Use of Rutabaga (*Brassica napus* var. *napobrassica*) for the Improvement of Canadian Spring Canola (*Brassica napus*)

By:

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Abstract

Spring-type oilseed *Brassica napus* L., commonly known as canola, has become the cornerstone of agricultural production in Western Canada, with the total acreage seeded increasing in each production year over the past two decades. However, the narrow genetic base of spring *B. napus* canola coupled with the ever-increasing acres planted have led to the emergence of clubroot disease, caused by *Plasmodiophora brassicae*, in the canola production areas. *Brassica napus* var. *napobrassica,* or rutabaga, is a biennial fodder-type *Brassica* species that has the potential to not only serve as a source of genetic diversity for *B. napus*, but also to provide strong resistance to *P. brassicae* pathotypes prevalent in the canola fields in Western Canada. An F_2-derived population of Rutabaga-BF × A07-26NR and a three-way cross-derived population of (A07-45NR × Rutabaga-BF) × A07-26NR were evaluated for different agronomic and seed quality traits, including resistance to *P. brassicae* pathotypes prevalent in Western Canada.

The three-way cross and F_2-derived populations both produced families that exceeded the checks for agronomic and seed quality traits for both the 2013 and 2014 yield trial experiments. The three-way cross-derived population produced several families with stable, non-segregating resistance to *P. brassicae* pathotype 3, as well as newly emerging pathotypes found in northern Alberta. Genetic diversity analysis showed that both the three-way cross and F_2-derived populations produced families of canola-quality *B. napus* plants with spring growth habit that were genetically similar to the parent Rutabaga-BF, indicating that rutabaga is a viable germplasm source for broadening the narrow genetic base of spring-type *B. napus*.

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Chapter 1: Literature Review

1.0 Introduction

Brassica napus, commonly known as rapeseed, is an important oilseed crop in agricultural production, with production increasing worldwide. Towards the end of the 20th century, a Canadian derivation of rapeseed with improved seed oil and meal quality known as canola was developed and released for commercial production. The development of this derivation has led to increased production acres, specifically in Canada but also on the world scale. Its high oil content and desirable fatty acid profile and the use of its meal as a source of protein for animal feed have made this crop profitable for producers. Specifically, canola is a type of *B. napus* containing less than 2% erucic acid in its seed oil and less than 30 μmoles of total aliphatic glucosinolate per gram of seed meal (Canola Council of Canada 2012).

Breeding advances have led to improvements in many agronomic, seed quality and disease resistance traits in *B. napus*. However, the intensive breeding within a restricted gene pool has also narrowed the genetic diversity in this crop species. Intensive breeding coupled with intensive cultivation have led to increased disease pressures on this crop. In Western Canada, the pathogen *Plasmodiophora brassicae,* which causes clubroot disease, has become a significant threat to canola production. First identified near St. Albert, Alberta in 2002 (Tewari et al. 2005), this pathogen has spread throughout most of Alberta, with confirmed cases identified in Saskatchewan (Dokken-Bouchard 2011), Manitoba (Canola Council of Canada 2011) and North Dakota (Markell, Lubenow and Beneda 2014). As a result, in addition to broadening genetic diversity for agronomic and other plant traits, *B. napus* breeding programs have focused on

improved resistance to diseases, namely blackleg, sclerotinia and more recently, clubroot. A primary gene pool source for broadening genetic diversity in *Brassica napus*, along with stable and durable resistance to clubroot, can be found in rutabaga, *Brassica napus* var. *napobrassica*, a fodder-type brassica species (reviewed in Rahman et al. 2014). This project will investigate whether *B. napus* var. *napobrassica* can be used as a source of germplasm for broadening genetic diversity in canola and introgression of clubroot resistance into this crop.

1.1 Brassica napus

1.1.1 Origin of *Brassica napus*

Brassica napus is one of 51 genera in the family *Brassicaceae*. Among the different *Brassica* species, *B. napus* is the most extensively cultivated (reviewed in Rakow 2004). Records of cultivation of Brassica crops, particularly *Brassica rapa*, go as far back as 1500 BC in India, 1100 BC in China and the middle ages in Europe (reviewed in Hayword (2012).

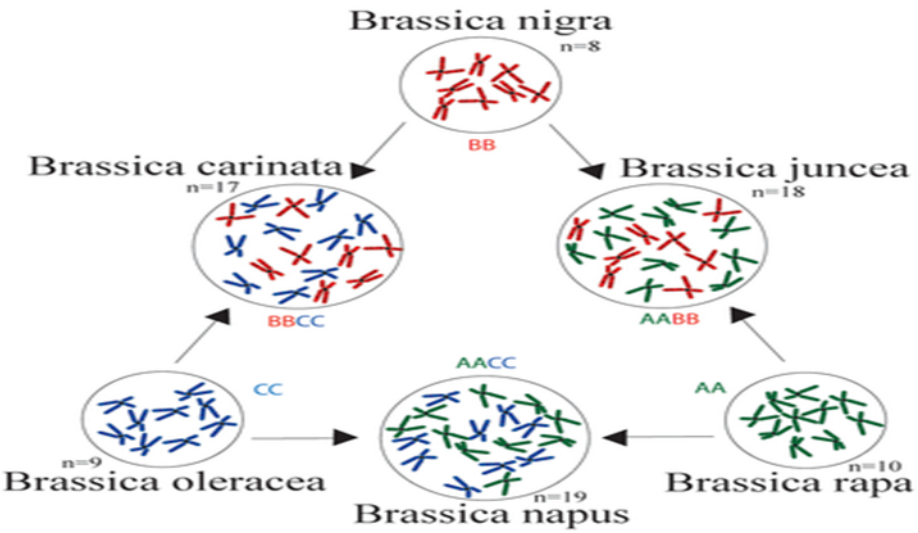

Figure 1.1. Triangle of U describing the relationship among *Brassica* species. Adapted from Nagahuru U (1935)

B. napus is an amphiploid species (AACC, 2n = 38) carrying the A and C genomes. This species originated from the diploid species *Brassica rapa (*AA, 2n = 20) and *Brassica oleracea* (CC, 2n = 18) through interspecific hybridization. Evolution likely occurred in the Mediterranean, where its two progenitor species overlapped (Prakash et al. 2012). U (1935) first hypothesized that *B. napus* is derived from the interspecific cross *B. rapa* × *B. oleracea* and described the relationships among six *Brassica* species in the form of a triangle, which is commonly known as the "U Triangle". Molecular marker analysis in the late 20th century confirmed that *B. napus* was generated from a cross between *B. rapa*, the donor of the A genome and *B. oleracea,* the donor of the C genome. Whether *B. oleracea* or *B. rapa* served as the maternal parent during the evolution of *B. napus* is not clear; however, evidence supports that the parent was *B. oleracea* (Allender and King 2010). Evidence suggests that several hybridization and/or domestication events occurred in several geographic areas rather than a single crossing event between the A and C genome species at a single location (Song and Osborn 1992, Song et al. 1988).

The *Brassica* genus is complex, as it includes a large number of species and abundant genetic variation within the genus. Wild-type *B. napus* does not exist. Although there is evidence of cultivation of *Brassica* species dating back to 2000 BC in both Asia and the Mediterranean, *B. napus* did not come into large-scale agricultural production until the 16th century, when it was produced in the form of oilseed and root-forming rutabaga types for use as food and fodder (Prakash et Al. 2012, Warwick 2011). The genetic diversity in this species remains narrow compared to crop species in cultivation for thousands of years (reviewed in Bonnema 2012).

Originally, *B. napus* seeds were used to produce lamp oil or, if necessary, edible oil in poorer areas (Gupta and Pratap 2007). Since the erucic acid and glucosinolate contents,

respectively, from the seed oil and meal were reduced with the development of canola-quality *B. napus*, this crop has become a quality source of oil for human consumption, with the meal providing a source of protein for a range of animal livestock and aquaculture species. Canola meal is also used as an organic fertilizer in some Asian countries (Bonnardeau 2007). Production of biodiesel is another end use of this oil. Biodiesel, which is produced through a trans-esterification process, can be blended with petroleum diesel. Biodiesel burns more cleanly and degrades more quickly than petroleum diesel (for details, see, Canola Council of Canada 2011, http://www.canolacouncil.org/canola-biodiesel/canola-biodiesel/biodiesel-basics/).

The ability of *Brassica* oilseed plants to germinate, grow and thrive in cool temperatures allows them to be successfully cultivated across most temperate regions worldwide. Compared to most cereals, the fertilization needs of *B. napus* are significantly greater, requiring significant amounts of nitrogen, phosphorous, potassium and sulfur, as well as several micronutrients. The nutritional requirements of canola are reviewed in detail by Grant and Bailey (1993).

B. napus can be divided into two separate types: oil yielding, which is divided into spring and winter growth habit types, and the tuber-forming type rutabaga, or *Brassica napus* var. *napobrassica*, which is most commonly used as a fodder crop (Canadian Food Inspection Agency 2012).

1.1.2. Worldwide Production of *Brassica napus*

Table 1.1. World production of major oilseeds crops. (Table adapted from FAO Food Outlook 2012. www.fao.org)

Oilseed	2011/2012 Production	2012/2013 Estimation	2013/2014 Forecast
Soybean	240.0	267.0	281.6
Rapeseed	61.7	64.1	67.6
Cottonseed	47.2	45.2	43.7
Groundnuts (unshelled)	37.2	38.3	39.6
Sunflower Seed	39.0	36.1	38.7
Palm Kernels	13.3	13.9	14.4
Copra	5.3	5.35.5	5.5
Total	443.8	470.1	491.1

Since the turn of the century, canola production has reached over 60 million metric tons per year (Table 1.1). Among oilseed crops, canola is second only to soybean in terms of total production, contributing approximately 13% of the world vegetable oil supply (USDA 2014a, Rahman et al. 2013). Production leaders include the European Union, followed by Canada, China and Australia (Table 1.2).

Table 1.2. Top 10 rapeseed-producing countries based on production in the 2013 growing season. Table courtesy of USDA (2014)

Country	Production (1,000 MT)
EU-27	20,850
Canada	18,000
China	14,200
India	7,000
Australia	3,400
Ukraine	2,350
Russia	1,400
United States of America	1,004
Belarus	700
Pakistan	320

1.1.3 *Brassica napus* Production in Canada

Prior to the Second World War, *Brassica* oilseed production in Canada was restricted to research plots. During the war, with the blockades of Europe and Asia, Canada, faced with a shortage of vegetable oil, needed to increase domestic production to supply the war effort. Following the end of the war, the acreage of Brassica oilseed crops (*Brassica napus, Brassica rapa*) and research on these crops increased to meet the growing demand.

In Canada, intensive research has improved the quality of *Brassica* seed oil and meal. Specifically, the contents of erucic acid from seed oil and glucosinolates from seed meal have been reduced (Canadian Food Inspection Agency 2012; http://www.inspection.gc.ca/plants/plants-with-novel-traits/applicants/directive-94-08/biology-documents/brassica-napus-l-/eng/1330729090093/1330729278970#A5:). This effort has differentiated Canadian production from typical rapeseed production in other parts of the world. The first canola-quality cultivar was developed and released by Agriculture and Agri-Food Canada and the University of Manitoba in the early 1970s. The first canola-crushing plant was established in Canada soon after (Statistics Canada 2012).

In Canada, canola production per year has surpassed 7,500 hectares, with yields averaging 1,900 kg/ha (Statistics Canada 2012). Due to its high profit margin compared to other crops, farmers have shortened their crop rotations to grow canola more frequently, enabling them to take advantage of increasing world demand for vegetable oil. Farmers commonly incorporate a 1 in 2 crop rotation instead of the recommended 1 in 4 crop rotations, which would help keep disease pressures at a low level (Hartman 2010). As a result, pressures from all pathogens and pests of *B. napus* have increased, including *P. brassicae*.

