTGTGCCTGCTAAACC | GCATCTCGATTCAATCCTCC | 310 |
| 2301 | sN3569F | C1 | Nap | AAFC | CACGACGTTGTAAAACTGTAC GTGCACCACGTTTTT | CTTCGATTACTCGGTGGCAT | 189 |
| 2310 | sN11675 | C1 | Nap | AAFC | CACGACGTTGTAAAACATATT GGGGGTCCTGGAGTC | TCCTTGCTTGAGCCTTTCAT | 263 |
| 4236 | *PBC*$_1$ | C1 | Nap | Farid et al. (2020) | AGTGGTTATCGGTATTGGATG G | CACAGACGAAGAATTGCTCAA C | 164 |
| 2297 | sN11657 | C1, C4 | Nap | AAFC | CACGACGTTGTAAAACCAGGT TGGTTTGACATGGTG | GCACACAGAGTGACGTTTGG | 248 |
| 624 | sN0758aN M | C1, C5, C7 | Nap | AAFC | CACGACGTTGTAAAACGACAT TCAGCGTCTGATGCAGTG | ATGGGGTAATGCACCAAAAA | 346 |
| 262 | sR10417 | C2 | Nap | AAFC | CACGACGTTGTAAAACGACCG GAGAAGAAACGAGCATTC | TAGGGTTTCTGACCCGATTG | 227-256 |
| 2059 | sNRE74 | C2 | Nap | AAFC | CACGACGTTGTAAAACGACCA ATCATGAATATCGGCAACA | CGTCATTCCAAACTTTAGGTCA | 158 |
| 2072 | sS2206 | C2 | Nap | AAFC | CACGACGTTGTAAAACGACTT TCATCATTTCGACTCACCC | TTATCTTCTCTCATTTCGCCG | 120 |
| 2222 | BnGMS633 | C2 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACCC AGTTCCATTCTCAATCAG | TATTTGTGTTCTCACGATGG | 342 |
| **2062** | **sN1825** | **C2, C3** | **Ole and Nap** | **AAFC** | **CACGACGTTGTAAAACGACC CACTGAGCGGTAGAGAAGG** | **CGGACTTTTACGGTGTTCGT** | **185** |
| 2065 | sS2268 | C2, C3 | Nap | AAFC | CACGACGTTGTAAAACGACCT TCTGCTCTGGCTGAAACA | TGATGTCTTCGCTGCTGTCT | 185 |
| 2075 | sORE66 | C2, C5, C8 | Nap | AAFC | CACGACGTTGTAAAACGACCG AGGTGGGAGAGATGAGAG | ATGGAACGCCAAAACAAAAA | 322 |
| 2063 | sN1937 | C2, C6 | Nap | AAFC | CACGACGTTGTAAAACGACCC CGCACTTTCTTCCTATTG | GGTGATGGTAACGAGCGATT | 281 |
| 435 | CB10036A | C3 | Nap | Piquemal et al. (2005) | CACGACGTTGTAAAACGACAT TCATCTCCTGCTCGCTTAG | AAACCCAAACCAAAGTAAGAA | 151 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 439 | CB10057 | C3 | Nap | Piquemal et al. (2005) | CACGACGTTGTAAAACGACCT AGGCTAAGGAAGATTGTCA | TAGTTTCTTCCTCCTGCTATC | 190 |
| 1082 | BoGMS0819 | C3 | Nap | Li et al. (2011) | CACGACGTTGTAAAACGACA GGGAGATGGACACATTTAG | GAGAGAGGGCAAAGAAGATAG | 114 |
| 4105 | PbC3.1 | C3 | Nap | Farid et al. (2020) | CACGACGTTGTAAAACGACAT GATAGGAGCCGGTCACAG | ACACGCAGCAGCAGTTAAGA | 198 |
| 302 | sS2277 | C4 | Nap | AAFC | CACGACGTTGTAAAACGACG ATCTGCGGTAGGAATCGAA | CGTGCTACATAATAGGGAAAA ACC | 219-225 |
| 731 | CB10109B | C4 | Nap | Piquemal et al. (2005) | CACGACGTTGTAAAACGACGT GTAGCCAGCTTGATCCT | CTTCTTCTGATGCAGCAGTG | 281 |
| 982 | MR140 | C4 | Nap | BBSRC | CACGACGTTGTAAAACGACCC CATATTCTAATCGTTCCA | TTCACTCATTCTTTGCTCATT | 143 |
| 990 | BRAS061 | C4 | Nap | Piquemal et al. (2005) | CACGACGTTGTAAAACGACGC AGCCTTCAACTCCCATAGA | TGGGTTCGAGCAGGGTTC | 210 |
| 994 | CB10493 | C4 | Nap | Piquemal et al. (2005) | CACGACGTTGTAAAACGACTG ACGTGTGAGCAACAGA | CTGAGTCACAAGCCGAGT | 222 |