1.1.4 Canola Production in Western Canada

Table 1.3. Major field crop production in Canada (thousand tons) during 2011–2013. Table adapted from Statistics Canada 2013 (www.statcan.gc.ca/daily-quotidien/131204/t131204b001-eng.htm)

Crop	2011	2012	2013
Wheat (Total)	25,288	27,205	37,530
Canola	14,608	13,869	17,960
Grain Corn	11,359	13,060	14,194
Barley	7,892	8,012	10,237
Soybeans	4,298	5,086	5,198
Oats	3,158	2,812	3,888
Field Peas	2,502	3,341	3,849
Lentils	1,574	1,538	1,881
Flax	399	489	712

Despite only being in significant production since the 1970s, canola is second only to combined spring, durum and winter wheat crops in terms of total production. Record numbers of acres have been sown in each of the past several years, with total production increasing each year (Statistics Canada 2013).

Canola-quality *B. napus* is one of several oilseed crops grown in Western Canada; others include flax (*Linum usitatissimum*), mustard (*Brassica juncea*), safflower (*Carthamus tinctorius*), soybean (*Glycine max*) and sunflower (*Helianthus annuus*) (Canadian Grain Commission 2013). Although other oilseed crops have strong niche markets, large-scale production of the majority of these crops is limited, and they are confined to specific areas of Canada due to climate, soil

and/or precipitation requirements. *B. napus* is the only oilseed crop that thrives across all growing areas of Western Canada.

Table 1.4. Canadian oilseed production in 2012–2013. Adapted from Canada: Outlook for Principal Field Crops (2013-12-20). Agriculture and Agri-Food Canada retrieved from http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/by-product-sector/crops/crops-market-information-canadian-industry/canada-outlook-for-principal-field-crops/canada-outlook-for-principal-field-crops-2013-12-20/?id=1387814218931#a3

Crop	Canola	Flaxseed	Soybean	Total Oilseed Production	Percentage of Oilseed Production for Canola
Area Seeded (kha)	8,912	397	1,680	10,989	81%
Area harvested (kha)	8,799	384	1,678	10,861	81%
Yield (t/ha)	1.58	1.27	3.03	1.79	88%
Production (kt)	13,869	489	5086	19,444	71%

Intensive production of *B. napus* in Western Canada has exacerbated disease pressures, which continue to reduce resistance in developed canola cultivars. Several diseases, such as sclerotinia stem rot caused by *Sclerotinia sclerotiorum*, blackleg caused by *Leptosphaeria maculans*, root rot caused by *Pythium* sp. and several other fungal diseases must be monitored and managed during the cropping year. Through sound agronomic practices and chemical/cultural controls, it is possible to control the majority of these pathogens year to year (Kharbanda and Tewari 1996).

Open-pollinated *B. napus* cultivars dominated the western Canadian canola acres until the end of the 20th century. With hybrid production becoming more commonplace and almost completely replacing open-pollinated cultivar production in the beginning of the 21st century (Canadian Canola Council 2010), the narrow genetic base of *B. napus* has been further compromised due to the constraints of developing inbred parental lines suitable for hybrid production.

Worldwide, consumer demand keeps pace with increasing production. Major canola oil importers include Japan, USA and China. Major importers of canola meal include the USA, the European Union and Vietnam (Canadian Canola Council 2013).

1.2. Genetic Diversity of Brassica napus

B. napus belongs to the family *Brassicaceae*, consisting of approximately 350 genera and 3,500 species. A wide range of morphological types exists within this species (Rich 1991). There is considerable diversity within the family; however, genetic diversity within *B. napus* canola is considerably narrow (reviewed by Rahman 2013). *B. napus* is treated as an inbreeding species, although approximately 21% outcrossing can occur under field conditions; the exact amount of cross-pollination in this crop depends on varying environmental factors (Cuthbert and McVetty 2001).

Genetic diversity in *B. napus* has been studied in some detail, and the germplasm has been placed into distinct groups, including spring oilseed and fodder, winter oilseed, winter fodder and vegetable genotypes (Hasan et al. 2006). These four distinct gene pools most likely resulted from domestication and breeding within different geographic areas. Of these, the spring-type oilseed *B. napus* has the least genetic diversity, as identified by Hasan et al. (2005), followed by the winter type. The narrow genetic diversity within the spring canola gene pool places a major constraint on the development of competitive commercial hybrid cultivars and the continued improvement of this crop (reviewed in Rahman 2013). Winter canola, primarily grown in Europe, has the same restrictions as spring canola, where development of a distinct heterotic pool for the development of hybrid cultivars has become limited due to the lack of genetic diversity in adapted germplasm (Gehringer et al. 2007). Contributing to the lack of genetic

diversity is the complete and total lack of wild-type *B. napus* present today. However, due to the diversity that exists in the *Brassica* family, various *Brassica* species represent a good source of genetic material for expanding the genetic diversity of *B. napus*. Introgression of both A and C genome components from allied *Brassica* species into *B. napus* via interspecific hybridization has proven successful in producing new and genetically distinct *B. napus* lines (Bennett et al. 2012). Genetic differentiation based on geographic location has the potential to be exploited in the breeding of spring canola to increase genetic diversity in this crop. For example, European *B. napus* canola is known to be genetically distinct from Chinese semi-winter and spring-type *B. napus* (Hu et al. 2007, reviewed in Rahman 2013). Some efforts have already been made to use these distinct gene pools in breeding winter and spring canola (Li et al. 2012, Kebede et al. 2010). However, little research has been conducted investigating the use of rutabaga (*Brassica napus* var. *napobrassica*) in breeding spring canola. Of all the *B. napus* variants, rutabaga is the most similar to winter-type *B. napus,* requiring vernalization to induce flowering. Rutabaga is genetically distinct from spring-type *B. napus* (Diers and Osborn 1994, Bus et al. 2011) and can be used as a source of new and variable germplasm for the improvement of spring *B. napus* canola. According to Soengas et al. (2006), some rutabaga germplasm appears to be more closely related to forage rape; however, this is not generally the case. Further research by Soengas et al. (2008) confirmed that although they may share an evolutionary past with forage rape, oilseed rape and rutabaga do not share a common evolutionary line and thus, rutabaga and canola/rapeseed are genetically distinct from each other.

Broadening the genetic diversity in canola germplasm can help prevent the breakdown of resistance to diseases and can increase the agronomic performance of this crop. According to Cowling (2007), the loss of genetic diversity in Australian spring canola lines has resulted in the

loss of resistance to the disease caused by *Leptosphaeria maculans*, commonly known as blackleg. Extensive breeding efforts in Canada have led to a decline in agronomic performance of Canadian spring-type *B. napus* (Fu and Gugel 2009). Therefore, efforts must focus on increasing genetic diversity in Canadian spring *B. napus* canola germplasm (reviewed in Rahman 2013).

It is possible to introgress genetic diversity and specific traits into *B. napus* canola from its progenitor species, *B. oleracea* and *B. rapa* (Bennett et al. 2012). Evaluation of *B. oleracea* germplasm has revealed significant sources of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) resistance in the C genome; however, introgression of resistance from *B. oleracea* into *B. napus* may delay flowering time due to the negative association between the two traits (Mei et al. 2012). Favorable traits and alleles can successfully be introgressed from this type of unadapted germplasm into adapted *B. napus* germplasm, as demonstrated by Udall et al. (2004).

1.3 Rutabaga

Despite the challenges in achieving standard agronomic and seed quality traits in canola, rutabaga shows potential for increasing both the genetic diversity and disease resistance of this crop. Significant variation in agronomic traits has been found in advanced generation populations of a *B. napus* × rutabaga cross, indicating that canola-quality lines are likely to be found in the segregating populations (Rahman et al. 2014). Resistance genes from stubble turnips of *B. rapa* origin are the most effective and widely used genes in clubroot resistance breeding of various *Brassica* crops (Diedrichsen et al. 2009). Rutabaga carries resistance to several clubroot pathotypes found in Canada (Hasan et al. 2012), and breeding efforts at the University of Alberta are focused on introgressing this resistance into elite canola cultivars (Rahman et al. 2014).

Currently, most clubroot-resistant *B. napus* cultivars only show resistance to pathotypes endemic to the areas of their release. *B. napus* var. *napobrassica* provides a source of genetic diversity, and it also provides a novel source of genes for *P. brassicae* resistance to multiple pathotypes for incorporation into breeding programs (Lüders et al. 2011)

1.4 Clubroot

Clubroot is a disease caused by the soil-borne obligate parasite *Plasmodiophora brassicae*. This protist belongs to the supergroup Rhizaria within the class Phytomyxea (Hwang et al. 2012). Although an obligate parasite, *P. brassicae* has the ability to persist in the soil profile via long-lived resting spores (Hartman et al. 2011). These spores have a half-life of four years and can last upwards of 19 years and remain viable in soil lacking a suitable host (Rastas et al. 2012). *P. brassicae* does not have airborne-specific spores, but its resting spores can be transported by wind, water erosion, field machinery and living organisms. The pathogen prefers wet acidic soils with soil temperatures upwards of 20°C with poor drainage, namely low-lying areas of fields, fields tending to be heavy clay in composition and acidic in nature, or those with a pH less than 6.5 (Hartman 2011). Cultural controls, such as adjusting soil pH via the use of soil amendments (Hwang et al. 2011), have had minimal success in controlling the pathogen on a large scale. Anecdotal evidence suggests that application of Boron nutrient to both mineral and organic fields can be successful, but when applied on a trial-wide scale, excess Boron did not successfully suppress *P. brassicae*, leading to only varying degrees of phytotoxicity in some *Brassica* plants (Deora et al. 2014). Therefore, efforts have focused on finding resistance genes and incorporating them into existing or new cultivars (Some et al. 1996).

Clubroot has been documented in vegetable brassicas since the Middle ages in Europe, and anecdotal evidence traces this disease to ancient Rome. *P. brassicae* in Russian cabbage production was first reported in 1869, soon followed in Great Britain, and notable losses were reported in the United States by 1893 (DeWolfe 1962). *P. brassicae* most likely spread to North America via fodder used for livestock feed, and it most likely spread to China and Japan during archeological times. *P. brassicae* has now been confirmed on every continent in which *Brassica* crop production occurs (Dixon 2009). The first confirmed infection of agricultural fields near St. Albert occurred in 2002, and infection was confirmed to be widespread across northern Alberta within the next several years (Howard et al. 2010). This disease has since spread across Alberta and into parts of Saskatchewan (Tewari et al. 2005, Dokken-Bouchard 2011). Most important to western Canadian agriculture, clubroot disease has a significant impact on crop yield; there is a distinct relationship between disease incidence and disease severity, as well as between yield and disease/soil infection (Wallenhammar 1999).

Populations of *P. brassicae* consist of several different pathotypes. At least 3–4 pathotypes can be found in canola fields in Alberta; however, certain pathotypes can become more prevalent in the population compared to others. Therefore, the rare pathotypes must also be taken into account. Rare pathotypes can quickly establish dominance if susceptible *B. napus* crops are continually grown (Xue et al. 2008). Typically, two pathotypes, designated pathotype 3 and 5 as per Williams' classification (Strelkov et al. 2008), are present in canola fields in Alberta. Recently, hybrid cultivars with clubroot resistance have been released in Western Canada. Growing of resistant cultivars will have to be managed carefully, as local populations of *P. brassicae* are diverse and virulence patterns can shift swiftly when faced with selection pressure (Strelkov et al. 2011). *P. brassicae* is a genetically diverse pathogen, in contrast to the

lack of genetic diversity present in *B. napus*. Pathotypes are discrete and specific to their area of origin due to limited gene flow, their slow method of dispersal and selection pressures specific to a localized area (Strehlow et al. 2013). Most *B. napus* accessions show complete susceptibility to pathotype 3, and moderate resistance can be found in a small number of accessions (Hasan et al. 2012, Peng et al. 2013).