| 1085 | BoGMS0767 | C4 | Nap | Li et al. (2011) | CACGACGTTGTAAAACGACA AACAAGTCAGATTCACCAAA | CTCTTCACCACTACCACAGTC | 114 |
| 2099 | sR0357 | C4 | Nap | AAFC | CACGACGTTGTAAAACGACCC GGCTCTTGTTTTATGGTT | AACACCGTTTCATCTTTGGC | 376 |
| 2113 | sN3685R | C4 | Nap | AAFC | CACGACGTTGTAAAACGACCC GCAAGCTCTTAACTCCAC | AACTGCATTCGTCCAGCTCT | 285 |
| 2115 | sN3817 | C4 | Nap | AAFC | CACGACGTTGTAAAACGACCC TGCCGTAACGTTCTTGTT | ATCTTCGAAGCAATCTCGGA | 169 |
| 2200 | BnGMS347 | C4 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACTC ACACAAATCTCCTCCTCT | AGGTATCAGCCAATGACTTC | 273 |
| 2225 | BnGMS681 | C4 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACGT CGAAGATTGTTGTCAGGT | TTCACGAAGAACCCTAGAAA | 131 |
| 2379 | sN12743J | C4, C6, C8, C9 | Nap | AAFC | CACGACGTTGTAAAACGACCT AGCCACCATGAAAGGAGC | AAACCAAGCAAACCCATCAG | 370 |
| 2102 | sNRG34 | C4, C9 | Nap | AAFC | CACGACGTTGTAAAACGACTC TCATTTTTCCTCAAGCTCC | CCACCAGCCATAGTCATCCT | 294 |
| 616 | sORA84a | C5 | Nap | AAFC | CACGACGTTGTAAAACGACCA AGAAACACCATCATTTCTCAA | GGCCCATTGATATGGAGATG | 178 |
| 621 | sN2052*n*P | C5 | Nap | AAFC | CACGACGTTGTAAAACGACGC TCCCAAGAGCAACAC | TCACAGTTGATCCCTGTTAAT | 417 |
| 721 | sN0761a | C5 | Nap | AAFC | CACGACGTTGTAAAACGACCG GAATTAGTGGAGTGGGAA | TATCACTGTTGTCTGCCCCA | 298 |
| 1056 | BoGMS0590 | C5 | Nap | Li et al. (2011) | CACGACGTTGTAAAACGACTG GTTTATCTTCATTCTTTGG | TATTGAGTTGTCGCACTTGA | 399 |
| 2445 | sORB17 | C5 | Nap | AAFC | CACGACGTTGTAAAACGACAC CATTGAGGTTTGTCGGAG | AAAGCTTCGGCAATAATGGA | 414 |

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|---|---|---|---|---|---|---|---|
| 2448 | sN12153I | C5 | Nap | AAFC | CACGACGTTGTAAAACGACCTCTCCCTTGGCTCTTCTT | CTGAGGAGAGGGTTTAGCGG | 181 |
| 2453 | sN12503 | C5 | Nap | AAFC | CACGACGTTGTAAAACGACACGGAGGAACAGAGGAGAG | TCCCACTGGCCATAGTTAGG | 290 |
| 607 | sN7410a | C5, C8 | Nap | AAFC | CACGACGTTGTAAAACGACAGATGGGAAGAGCAAAAGC | ATGCCCTGGAGTCAATGTTC | 155 |
| 733 | CB10211 | C6 | Nap | Piquemal et al. (2005) | CACGACGTTGTAAAACGACAGCAGAGATCGATGGAG | ATAGAAGGCTGCCCCTC | 150 |
| 756 | Na10-C06 | C6 | Nap | BBRC | CACGACGTTGTAAAACGACTGGATGAAAGCATCAACGAG | ATCAATCAACACAAGCTGCG | 223 |
| 991 | BRMS-015 | C6 | Nap | Suwabe et al. (2002) | CACGACGTTGTAAAACGACTCGCCAATAGAACCCAAAACTT | CATCTCCATTGCTGCATCTGCT | 263 |
| 2213 | BnGMS491 | C6 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACAAGTGTGTATTAGGGACGAGT | TCCCGTACTTCAAGCTGTAT | 161 |
| 2365 | sN11904 | C6 | Nap | AAFC | CACGACGTTGTAAAACCAATGGATCGGATGGAGATT | GTCTTGTCTTCATGGTCGGG | 239 |
| 2366 | sN3815 | C6 | Nap | AAFC | CACGACGTTGTAAAACTTCAAGCTATGCAGTGTGGC | GGTCTGGAAATCGCTGCTT | 482 |
| **2380** | **sN9539** | **C6** | **Ole** | **AAFC** | **CACGACGTTGTAAAACGACCTCGTAAACTGGCAAGCCTC** | **AAGTTTTGGGCTGACCATGA** | **168** |
| 4237 | *PbC6.2* | C6 | Nap | Farid et al. (2020) | CCTAATCCTAAGTCGGCCAAG | AACCAAAACCAGAAGAGAAGGC | 241 |
| 2122 | sNRD41 | C7 | Nap | AAFC | CACGACGTTGTAAAACGACAAAGGGCGGTCTAGCATCTT | CGTCAATGCTCAAATCCCTT | 241 |
| 2205 | BnGMS386 | C7 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACTTGGCTCATCAATGACAATA | ACAATGTGGTAAACACGAAA | 220 |
| 2242 | BoGMS1065 | C7 | Nap | Li et al. (2011) | CACGACGTTGTAAAACGACGGGTTGATTGGGAAGTGT | CTTAGCACCATTTGTTTGTATT | 209 |
| 2391 | sN2564 | C7 | Nap | AAFC | CACGACGTTGTAAAACGACGAATTCCTTCTGGGCTTTCC | CTAAATGAGGATGGGAGCGA | 348 |
| 2393 | sORF37 | C7 | Nap | AAFC | CACGACGTTGTAAAACGACGAAGGCTCAACAAAAATGGG | AAGCCCAAAGGTAAGGAAGG | 100 |
| 2410 | sN1975 | C7 | Nap | AAFC | CACGACGTTGTAAAACGACTCCCTTGCCTTCTCTTCTTG | TCGGCCAAGCATCTCTAACT | 140 |
| 2420 | sNRD41 | C7 | Nap | AAFC | CACGACGTTGTAAAACGACAAAGGGCGGTCTAGCATCTT | CGTCAATGCTCAAATCCCTT | 241 |
| 2428 | sN3825J | C7 | Nap | AAFC | CACGACGTTGTAAAACGACCTGCGTCGTCGAAGTTCATA | TCTCCTTGAAAAACACAGCG | 307 |
| 2431 | sN0706 | C7 | Nap | AAFC | CACGACGTTGTAAAACGACTCCGACGGTCAAGATTAAGG | GGCTGTGGTGGATCTAGGAA | 401 |
| 3519 | PbC7.2 | C7 | Nap | Parkin et al. (2014) | GATAACAATGCCAAGCCGACC | TCAATGCGTATTGTCGTCGT | 4854 |

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|---|---|---|---|---|---|---|---|
| 4350 | PbC7.2 | C7 | Nap | Farid et al. (2020) | CTAAATTCCCAACACTAACCA | ACGAGGAAATTACGACGATA | 167 |
| 240 | sN2557 | C8 | Nap | AAFC | CACGACGTTGTAAAACGACGC ATCACTCTAGGGTTTCCG | CAAAGCAACCGACAAGAACA | 456 |
| 992 | CB10028 | C8 | Nap | Piquemal et al. (2005) | CACGACGTTGTAAAACGACCT GCACATTTGAAATTGGTC | AAATCAACGCTTACCCACT | 199 |
| 2087 | sN11670 | C8 | Nap | AAFC | CACGACGTTGTAAAACGACA GTCGGGCTCGTATATCTCG | GTTTCGTGGCGGAAATTAGA | 100 |
| 2179 | BnGMS3 | C8 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACA AAGAGCCCACATGAAAGTA | TGAACTAGGCACCAAGAACT | 359 |
| 2246 | BoGMS0741 | C8 | Nap | Li et al. (2011) | CACGACGTTGTAAAACGACCT CAAACTCCGTCGCTCT | TCCTCCTCACTACTTTCTTCA | 286 |
| 2248 | BoGMS0868 | C8 | Nap | Li et al. (2011) | CACGACGTTGTAAAACGACA AATCCCAACGAGATAGGTAG | AGAAAGAAAGGAAGAAAGTGG | 226 |
| 751 | FITO095 | C9 | Nap | Iniguez-Luy et al. (2008) | CACGACGTTGTAAAACGACA GATTTCATCCACAGCCTC | TTTGATTCTTGCGTTCTCTC | 233 |
| 2193 | BnGMS213 | C9 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACGT AGTACGGAGATGCGTGAT | AAAGAACGAGTTGACTTTCG | 134 |
| 2204 | BnGMS385 | C9 | Nap | Cheng et al. (2009) | CACGACGTTGTAAAACGACTT TCATGACTTAGCCACCTT | CCAAGTATTCAATTTCTGGC | 197 |
| 3040 | 3040 | C9 | Nap | Li et al. (2011) | CACGACGTTGTAAAACGACTC AAACTTTTGACTTTGAATATCCC | AAACAATTTTCAAGTTTTGGTCA | 239 |

Note: Nap = *B. napus*; Ole = *B. oleracea*; AAFC = Agriculture and Agri-Food Canada