P. brassicae shows extensive genetic variation in the field, with numerous pathotypes showing adaptation to multiple growing areas and different pathotypes exhibiting varying degrees of virulence depending on the host plant (Manzanares-Dauleux et al. 2001). In Western Canada, established pathotypes 3 and 5 both show a high degree of virulence, most likely because they were specific to *B. napus* from the start (Strelkov et al. 2006). Pathotype 3 is still the predominant *P. brassicae* pathotype found specifically in Alberta, with 2, 5, 6 and 8 also present in various areas across the Canadian Prairies (Hwang et al. 2012a). Rutabaga carries resistance to these pathotypes and can be used in breeding clubroot-resistant spring-type *B. napus* cultivars for Western Canada.

1.4.1 Clubroot Life Cycle

The life cycle of *P. brassicae* is divided into three stages; soil survival, root hair infection and cortical infection. This pathogen is capable of infecting all brassica species, including weed populations in Western Canada, allowing spores to propagate in the soil even in years canola is out of rotation. *P. brassicae* overwinters as resting spores before germinating into zoospores in the spring. If suitable hosts are not present, the resting spores will remain dormant until the next growing season and can remain viable for at least five years without a host in the soil (Kageyama and Asano 2009).

Haploid zoospores travel via soil water until they come in contact with root hairs of a host plant and penetrate the root wall, forming primary plasmodia in the root cell cytoplasm. These plasmodium develop into zoosporangia, containing 4–16 zoospores. Zoospores are released into the soil or neighboring root cortical cells. Secondary zoospores infect the root tissues, providing consistent secondary infection throughout the growing season. Secondary infection leads to the formation of secondary plasmodium, which cause the characteristic galls prominently displayed on the root tissue. Without secondary infection, symptoms or yield/quality losses are relatively rare (Howard et al. 2010). Resting zoospores that become active later in the growing season provide a source of secondary inoculum, increasing the infection rate and severity (Feng et al. 2013). Galls deteriorate rapidly in the soil towards the end of the growing season, releasing resting spores in the soil, which can persist upwards of 18 years (Wallenhammar 1996, reviewed in Ingram and Tommerup 1972, McDonald et al. 2014).

Certain non-host plants such as perennial ryegrass (*Lolium perenne*) can promote resting spore germination; however, such germination is atypical outside of a controlled environment (McDonald et al. 2014). Early studies showed that clubroot disease developed on cabbage at temperatures ranging from 9ºC to 30ºC, although the optimum temperature was later found to be 23ºC. Increased soil moisture is also beneficial for pathogen development (reviewed in Gossen et al. 2014). The acidic nature of decaying and high organic matter soils promotes *P. brassicae* development; however, this is not the case in the absence of primary hosts (Friberg 2005).

1.4.2 Clubroot Symptoms

Several symptoms become prominent over the course of the growing season. Aboveground wilting is a prominent symptom; plants become heat stressed during the day, only to recover at night (Grabowski 2010). Symptoms are exacerbated in warm climates; the amount

of infection is positively correlated with increasing temperature. Visual symptoms start to appear at temperatures of 15°C or above, while no symptoms occur at temperatures below 10°C (Sharma et al. 2011). Leaf discoloration can occur, with leaves appearing bluish during early infection before becoming more chlorotic at advanced stages (Bhattacharya et al. 2013). Additional aboveground symptoms of clubroot include wilting, stunting, premature ripening and poor seed set. Once aboveground symptoms have been observed, clubroot can be differentiated from other diseases, nutrient deficiencies and environmental stress by examining the roots for characteristic galls. In susceptible rutabaga populations, swelling on the base of the bulb near the soil surface and along the taproot is generally observed. These galls choke off the supply of nutrients and water to the roots, resulting in aboveground symptoms (Miller et al. 2013). The majority of early infections are observed near field entrances, where machinery traffic is most intensive. The occurrence of infection decreases rapidly at 150 and beyond 300 meters from field entrances (Cao et al. 2009).

1.4.3 Control of Clubroot

Soil sterilants prove successful if applied at high rates (400 kg/ha) and to great depth (24 cm) (Buczacki and White 1979). Recently, sterilants such as Vapam have been effective at lower rates, but their use is still impractical outside of greenhouse and horticultural settings (Hwang et al. 2014). Liming soil to increase soil pH also helps reduce clubroot disease; however, it is extremely difficult to eliminate this disease completely with this soil treatment (Myers and Campbell 1985). A multi-faceted approach consisting of crop rotation, chemical control of weeds, soil amendments and pH modification can significantly reduce inoculum present in vegetable *Brassica* production (Donald and Porter 2009). Hwang et al. (2011) found that several soil treatments, including lime and wood ash, yielded positive results in reducing clubroot

inoculum. However, prohibitive efficiency and cost requirements, coupled with a lack of significant yield increase, renders this approach impractical for Western Canadian *B. napus* production. Varying seeding date, fungicide use, the use of soil drenches and fumigation have had some positive effects on vegetable production, but these techniques are largely impractical for large-scale production of spring canola in Western Canada (Gossen et al. 2013, Peng et al. 2011). Little research has been conducted regarding the biological control of *B. napus*, although the endophytic fungus *Heteroconium chaetospira* suppressed *P. brassicae* in growth cabinet trials. In these trials, *H. chaetospira* was able to colonize *B. napus* root tissues after inoculation and to suppress root hair infection by *P. brassicae*. While this method is far from practical for use in large-scale agricultural control of clubroot, early results are promising regarding its use to control clubroot in controlled settings (Lahlali et al. 2014).

1.4.4 Clubroot Resistance in *Brassica napus*

Since clubroot was first confirmed in Alberta in 2003, breeding efforts have focused on introducing *P. brassicae* resistance in *B. napus*. In 2009, resistant cultivars were released that showed great resistance and agronomic performance in clubroot-infested areas (Strelkov and Hwang 2013). To date, genetic resistance is the only control measure used in Western Canada. The long-term durability of resistance against the existing and developing pathotypes in Western Canada is currently unknown (reviewed in Gossen et al. 2013).

Generally, resistance to clubroot follows the gene-for-gene model due to the dominant nature of the major resistance genes (Feng et al. 2014). Most early clubroot-resistant *B. napus* cultivars typically carry a single gene and exhibit pathotype-specific resistance (for review, see Diederichsen et al. 2009). Several resistance loci have also been identified on different chromosomes of the *B. napus* genome, such as chromosomes A2, A3, A8, A9, C13, C15, C16

and C19. These loci, specifically on chromosomes A3 and A8, often confer resistance to specific pathotypes (reviewed in Piao et al. 2009, Manzanares-Dauleux et al. 2000, Werner et al. 2008). However, a locus in *B. oleracea* was found to confer resistance to more than one *P. brassicae* pathotype (reviewed in Diederichsen et al. 2009).

Resistance to *P. brassicae* can be found outside of spring-type *B. napus* germplasm, including the winter-type *B. napus* rutabaga, along with progenitor species *B. rapa* and *B. oleracea* (Hasan et al. 2012), where resistance can be under the control of simple Mendelian genetics (dominant/recessive inheritance) or quantitative gene loci (reviewed in Rahman et al. 2014). Research on *Arabidopsis thaliana,* a primitive ancestor of *Brassica* species, revealed a genomic region that shows co-linearity with the Brassica chromosome regions where clubroot resistance is located. This finding indicates that the clubroot resistance gene evolved in the ancestral genome and that the *Brassica* genomes received multiple resistance genes during their continued evolution from *A. thaliana* (Suwabe et al. 2005). Accessions of both the A and C genome species carry resistance to pathotypes tested under extreme pathogen pressure in controlled settings (Peng et al. 2011, Hasan et al. 2012). In general, *B. napus* germplasm are highly susceptible to different *P. brassica* pathotypes, such as 2, 3, 5, 6 and 8 (Hasan et al. 2012, Peng et al. 2013).

Fodder turnip typically carries strong resistance to different pathotypes of *P. brassicae*. Clubroot resistance has been introgressed with great success into Chinese cabbage cultivars from European fodder turnip; however, some of this resistance had been overcome by the pathogen after several years of cultivation (Kuginuki et al. 1999). Some of the genes conveying viable resistance to clubroot are located on different regions of the same chromosome, such as A3, while the others are located on entirely separate chromosomes (Li and McVetty 2013). The

genetics of resistance in rutabaga are more complex than those of turnip and winter canola. Rutabaga shows resistance to pathotype 3, the most prevalent pathotype in Western Canada, as well as pathotypes 2, 5, 6 and 8 in most cases (Hasan and Rahman 2013).

Detailed knowledge of the mechanism underlying resistance to clubroot disease in *Brassicaceae* is currently limited. In *B. napus* plants, resistance is generally exhibited during secondary infection. Primary infection typically occurs with root hair infection regardless of the presence of resistance in the host plant; the incidence of root hair infection can reach up to 50% even in resistant cultivars. Conversely, resistant plants exhibit no secondary infection compared to intermediate or completely susceptible *B. napus* plants (Deora et al. 2012). The delayed resistance response observed in greenhouse tests between primary and secondary infection was confirmed by PCR analysis, which showed that several *P. brassicae* and *B. napus* genes were upregulated at 7 days after infection, confirming a delayed resistance response along with the importance of specific genes conferring resistance to *P. brassicae* pathotypes *(*Feng et al. 2012). In susceptible plants, low sucrose content is found in leaves, as sucrose is exported to the roots of the plant to supplement gall formation, and most photosynthates are transported to (and accumulate in) the roots as well (for review, see Ludwig-Mŭller et al. 2009). Although the timing of the resistance response mechanism has been narrowed down to the onset of secondary infection, the molecular mechanism of the defense response is still unknown (Hatakeyama et al. 2013).

Successful infection leads to increased levels of the hormones cytokinin and auxins in plant root tissue, leading to an increase in plant cell division, as well as the division of *P. brassicae* plasmodium. In the later stages of infection, the high auxin levels cause the

plasmodium to hypertrophy and coincidentally increase the potential number of resting spores (for detailed review, see Diederichsen et al. 2013).

1.5 Challenges of Crossing *B. napus* × Rutabaga

The end uses for spring-type *B. napus* and rutabaga are dissimilar and therefore, there was previously little interest in using rutabaga in the breeding of spring *B. napus* canola. However, the identification of clubroot resistance in rutabaga has created interest in using this germplasm in the breeding of spring canola. Previous research conducted by the Canola Breeding Program at the University of Alberta confirmed that canola-quality progeny could be derived from a spring canola × rutabaga cross by the sixth generation, although flowering was delayed on average by two days and maturity was delayed six days compared to the *B. napus* parent (Rahman et al. 2014).

Flowering and maturity in *B. napus* can be influenced by both environmental and agronomic factors. Days to maturity can range from 95–125 days depending on growing degree day (GDD) accumulation (Canola Council of Canada 2014). In certain canola growing areas of Western Canada, there can be as few as 110 frost-free days (Dzikowski 1998). Problems associated with delayed maturity can be offset to some extent by early seeding in spring (Kirkland and Johnson 2000).

Flowering time is controlled by several quantitative trait loci (QTL) located on different linkage groups, such as A3, A4, A6, A7, C3, C4 C8 and C9, as well as epistatic interactions between the genes (Luo et al. 2014, Raman et al. 2014). The effect of the environment on this trait has also been confirmed in field and greenhouse studies (Raman et al. 2014). Little research

has been conducted on days to maturity. Additive gene effects were found to be more important than non-additive effects for this trait (Amiri-Oghan et al. 2009).

The progeny derived from a rutabaga × spring canola cross typically segregate for erucic acid content, as the seed oil of rutabaga contains high levels of this fatty acid (Hasan and Rahman 2014). Erucic acid content in *B. napus* is controlled by two gene loci acting in an additive manner. If one or both loci are homozygous recessive, the erucic acid levels in the seed oil will be virtually zero (Harvey and Downey 1962). Therefore, zero-erucic plants can be obtained from the progeny of rutabaga × canola crosses.

Glucosinolate content is high in rutabaga seeds (Velasco et al. 2008). This compound is nutritionally undesirable and makes the seed meal less palatable to animals. There are different types of glucosinolates, which collectively must be present at under 30 μmoles per gram in oil-free solid meal (Canadian Canola Council 2012). Glucosinolate content in seed meal is controlled by several QTL and genes acting in an additive manner (Toroser et al. 1995, Uzunova et al. 1995). *B. napus* accessions that are homozygous recessive at these loci would have low glucosinolate content in the seed (Howell et al. 2003).

A total of 46 QTL contributing to oil content have been identified on 16 of the 19 linkage groups of *B. napus* (Jiang et al. 2014). A single QTL can account for up to 20% variation for seed oil content. Most of these QTL exhibit additive and dominance gene effects, while epistatic interactions account for only a small portion of variation for this trait. Two of the major QTL contributing up to 50% of variation in oil content are located in the genomic region carrying the genes controlling erucic acid content in seed oil (Ecke et al. 1995, for review, see Javed et al. 2014).

1.6 Marker Assisted Breeding

Many tools are available to examine the extent of genetic diversity in *B. napus* germplasm, as well as members of the *Brassica* family such as *B. rapa* and *B. oleracea*. Simple sequence repeats (SSR) are sequences of DNA consisting of a tract of tandemly repeated DNA motifs (nucleotides) of one to a few nucleotides, which can be found within the eukaryotic genome (for review, see Tautz and Renz 1984). SSR markers have been used to assess genetic diversity between different *Brassica* populations and to differentiate between accessions within a specific population, such as *B. napus* (Hasan et al. 2006, Zhou et al. 2006). These markers have also been used to construct a genetic linkage map of *B. napus* (Piquemal et al. 2005). SSR markers associated with clubroot resistance in the *Brassica* A genome have been identified by various researchers (reviewed in Piao et al. 2009, Rahman et al. 2013). These markers can be used in marker assisted breeding (Rahman et al. 2013). In most rutabaga populations, genetic resistance to pathotypes 2 and 3 is conferred by a single major gene (Ayers and Lelacheur 1972). Although rutabaga (*B. napus* var. *napobrassica*) accessions with almost complete resistance to all major *P. brassicae* pathotypes are found in the available germplasm (Hasan et al. 2012), the genomic locations of resistance genes have yet to be identified.

1.7 Research Objectives

The objective of this research is three fold:

1) Evaluate the agronomic performance of canola-quality *B. napus* lines derived from F_2 and a three-way cross of spring *B. napus* canola × rutabaga crosses.

2) Evaluate the canola-quality *B. napus* lines derived from F_2 and three-way cross populations of *B. napus* canola × rutabaga for resistance to *P. brassicae* pathotype 3 as well as four newly discovered *P. brassicae* pathotypes found in northern Alberta in 2014.
3) Estimate genetic diversity in families derived from F_2 and a three-way cross of *B. napus* canola × rutabaga using SSR markers.

With these objectives in mind, three hypotheses will be tested in this Master's thesis project:

1) The performance of spring-type *B. napus* canola families developed from both F_2 and three-way cross of spring *B. napus* canola × rutabaga will exceed that of the *B. napus* parent.
2) Resistance to *P. brassicae* pathotype 3 can be found in spring-type *B. napus* canola families developed from both three-way cross and F_2 of spring *B. napus* canola × rutabaga crosses.
3) Canola-quality families derived from the progeny of three-way cross and F_2 of *B. napus* canola × rutabaga crosses will be genetically distinct from the *B. napus* parent.

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Chapter 2:

Evaluation of the Agronomic Performance of Three-way Cross and F_2-Derived Families of Spring Canola × Rutabaga Crosses

2.0 Introduction

Canola (*Brassica napus*) has become a valuable crop, with production steadily increasing both in Canada and worldwide (Statistics Canada 2012, USDA 2014). The length of the growing season must be accounted for when sowing *B. napus*. On the Canadian Prairies, the frost-free season can vary from 80 to 120 days in certain areas (Government of Canada 2013). The growing duration for spring-type *B. napus* can range from 95–125 days, which varies depending on cultivar, location and environmental factors (Canola Council of Canada 2014). Spring *B. napus* canola × rutabaga (*B. napus* var. *napobrassica*) crosses can be expected to produce biennial progeny (Howatt 2005) with a significant delay in flowering and maturity. Indeed, a delay in flowering (2 days) and maturity (6 days) was observed in advanced generation progeny derived from spring-type *B. napus* canola × rutabaga crosses (Rahman et al. 2014).

To offset the delayed flowering and maturity observed in the progeny of *B. napus* × rutabaga cross, early spring seeding should ideally be performed, as it has no significant effect on yield or seed quality compared to normal or late spring seeding (Clayton et al. 2004). The ability of *B. napus* to withstand mild frost in the early season, particularly past the cotyledon stage, would make early seeding feasible (Kirkland and Johnson 2000). Also, rutabaga is frost tolerant at the seedling stage (Undersander et al. 2013).

For genetic improvement of spring canola from a long-term perspective, it is important to use genetically diverse materials in breeding programs. Rutabaga, a subspecies of *B. napus*, is genetically distinct from spring canola (Diers and Osborn 1994) and provides resistance to *P. brassicae* (Hasan and Rahman 2013, Rahman et al. 2011).

The objective of this experiment was to evaluate the agronomic performance of a set of advanced generation families derived from a three-way cross and F_2 of spring canola × rutabaga crosses. It is expected that some of the families derived from both the three-way cross and F_2 will meet the seed quality standards of the spring-type *B. napus* parents, as well as exceeding the yields of the spring canola parents. The experiment was completed over two consecutive growing seasons in 2013 and 2014.

2.1 Materials and Methods

Two spring-type *B. napus* canola breeding lines, A07-26NR and A07-45NR, and one rutabaga line, Rutabaga-BF, were used to develop spring-type canola lines from canola × rutabaga crosses. The following single and three-way crosses were performed by the canola program at the University of Alberta (U of A): Rutabaga-BF × A07-26NR and (A07-45NR × Rutabaga-BF) × A07-26NR. In the fall of 2012, I received the following seed families from these crosses: three-way $F_{5:6S}$, three-way $F_{5:6B}$, $F_{4:5S}$, $F_{4:5B}$, $F_{5:6S}$ and $F_{5:6B}$. Generations followed by an 'S' indicates self-pollinated seeds derived from single plants, and 'B' indicates open-pollinated bulk seed harvested from several plants.

In the fall of 2012, the three-way $F_{5:6S}$, $F_{4:5S}$ and $F_{5:6S}$ families were grown in a greenhouse provided by the Crop Diversification Centre-North of the Government of Alberta. Self-pollinated seeds from this planting were seeded in the nursery trial in 2013, while the open-pollinated bulk seed families that were received from the University of Alberta canola program

were grown in the yield trial. Details about the different generation families generated during the course of this study and evaluated in different experiments are summarized in Tables 2.1 and 2.2.

Table 2.1. List of different generation families generated and used for different purposes. These families were derived from a three-way cross of (A07-45NR x Rutabaga-BF) × A07-26NR involving spring-type *B. napus* and rutabaga parents. Family designation followed by 'S' indicates self-pollinated seeds harvested or used, and 'B' indicates open-pollinated bulk seed harvested or used.

Growing Season	Seed Increase	Nursery Trial	Yield Trial
Seeded 2012–13	Three-way $F_{5:6S*}$		
Harvested 2012–13	Three-way $F_{5:7S*}$		
Seeded 2013		Three-way $F_{5:7S}$	Three-way $F_{5:6B}$
Harvested 2013		Three-way $F_{7:8S}$	
Seeded 2013–14	Three-way $F_{7:8S*}$		
Harvested 2013–14	Three-way $F_{7:9B*}$		
Seeded 2014		Three-way $F_{7:8S}$	Three-way $F_{7:9B}$
Harvested 2014		Three-way $F_{8:9S}$	Three-way $F_{9:10B}$

* Seed increase was performed in winter 2012–13 in a greenhouse at the Crop Diversification Centre North (CDC-N), in Edmonton, Alberta, and in 2013–2014 in an off-season nursery in Chile

Table 2.2. List of different generation families generated and used for different purposes. These families were derived from an A07-26NR × Rutabaga-BF cross involving spring-type *B. napus* and rutabaga parents. Family designation followed by 'S' indicates self-pollinated seeds harvested or used, and 'B' indicates open-pollinated bulk seed harvested or used.

Growing Season	Seed Increase	Nursery Trial	Yield Trial
Seeded 2012–13	$F_{4:5S*}$, $F_{5:6S}$*		
Harvested 2012–13	$F_{4:6S*}$, $F_{5:7S*}$		
Seeded 2013		$F_{4:6S}$, $F_{5:7S}$	$F_{4:5B}$, $F_{5:6B}$
Harvested 2013		$F_{6:7S}$, $F_{7:8S}$	
Seeded 2013–2014	$F_{6:7S}$*, $F_{7:8S*}$		
Harvested 2013–14	$F_{6:8B}$*, $F_{7:9B*}$		
Seeded 2014		$F_{6:7S}$, $F_{7:8S}$	$F_{6:8B}$, $F_{7:9B}$
Harvested 2014		$F_{7:8S}$, $F_{8:9S}$	$F_{8:9B}$, $F_{9:10B}$

*Seed increase was performed in winter 2012–13 in a greenhouse at the Crop Diversification Centre North (CDC-N) in Edmonton, Alberta, and in winter 2013–14 in an off-season nursery in Chile

2.2 Experimental Design

Agronomic performance was evaluated in two stages over two growing seasons in nursery and yield (YT) trials. Yield trials were seeded in full (1.5 x 6 m) plots, and nursery trials were seeded in half plots (1.5 x 3m). Nursery trial plots in 2013 were seeded using the seed increased in the greenhouse in winter 2012–13. The three-way cross and F_2-derived families were seeded in an unreplicated trial in separate blocks, with checks seeded every 10^{th} plot. Checks used were A07-26NR and A07-45NR, the spring-type *B. napus* parents of the crosses. Due to low levels of self-pollinated seed harvested from the 2013 nursery plots, the 2014 nursery was converted to 2 meter long single row plots, with parental checks still seeded every 10^{th} plot.

The yield trials were seeded at three locations over two successive years using open-pollinated bulk seed (Table 2.1, Table 2.2). In 2013, the three-way cross-derived families were seeded in two replications per location, while the F_2-derived families were seeded in one replication per location. The three-way cross and F_2-derived families were seeded in separate blocks, with one placed on top of the other following alpha lattice design. The sites used were the Edmonton Research Station (ERS), St. Albert Research Station and a field site near Killam, Alberta courtesy of a local cooperator. Due to the significant difference in the number of families between the three-way cross and the F_2-derived population, the *B. napus* parents were used as filler plots. Both *B. napus* parents were used as checks, as in the nursery trial. For the 2014 growing season, families harvested from contra production in Chile (Table 2.1, Table 2.2) were seeded in three trials, with two at separate seeding dates for the ERS site and one for the Killam field site. Each trial had two replications, with the three-way cross and F_2-derived families blocked one on top of the other as in the 2013 trial.

Both nursery and yield trials were fertilized prior to seeding by the University of Alberta Canola Group, and plots were managed throughout the year with herbicide, fungicide and pesticide as required. Yield trial plots were harvested using a combine, with measurements taken for seed moisture and plot yield (kg/ha), while the nursery plots were harvested manually.

2.2.1 Data Collection and Self-Pollination in Nursery Trial

Data on the agronomic traits, days to flowering (DTF) and days to maturity (DTM) were collected for each plot. DTF notes were taken at the onset of flowering: when 50% of the plants in a plot had a single open flower, the DTF was recorded. At the time of flowering, three plants representative of the entire plot were selected and self-pollinated using self-pollination bags (Vilutis & Co., Frankfurt, IL USA). DTM notes were taken by harvesting representative siliques from 2/3 up the main raceme of 4–5 plants in the plot. If approximately 60% of seeds had evidence of change in color from green to brown, the plot was considered to be mature.

At maturity, the self-pollinated plants were harvested and hung to dry for a minimum of five days before threshing. If fewer than three self-pollinated plants survived to maturity in a plot, a representative open-pollinated plant was harvested in its place. Open pollinated bulk seed was harvested from each plot for chemical analysis. This analysis was conducted at the Analytical Laboratory of the canola program of the University of Alberta. Analysis for seed oil, protein and glucosinolate contents was performed using near-infrared spectroscopy (NIRS; FOSS NIR System, Model 6500). Selections were made to advance superior families to the next generation based on agronomic and seed quality data.

2.2.2 Data Collection in Yield Trial

The following agronomic notes were taken throughout the course of the growing season: DTF, DTM, silique length, seeds per silique and seed yield. DTF and DTM were recorded as described for the nursery trials. Data on silique length and number of seeds per silique were collected only in 2014 from each plot at the Killam site and for one of the trials located at ERS. For this collection, 25 siliques from five plants (5 x 5 = 25) were harvested from the upper middle third of the main raceme and the length (mm) from the base of the silique to the base of the beak (i.e., length of the silique excluding the beak) was measured. Seeds were harvested from these siliques, and the average values were used for statistical analysis.

2.3 Statistical Analysis Methods

2.3.1 Nursery Trial

The PROC GLM procedure of SAS was used to determine if the three-way cross and F_2-derived populations were similar to each other and to compare these two populations to the check. In 2013, only DTF and seed quality data (oil, protein and glucosinolate content) were analyzed, as DTM data were compromised by a root maggot infestation. In 2014, DTF, DTM and all three seed quality traits (oil, protein and glucosinolate content) were compared between the three-way cross and F_2-derived populations as well as with the checks. One-way ANOVA with a Student-Newman-Keuls (SNK) test was performed to determine if the two populations were significantly different from each other and from their respective check. The three-way cross-derived population was compared with the mean of the two checks (A07-45NR and A07-26NR), and the F_2-derived population was compared with A07-26NR. The following formulas were used:

$$y_{ijkl} = \mu_j + \varepsilon ijkl$$

, where y is the independent variable, μ_j is the mean observation for the jth treatment group and $\varepsilon ijkl$ is residual error.

$$q = \frac{\overline{X_A} - \bar{X}_B}{\sqrt{\frac{MSE}{2}\left(\frac{1}{n_A}+\frac{1}{n_B}\right)}}$$

, where q = the difference between the largest and smallest data point in a sample, $\overline{X_A}$ and $\bar{X}_B$ are the largest and smallest sample means within a range, respectively, MSE is the error of variance and n is the sample size; n_A and n_B represent the sample sizes of the two sample means.

2.3.2 Yield Trial

A two-way ANOVA was performed with 2014 yield trial data for agronomic and seed quality traits (seed oil, protein and glucosinolate content) using the PROC GLM procedure of SAS. The interaction between populations and sites was also examined. ANOVA was ran for each trait for both the three-way cross and F_2-derived cross populations separately, as well as by including the two populations in the analysis for genotype × site interaction. The two-way ANOVA formula was as follows:

$$y_{ijk} = \mu + a_i + \beta_i + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

, where μ is the overall mean, a_i is the effect of genotype on a trait, β_i is the effect of site on a trait, $(\alpha\beta)_{ij}$ is the effect of the interaction between genotype and site and ε_{ijk} is the residual

error. ANOVAs were analyzed using Type 1 sum of squares $P < 0.05$. Although Type 1 error was possible, given the tentative bias to expect variation between two populations, extremely low p-values would tend to alleviate concerns of Type 1 error. LS mean values were calculated for each trait.

Silique length and number of seeds per silique for both three-way cross and F_2-derived populations were compared to their respective checks using the *t*-test for unequal variances in Microsoft Excel. Correlations between silique length and number of seeds per silique, and their relationship with seed yield, were calculated using Microsoft Excel.

2.4 Results

2.4.1 Nursery Trial

During the course of the 2013 growing season, a root maggot infestation had a severe impact on the entire nursery trial, leading to significant plant death. Therefore, DTM notes were not taken as they were deemed to be unreliable. Data for all other traits (seed oil, protein, glucosinolate) were recorded. During the 2014 growing season, data for all traits were collected.

DTF

In the 2013 growing season, the DTF of the three-way $F_{5:7S}$ population was similar to the check mean, as well as to both A07-45NR and A07-26NR separately; however, the $F_{4:6S}/F_{5:7S}$ populations flowered significantly later than the *B. napus* parent A07-26NR. In 2014, no significant difference in DTF was found between the checks and the populations derived from *B. napus* × rutabaga crosses.

DTM

In 2014, no significant difference in DTM was found between the checks and the populations derived from *B. napus* × rutabaga crosses.

Seed Oil

In 2013, seed oil content in the three-way $F_{5:7S}$ population was significantly higher than the check mean, while the $F_{4:6S}$ /$F_{5:7S}$ populations were statistically similar to A07-26NR. The three-way $F_{5:7S}$ population had significantly higher oil content than the $F_{4:6S}$/$F_{5:7S}$ populations; however, in 2014, all populations (three-way $F_{7:8S}$, $F_{6:7S}$/$F_{7:8S}$ and checks) were statistically similar to each other.

Seed Protein

In 2013, the seed protein contents of the three-way $F_{5:7S}$ population and the $F_{4:6S}$/$F_{5:7S}$ populations were statistically similar to their check mean or to the parent A07-26NR. Likewise, no significant difference between these populations was detected in 2014.

Glucosinolate

In 2013, there was no significant difference in glucosinolate content between the three-way $F_{5:7S}$ population and the check mean, while the $F_{4:6S}$/$F_{5:7S}$ populations had significantly higher glucosinolate content than A07-26NR and three-way $F_{5:7S}$. In 2014, the three-way $F_{7:8S}$ population was not statistically distinct from the check mean, but it was marginally distinct from the $F_{6:7S}$/$F_{7:8S}$ populations.

Table 2.3. Agronomic and seed quality traits of the three-way cross and F_2-derived populations of spring *B. napus* canola × rutabaga crosses evaluated in the nursery in the 2013 growing season.

Population	No. families	DTF[1]		% Seed oil content		% Protein		Glucosinolate (µmol/g)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Three-way $F_{5:7S}$	240	51.0 ± 2.36b	48-59	47.2 ± 1.73a	42.9-54.6	26.8 ± 1.42b	21.7-30.1	14.2 ± 3.38b	8.3-40.3
$F_{4:6S}$, $F_{5:7S}$	59	53 ± 3.62a	48-61	46.1 ± 1.84bc	41.5-49.9	27.7 ± 1.19ab	24.7-29.4	21.8 ± 13.89a	10.9-67.9
A07-26NR	18	50.0 ± 2.09b	48-56	46.9 ± 1.22ab	44.5-49.5	27.1 ± 1.27b	24.1-28.6	13.6 ± 0.81b	12.3-14.9
A07-45NR	16	51.0 ± 1.31b	49-54	45.3 ± 0.86c	44.1-47.0	28.0 ± 0.98a	25.8-28.9	12.7 ± 1.47b	9.9-14.4
Check Mean	34	50.0 ± 1.77b	48-56	46.1 ± 1.34bc	44.1-46.41	27.5 ± 1.22ab	24.1-28.9	13.2 ± 1.23b	9.9-14.9

[1]DTF = Days to Flowering; Mean ± SD values followed by same letter are not significantly different at $p < 0.05$. DTF means are rounded to the nearest full day.

Table 2.4. Agronomic and seed quality data from the three-way cross and F_2-derived populations of spring *B. napus* canola × rutabaga crosses evaluated in the nursery in the 2014 growing season.

Population	No. Families	DTF[1]		DTM[2]		% Seed Oil		% Protein		Glucosinolate (µmol/g)	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Three-way $F_{7:8S}$	73	44.0 ± 1.37a	42-47	92.0 ± 2.55a	88-98	43.7 ± 1.03a	41.7-46.5	29.9 ± 1.26a	26.1-32.3	16.5 ± 1.89b	11.8-22.2
$F_{6:7S}$, $F_{7:8S}$	32	43.0 ± 2.22a	41-50	93.0 ± 3.23a	87-98	43.7 ± 1.67a	39.1-45.7	30.0 ± 0.96a	28.2-33.2	18.5 ± 1.03a	17.0-22.2
A07-26NR	7	42.0 ± 0.44a	42-43	93.0 ± 3.21a	88-98	44.4 ± 1.28a	43.2-46.8	29.2 ± 1.11a	26.9-30.2	17.9 ± 1.11ab	16.4-19.5
A07-45NR	8	43.0 ± 1.31a	42-46	94.0 ± 3.09a	91-99	43.2 ± 0.99a	41.3-44.4	29.0 ± 0.81a	27.9-30.3	16.5 ± 1.18b	15.4-19.1
CK Mean	15	43.0 ± 1.18a	42-46	93.0 ± 3.16a	88-99	43.8 ± 1.24a	41.3-46.8	29.1 ± 0.93a	26.9-30.3	17.1 ± 1.32ab	15.4-19.5

[1]DTF = Days to Flowering; [2]DTM = Days to Maturity; DTF and DTM means are rounded to the nearest full day. Mean ± SD values followed by same letter are not significantly different at $p < 0.05$

2.4.2 Yield Trial

The 2013 growing season was met with numerous challenges. The ERS site provided DTF data, although the rest of the data for the other five traits had to be discarded due to root maggot attack. At the St. Albert site, the population derived from F_2 had to be discarded due to seeding error. Also, there was a hail event at the onset of flowering in 2013, although it was not significant enough to affect the data quality. In 2014, all data were collected successfully from all sites. The range of variation for DTF, DTM and seed yield is summarized graphically in Figures 2.1, 2.2 and 2.3, respectively. Data for these agronomic traits, along with data for seed quality traits (oil, protein and glucosinolate), are presented in Tables 2.5, 2.6 and 2.7.

Analysis of Variance (ANOVA)

Analysis of variance was performed to determine if significant variation existed within the three-way cross and F_2-derived populations for the six traits (DTF, DTM, yield, seed oil, seed protein and glucosinolates) and to investigate the extent of interaction of these traits with the environment. Since there were a significant number of data points missing in 2013, ANOVA for the 2013 season was not performed, and only 2014 yield trial data were used for this analysis (Tables 2.5, 2.6).

ANOVA revealed that significant variation existed within the three-way cross and F_2-derived populations, as well as the combined populations, for all traits. Genotype × site interaction was significant in all cases, except for DTM for both the three-way cross and the F_2-derived populations and seed oil and protein contents for the three-way cross-derived population.

Killam

DTF

In both 2013 and 2014, the DTF data for the three-way cross and F_2-derived populations were statistically similar to each other; however, both populations were distinct from the check mean (average of A07-26NR and A07-45NR), as well as A07-26NR itself. These two populations took approximately 47–48 days to flower in 2013 and 49–50 days in 2014.

DTM

In both 2013 and 2014, the DTM data for the three-way cross and F_2-derived populations were similar to each other, as well as to the check mean and A07-26NR, respectively.

Yield

Seed yields of these two populations were similar in 2013; however, in 2014, the three-way cross-derived population produced lower yields than the F_2-derived population at this site.

Seed Oil

Oil contents in seeds harvested from Killam were generally higher than those from the other two locations. In 2013, the F_2-derived population had significantly lower seed oil content than the three-way cross-derived population (51.8 ± 2.82 vs. 52.5 ± 1.14) and the check A07-45NR. However, these two populations were statistically similar for this trait in 2014. In 2014,

Table 2.5. Summary of agronomic and seed quality traits of the three-way cross and F_2-derived populations of *B. napus* canola × rutabaga crosses evaluated in yield trials at three locations in 2013. Traits with the same letter are not significantly different ($p < 0.05$).

		Three-way $F_{5:6B}$			$F_{4:5B}$, $F_{5:6B}$			A07-26NR			A07-45NR			Check Mean		
Trait		Killam	ERS	St. Albert	Killam	ERS	St. Albert	Killam	ERS	St. Albert	Killam	ERS	St. Albert	Killam	ERS	St. Albert
No. Families		32	32	32	9	9	9	17	17	6	18	18	6	35	35	12
DTF	Mean ± SD	48.0 ± 1.61a	51.0 ± 2.38	52.0 ± 2.61a	47.0 ± 1.84a	51.0 ± 3.00a	-	46.0 ± 1.30b	50.0 ± 0.77c	49.0 ± 1.80a	46.0 ± 1.10b	50.0 ± 0.80bc	50.0 ± 1.70a	46.0 ± 1.14b	50.0 ± 0.84bc	50.0 ± 1.67a
	Range	46-52	48-65	48-87	45-52	49-57	-	45-50	49-51	48-52	45-49	48-52	48-52	45-50	49-52	48-52
DTM	Mean ± SD	101.0 ± 2.49a	-	109.0 ± 2.1a	100.0 ± 2.60a	-	-	100.0 ± 2.4a	-	110.0 ± 1.60a	100.0 ± 2.40a	-	110.0 ± 1.90a	100.0 ± 2.40a	-	110.0 ± 1.64a
	Range	97-106	-	104-114	97-106	-	-	97-106	-	108-112	97-105	-	108-113	97-106	-	108-113
Yield	Mean ± SD	3244 ± 490.1a	-	3439 ± 479.0c	3417 ± 313.0a	-	-	3561 ± 331.6a	-	3778 ± 215.3b	3396 ± 351.5a	-	4505 ± 262.2a	3464 ± 349.3a	-	4215 ± 469.6ab
	Range	2399-4443	-	2280-4838	2592-3821	-	-	2868-4213	-	3459-3982	2784-4024	-	4170-4838	2784-4213	-	3459-4838
% Seed Oil	Mean ± SD	52.5 ± 1.14 ab	-	47.0 ± 1.01a	51.8 ± 2.82c	-	-	53.0 ± 1.16a	-	47.4 ± 1.18a	51.8 ± 1.20b	-	46.1 ± 1.60a	52.4 ± 1.32ab	-	46.8 ± 1.48a
	Range	49.4-55.0	-	44.2-49.3	46.5-54.8	-	-	51.1-54.8	-	46.2-49.0	49.6-54.0	-	44.2-48.1	49.6-54.8	-	44.2-49.0
% Protein	Mean ± SD	20.5 ± 1.39ab	-	23.9 ± 0.97a	20.2 ± 1.85a	-	-	20.0 ± 1.16b	-	25.5 ± 1.21a	19.6 ± 1.12b	-	25.2 ± 1.27a	19.8 ± 1.15b	-	25.3 ± 1.16a
	Range	16.9-24.1	-	23.8-28.1	18.0-24.0	-	-	18.0-21.7	-	23.8-26.6	17.7-21.2	-	23.8-26.8	17.7-21.7	-	23.8-26.8
Glucosinolate	Mean ± SD	13.1 ± 1.68b	-	16.2 ± 2.11a	17.1 ± 8.49a	-	-	13.3 ± 0.47b	-	16.8 ± 0.71a	10.4 ± 0.66c	-	13.4 ± 1.90b	11.9 ± 1.60bc	-	15.1 ± 2.29ab
(µmol/g)	Range	9.8-17.4	-	10.1-19.5	9.1-43.0	-	-	12.3-14.2	-	16.1-17.8	9.1-11.5	-	11.6-15.8	9.10-14.20	-	11.6-17.8

DTF = Days to flowering DTM = Days to maturity

Table 2.6. Summary of agronomic and seed quality traits of the three-way cross and F_2-derived populations of *B. napus* canola × rutabaga crosses evaluated in yield trials at three locations in the 2014 growing season. Traits with the same letter are not significantly different ($p < 0.05$).

		Three-way $F_{7:9B}$			$F_{6:8B}$, $F_{7:9B}$			A07-26NR			A07-45NR			Check Mean		
Trait		Killam	ERS	St. Albert	Killam	ERS	St. Albert	Killam	ERS	St. Albert	Killam	ERS	St. Albert	Killam	ERS	St. Albert
No. Families		75	75	75	32	32	32	12	12	12	11	11	11	23	23	23
DTF	Mean ± SD	49.0 ± 1.21a	41.0 ± 1.21ab	40.0 ± 0.09a	50.0 ± 1.49a	41.0 ± 1.34ab	39.0 ± 1.20b	49.0 ± 0.40b	41.0 ±0.50b	39.0 ±0.70c	48.0 ± 0.60b	42.0 ± 0.90a	38.0 ± 0.70c	49.0 ± 0.60b	10.0 ± 0.90ab	38.0 ± 0.7c
	Range	47-54	40-45	38-44	47-55	40-45	37-44	48-49	40-41	38-40	47-49	40-43	37-40	47-49	40-43	37-40
DTM	Mean ± SD	96.0 ± 2.60ab	88.0-2.76b	90.0 ± 3.53ab	95.0 ± 2.46b	87.0 ± 2.59b	90.0 ± 3.26ab	95.0 ±1.90b	87.0 ±1.60b	88.0 ± 2.4b	96.0 ± 1.70a	91.0 ±3.10a	91.0 ± 3.40a	96.0 ± 2.10ab	89.0 ± 3.10ab	90.0 ± 3.3ab
	Range	91-102	84-94	85-99	91-102	84-95	86-100	91-98	85-90	86-95	93-100	85-95	85-98	91-100	85-95	85-98
Yield	Mean ± SD	2674 ± 308.0b	2711 ± 311.0a	2614 ± 333c	2943-354.0a	2560 ± 330.0a	2908-358.0b	2885 ± 245.0a	2712 ± 329.6a	3110 ± 156.4a	2821 ± 325.7ab	2667 ± 380.7a	3179 ± 179.4a	2842 ± 292.5ab	2688 ± 349.8a	3146 ± 170.6a
	Range	1879-3997	1900-3543	1824-3341	2014-3583	1977-3260	1669-3416	2378-3205	2182-3257	2719-3353	1677-3290	1851-3242	2875-3541	1677-3290	1851-3257	2719-3541
% Seed Oil	Mean ± SD	53.1 ± 1.24a	47.0 ± 1.23a	47.3 ± 1.23b	53.0 ± 1.68a	46.8 ± 1.75a	48.2 ± 1.86a	53.3 ± 0.80a	47.7 ± 1.13a	48.0 ± 0.56a	51.4 ± 0.69c	45.5 ± 1.11b	46.8 ± 0.83b	52.3 ± 1.19b	46.6 ± 1.55a	47.4 ± 0.93b
	Range	50.0-55.3	44.3-50.0	43.7-50.1	47.8-55.4	42.7-49.1	42.4-50.6	52.0-54.6	46.1-49.2	46.9-48.8	50.1-52.6	44.0-47.4	45.2-48.7	50.0-55.0	44.0-49.2	45.2-48.8
% Protein	Mean ± SD	19.1 ± 1.24a	25.8 ± 1.46a	26.4 ± 1.26a	18.7 ± 1.24a	25.9 ± 1.46a	25.2 ± 1.35b	18.5 ± 0.97a	24.6 ± 1.42a	25.5 ± 0.66b	18.9 ± 0.70a	25.2 ± 1.30a	23.2 ± 3.50b	18.7 ± 0.85a	24.9 ± 1.35a	25.3 ± 0.75b
	Range	16.8-22.7	22.2-28.9	23.8-29.9	16.4-21.9	23.2-29.2	23.2-29.7	16.9-20.0	22.8-26.7	24.2-27.5	17.6-20.0	22.8-27.3	17.6-26.4	16.9-20.0	22.8-27.3	23.1-27.5
Glucosinolate	Mean ± SD	12.0 ± 1.32b	14.4 ± 1.41b	15.3 ± 1.52b	13.0 ± 0.64a	15.4 ± 0.75a	16.0 ± 1.39a	12.5 ± 0.50b	14.2 ± 0.96b	15.3 ± 0.91b	11.1 ± 0.58c	14.4 ±0.60b	13.0 ± 1.45c	11.6 ± 0.73b	14.3 ± 0.79b	14.6 ±0.97c
(µmol/g)	Range	8.1-15.2	11.8-19.4	11.8-20.7	11.5-14.7	13.9-16.9	13.5-20.1	11.3-12.8	13.1-16.4	13.5-17.0	10.2-12.2	13.4-15.6	10.4-15.1	10.2-12.9	13.1-16.5	12.8-17.0

DTF = Days to flowering DTM = Days to maturity

the three-way cross-derived population had significantly higher oil content than A07-45NR and the check mean.

Protein

Protein contents were generally lower in seeds harvested from Killam compared to the two other locations. In 2013, the three-way cross and F_2-derived populations were statistically similar to each other but distinct from the checks. However, in 2014, both three-way cross and F_2-derived populations had similar protein contents, and these populations were also statistically similar to the checks.

Glucosinolate

In 2013, the F_2-derived population had significantly higher glucosinolate content than A07-26NR and the three-way population, while the three-way cross-derived population was similar to the check mean. In 2014, both populations had glucosinolate levels below 15 μmol/g, with the three-way cross-derived population statistically similar to the check mean and the F_2-derived population distinct from A07-26NR.

ERS Site

DTF

The DTF note was the only note successfully taken at the ERS site in 2013 due to severe root maggot pressure. Both the three-way cross and F_2-derived populations flowered approximately one day later than A07-26NR. However, in 2014, no significant difference between the three-way cross and F_2-derived populations was found, and these two populations were statistically similar to A07-26NR.

DTM

In 2014, the DTM values of the three-way cross and F_2-derived populations were not significantly different from each other or from A07-26NR. These populations took approximately 87–88 days to mature.

Yield

The average seed yield of all populations was statistically similar in 2014; however, wide variation between the families was found in both three-way cross and F_2-derived populations. This result suggests that canola lines with seed yield comparable to A07-26NR or A07-45NR can be selected from these two populations (Table 2.7, Figure 2.3).

Oil

Like seed yield, the oil contents of the three-way cross and F_2-derived populations were statistically similar to each other, as well as to A07-26NR and the check mean.

Protein

There was no significant difference in seed protein content between the three-way cross and F_2-derived populations or between these populations and their respective checks.

Glucosinolate

The three-way cross-derived population had glucosinolate content similar to the check mean; however, the F_2-derived population had higher mean glucosinolate content than the three-way cross-derived population and the checks.

St. Albert

DTF

In 2013, the three-way cross-derived population took approximately two days longer to flower than the mean of the two *B. napus* parents; however, this difference was not statistically significant. In 2014, both the three-way cross and F_2-derived populations were statistically distinct from each other as well as from the checks.

DTM

The three-way cross-derived population was statistically similar to the check mean in both 2013 and 2014. The F_2-derived population was statistically similar to the three-way cross-derived population in 2014; however, this population matured an average of two days later than A07-26NR.

Yield

The average seed yield of both the three-way cross and F_2-derived populations was significantly lower than that of A07-26NR as well as the check mean in both 2013 and 2014. However, variation for seed yield was found among the families of both populations, suggesting that canola-quality lines with seed yield similar to that of A07-26NR can be achieved from both populations (Table 2.7, Figure 2.3).

Oil

The average oil content of the three-way cross-derived population in 2013 was 47.0 ± 1.01, which is statistically similar to the oil content of the check mean. In 2014, the three-way cross and F_2-derived populations were statistically similar to the check mean and A07-26NR, respectively; however, the F_2-derived population had a higher oil content than the three-way

cross-derived population. The difference between the three-way cross and F_2-derived populations for this trait was not observed at the Killam or ERS site.

Protein

The three-way cross-derived population was statistically similar to the check mean in 2013 (23.9 ± 1.0% vs. 25.3 ± 1.2%); however this population had significantly higher protein content than the F_2-derived population in 2014 and was statistically distinct from both *B. napus* parents.

Glucosinolate

The average glucosinolate content of the three-way cross and F_2-derived populations was comparable to that of A07-26NR, as was found at both the Killam and ERS sites. This finding suggests that most of families of these two populations were of canola quality in regards to glucosinolate content.

Least Squares Means of Agronomic Traits

Least Squares means (LS means) were calculated for the agronomic and seed quality trait data collected from the three trials in the 2014 yield trial season. For the DTF trait, there was no significant difference between the three-way cross and F_2-derived populations; however, these two

Table 2.7. Summary of Least Squares means (LS means) ± Standard Error for all six agronomic traits across all three sites in 2014

Population	Entries	DTF	DTM	Yield	Seed Oil	Seed Protein	Glucosinolate
Three-way $F_{7:9B}$	75	43.4 ± 0.06a	91.4 ± 0.16ab	2709.8 ± 17.08a	49.2 ± 0.07ab	23.7 ± 0.07a	13.9 ± 0.06a
$F_{6:8B}$, $F_{7:9B}$	32	43.3 ± 0.09a	90.6 ± 0.24ac	2803.9 ±26.16ab	49.4 ± 0.11ab	23.3 ± 0.10b	14.9 ± 0.10b
A07-26NR	12	42.7 ± 0.11b	91.5 ±0.28ab	2892.1 ± 30.85ab	48.8 ± 0.13c	23.0 ± 0.12b	13.5 ± 0.11c
A07-45NR	11	42.7 ± 0.15b	90.1 ± 0.41ac	2902.0 ± 44.61ac	49.7 ± 0.19b	22.9 ± 0.17b	13.9 ± 0.17ac
Check Mean	23	42.8 ± 0.15b	92.9 ± 0.39d	2882.9 ± 42.71ac	47.9 ± 0.18b	23.1 ± 0.16b	13.2 ± 0.16c

DTF = Days to Flowering DTF = Days to Maturity.
Least Squares mean ± Standard Error values followed by the same letter are not significantly different at $p < 0.05$.

populations flowered later than the checks. For DTM, the three-way cross-derived population was statistically similar to the check mean, and the F_2-derived population was similar to A07-26NR. For seed yield, no significant difference was found between the three-way cross and F_2-derived populations, and these two populations were also similar to both A07-26NR and A07-45NR.

For seed oil content, both three-way cross and F_2-derived populations were similar to each other, as well as to the check A0-26NR, and these two populations had significantly higher oil content than A07-45NR. On the contrary, the three-way cross-derived populations had higher protein content than the F_2-derived population and the checks. The F_2-derived population had higher glucosinolate content than the three-way cross-derived population.

Silique Length and Seeds per Silique

Three-way cross-derived population

Data for silique length and number of seeds per silique were collected from the Killam and St. Albert sites in 2014. At the Killam site, siliques from the three-way cross-derived population, on average, were 6 mm shorter than the check mean (Table 2.8). This population also had significantly fewer seeds per silique than the check mean (Table 2.8; $p < 0.01$, $t = 1.98$). At

St. Albert, the three-way cross-derived population also had significantly shorter siliques than the check mean ($p < 0.001$, $t = 1.98$; Table 2.8). The check mean also had significantly more seeds per silique, on average, than the three-way population ($p < 0.001$, $t = 1.98$).

F_2-Derived Population

At Killam, the F_2-derived population was closer to A07-26NR than to the three-way cross-derived population (Table 2.7), with no distinct difference between the mean of the F_2-derived population and A07-26NR ($p = 0.08$, $t = 2.02$). Conversely, for number of seeds per silique, the F_2-derived population was statistically distinct from A07-26NR at Killam ($P = 0.04$, $t = 1.98$). At the St. Albert site, there was no distinct difference in the mean silique length ($p = 0.21$, $t = 2.00$) or the number of seeds per silique ($p = 0.08$, $t = 2.02$) between the F_2-derived population and A07-26NR.

Three-way cross vs. F_2-Derived Populations

At St. Albert, the three-way cross and F_2-derived populations were statistically distinct for silique length ($p = 0.02$, $t = 1.97$), with the silique length of the F_2-derived population averaging 2 mm longer than that of the three-way cross-derived population (Table 2.8). However, these two populations were statistically similar for number of seeds per silique ($p = 0.05$, $t = 1.97$). At Killam, the two populations were statistically distinct from each other for silique length ($p = 0.04$, $t = 1.98$), although they were statistically similar for number of seeds per silique ($p = 0.59$, $t = 1.97$).

Table 2.8. Silique length and number seeds per silique for the three-way cross and F_2-derived families of spring *B. napus* canola × rutabaga crosses evaluated in 2014 yield trials at two locations

	Silique Length (mm)				No. Seed per Silique			
	Killam		St. Albert		Killam		St. Albert	
Family	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Three-way $F_{7:9B}$	48.2 ± 5.15	34.4-60.8	56.5 ± 5.41	26.1-72.0	18 ± 3.5	11-27	23 ± 3.2	14-33
$F_{6:8B}$, $F_{7:9B}$	49.7 ± 4.81	39.0-59.4	58.5 ± 3.62	49.7-68.8	19 ± 3.4	9-25	24 ± 2.3	18-28
A07-26NR	51.8 ± 4.70	42.1-62.3	59.4 ± 2.54	54.4-64.5	21 ± 2.8	15-26	25 ± 2.2	21-29
A07-45NR	56.1 ± 5.51	45.1-65.0	63.6 ± 4.09	56.8-71.4	21 ±3.2	15-26	26 ± 2.1	23-31
Check mean	54.0 ± 5.53	42.1-65.0	61.6 ± 4.03	54.4-71.4	21 ± 3.2	15-26	26 ± 2.2	21-31

Correlation Analysis

Pearson correlations between silique length, number of seeds per silique and seed yield were estimated based on data from the three-way cross and F_2-derived families; a summary is presented in Table 2.9.

At Killam, there was moderate correlation between silique length and number of seeds per silique in the three-way cross ($r = 0.82$, $R^2 = 0.67$) and in the F_2-derived cross population ($r = 0.71$, $R^2 = 0.50$). However, there was no significant correlation between silique length and seed yield or between number of seeds per silique and seed yield in the three-way cross and F_2-derived populations.

Table 2.9. Summary of correlation analysis between silique length, number of seeds per silique and seed yield in three-way cross and F_2-derived populations tested at two locations in 2014.

		Silique Length vs. no. seeds per silique				Silique Length vs. yield				No. seeds per silique vs. yield			
Location	Family	R value	*df*	p value	R^2	R value	*df*	p value	R^2	R value	*df*	p value	R^2
Killam	Three-way cross derived	0.82	74	<0.0001	0.67	0.05	74	0.69	0.002	0.03	74	0.75	0.001
Killam	F_2 Derived	0.71	31	<0.0001	0.5	0.02	31	0.39	0.02	0.33	31	0.06	0.11
St. Albert	Three-way cross derived	0.66	74	<0.0001	0.44	0.1	74	0.39	0.01	0.03	74	0.77	0.001
St. Albert	F_2 Derived	0.28	31	0.31	0.03	0.22	31	0.54	0.01	0.16	31	0.36	0.03

At St. Albert, the correlation between silique length and number of seeds per silique in both the three-way cross ($r = 0.66$, $R^2 = 0.44$) and F_2-derived ($r = 0.18$, $R^2 = 0.31$) populations was not as strong as that detected at Killam. Almost no correlation between silique length and seed yield ($r = 0.10$, $R^2 = 0.01$, and $r = 0.11$, $R^2 = 0.01$) or between number of seeds per silique and seed yield ($r = 0.03$, $R^2 = 0.001$ and $r = 0.06$, $R^2 = 0.03$) was found in these two populations at St. Albert.

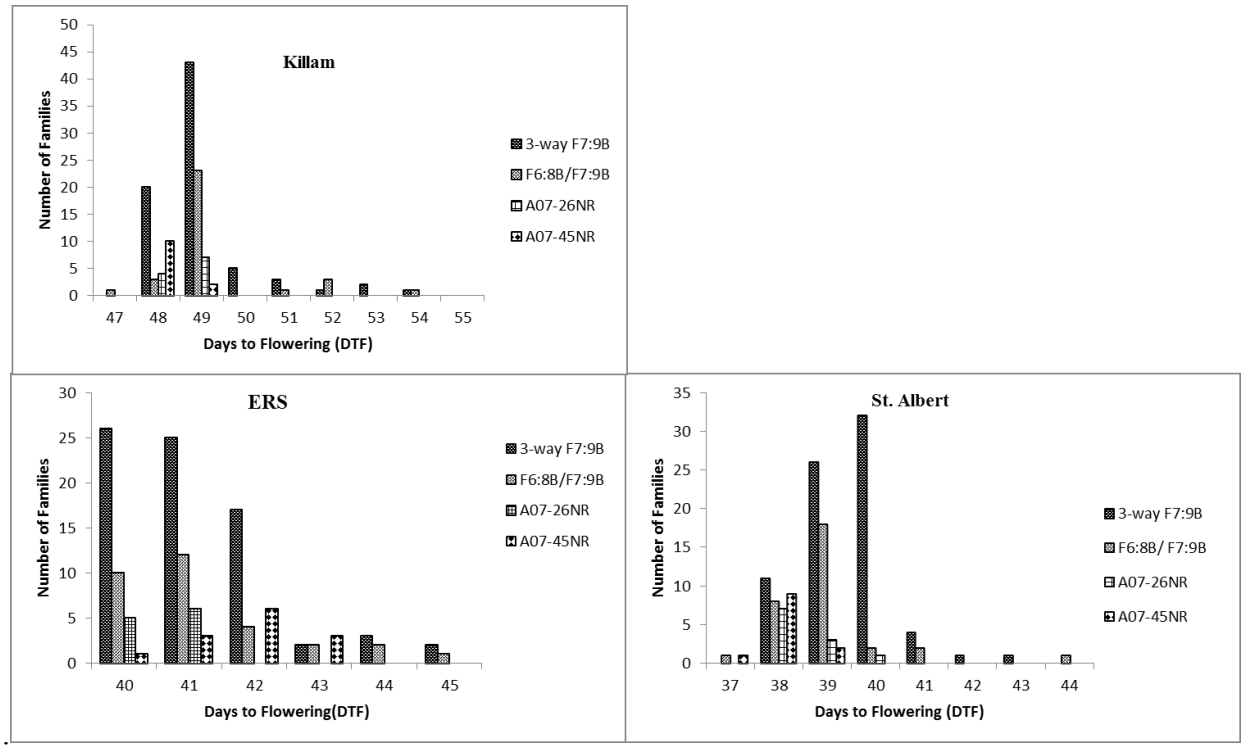

Figure 2.1. Days to flowering of the three-way cross (n = 75) and F_2 (n = 32) derived families of spring *B. napus* canola × rutabaga cross tested in yield trial in 2014.

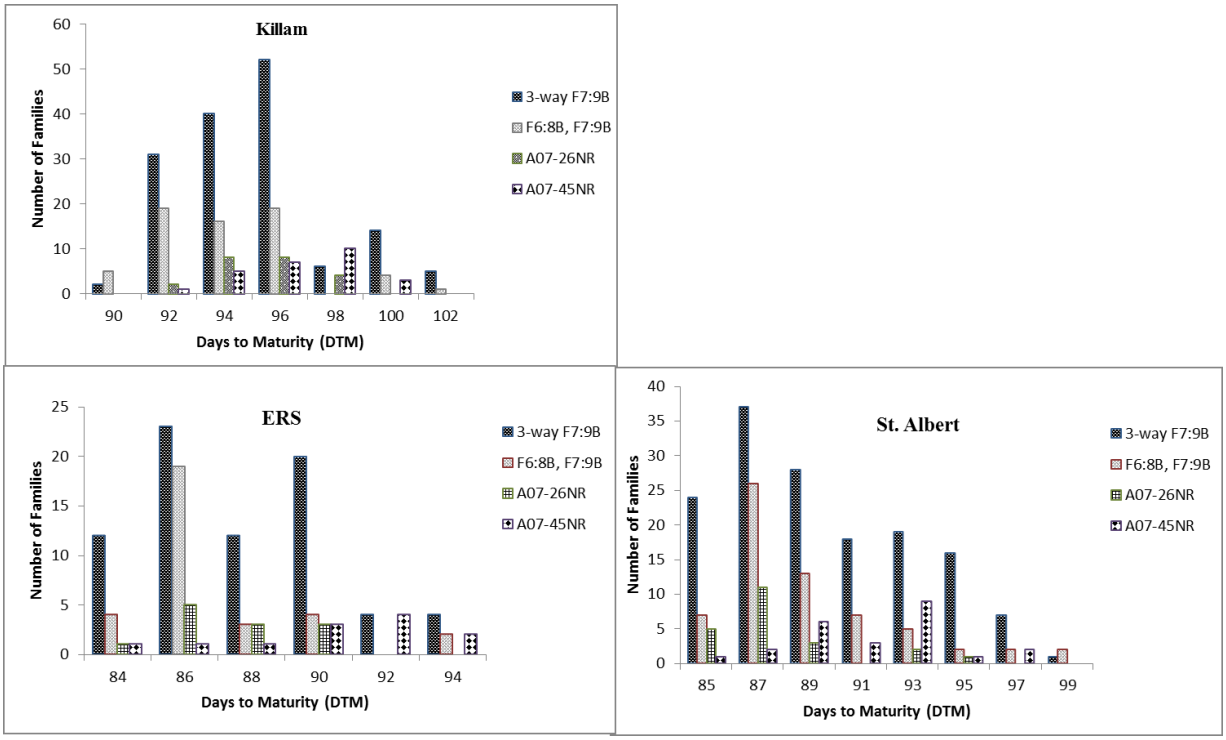

Figure 2.2. Days to maturity of the three-way cross (n = 75) and F_2-derived (n = 32) families of spring *B. napus* canola × rutabaga cross tested in yield trial in 2014.

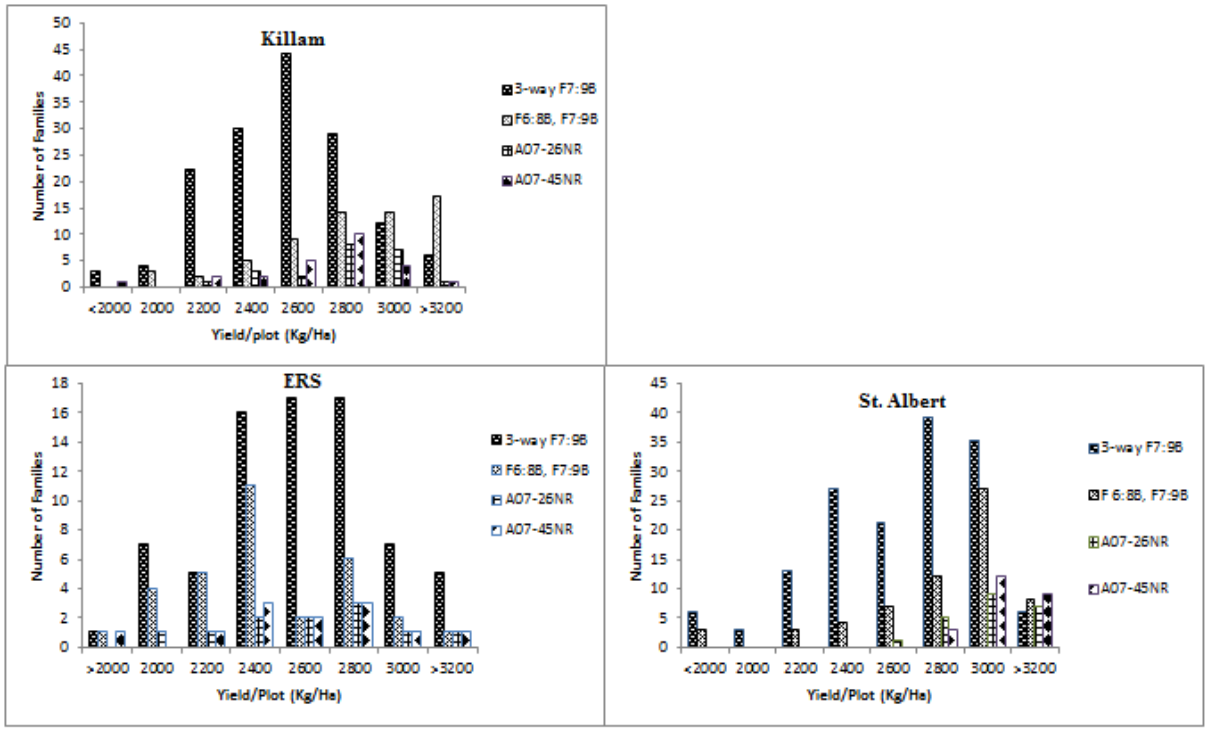

Figure 2.3. Seed yield data for the three-way cross (n = 75) and F_2-derived (n = 32) families of spring *B. napus* canola × rutabaga cross tested in yield trial in 2014.

Table 2.10. ANOVA showing statistical significance of genotype (families), site and genotype × environment interaction for DTF of the three-way cross and F_2-derived populations as well as the combined populations of *B. napus* × rutabaga grown in three trials in 2014

Population	Source of variation	Df	SS (%)	Mean square	F value	Pr > F
Three-way cross-derived	Genotype	74	4.2	4.57	17.34	<0.0001
	Site	2	94.6	3,814.04	14,483.70	<0.0001
	Genotype × site	148	1.2	0.66	2.50	<0.0001
F_2 derived	Genotype	31	4.5	5.73	16.66	<0.0001
	Site	2	93.3	1,824.08	5,306.40	<0.0001
	Genotype × site	62	2.2	1.38	4.03	<0.0001
Populations Combined	Genotype	106	4.3	4.87	16.94	<0.0001
	Site	2	94.1	5,632.41	19,599.00	<0.0001
	Genotype × site	212	1.6	0.92	3.20	<0.0001

Table 2.11. ANOVA showing statistical significance of genotype (families), site and genotype × environment interaction for DTM of the three-way cross and F_2-derived populations as well as the combined populations of *B. napus* × rutabaga grown in three trials in 2014

Population	Source of variation	Df	SS (%)	Mean square	F value	Pr > F
Three-way cross derived	Genotype	74	24.0	19.60	2.91	<0.0001
	Site	2	60.1	1,820.05	270.17	<0.0001
	Genotype × site	148	15.9	6.52	0.97	0.5787
F_2 derived	Genotype	31	19.5	14.84	2.69	0.0004
	Site	2	61.7	728.65	131.92	<0.0001
	Genotype × site	62	18.8	7.15	1.30	0.1530
Populations Combined	Genotype	106	23.4	1,872.06	2.94	<0.0001
	Site	2	60.0	2,547.08	399.62	<0.0001
	Genotype × site	212	16.6	6.66	1.04	0.3745

Table 2.12. ANOVA showing statistical significance of genotype (families), site and genotype × environment interaction for seed yield of the three-way cross and F_2-derived populations as well as the combined populations of *B. napus* × rutabaga grown in three trials in 2014

Population	Source of variation	Df	SS (%)	Mean square	F value	Pr > F
Three-way cross derived	Genotype	74	60.0	251,279.10	4.39	<0.0001
	Site	2	1.9	302,848.67	5.29	0.0060
	Genotype × site	148	38.1	79,735.92	1.39	0.0218
F_2 derived	Genotype	31	49.8	310,056.78	5.63	<0.0001
	Site	2	18.0	1,730,561.04	31.42	<0.0001
	Genotype × site	62	32.2	100,258.48	1.82	0.0093
Populations Combined	Genotype	106	57.9	286,749.18	5.07	<0.0001
	Site	2	3.1	816,889.11	14.44	<0.0001
	Genotype × site	62	39.0	9,462.18	1.70	<0.0001

Table 2.13. ANOVA showing statistical significance of genotype (families), site and genotype × environment interaction for seed oil content of the three-way cross and F_2-derived populations as well as the combined populations of *B. napus* × rutabaga grown in three trials in 2014

Population	Source of variation	Df	SS (%)	Mean square	F value	Pr > F
Three-way cross derived	Genotype	74	8.3	4.00	4.97	<0.0001
	Site	2	87.5	1,553.81	1,928.26	<0.0001
	Genotype × site	148	4.2	1.00	1.25	0.0894
F_2 derived	Genotype	31	23.9	12.09	29.43	<0.0001
	Site	2	70.3	551.86	1,343.90	<0.0001
	Genotype × site	62	5.8	1.48	3.60	<0.0001
Populations Combined	Genotype	106	13.2	6.41	9.32	<0.0001
	Site	2	81.5	2,090.72	3,040.49	<0.0001
	Genotype × site	212	5.3	1.28	1.85	<0.0001

Table 2.14. ANOVA showing statistical significance of genotype (families), site and genotype × environment interaction for seed protein content of the three-way cross and F_2-derived populations as well as the combined populations of *B. napus* × rutabaga grown in three trials in 2014

Population	Source of variation	df	SS (%)	Mean square	F value	Pr > F
Three-way cross derived	Genotype	74	6.9	4.68	5.20	<0.0001
	Site	2	90.3	2,281.32	2,537.52	<0.0001
	Genotype × site	148	2.8	0.96	1.07	0.3351
F_2 derived	Genotype	31	8.6	5.55	11.91	<0.0001
	Site	2	87.6	874.79	1878.54	<0.0001
	Genotype × site	62	3.8	1.24	2.65	<0.0001
Populations Combined	Genotype	106	7.9	5.27	6.85	<0.0001
	Site	2	88.6	3,142.33	4,083.95	<0.0001
	Genotype × site	212	3.5	1.16	1.51	0.0013

Table 2.15. ANOVA showing statistical significance of genotype (families), site and genotype × environment interaction for glucosinolate of the three-way cross and F_2-derived populations as well as the combined populations of *B. napus* × rutabaga grown in three trials in 2014

Population	Source of variation	Df	SS (%)	Mean square	F value	Pr > F
Three-way cross derived	Genotype	74	34.8	7.29	18.86	<0.0001
	Site	2	55.2	427.61	1,106.76	<0.0001
	Genotype × site	148	10.0	1.05	2.71	<0.0001
F_2 derived	Genotype	31	10.0	1.57	3.50	<0.0001
	Site	2	76.0	186.10	413.66	<0.0001
	Genotype × site	62	14.0	1.10	2.46	0.0002
Populations Combined	Genotype	106	32.7	6.65	16.40	<0.0001
	Site	2	56.9	613.60	1,513.70	<0.0001
	Genotype × site	212	10.4	1.06	2.60	<0.0001

2.5 Discussion

The aim of this study was to determine if spring-type *B. napus* canola could be developed from *B. napus* × rutabaga crosses and grown successfully in terms of agronomic and seed quality performance in Western Canada. Little research has been conducted on the use of rutabagas in the breeding of spring canola other than by the University of Alberta canola program. This program provided the two populations derived from spring-type *B. napus* × rutabaga crosses that were used in this experiment.

This study demonstrates that it is possible to obtain canola-quality families with acceptable agronomic traits from both a three-way cross and an F_2 of spring-type *B. napus* × rutabaga cross. However, fewer canola-quality families were obtained from the F_2 than from the three-way cross, which is due to the greater genome contribution of the canola-quality parents in the three-way cross-derived population than in the F_2-derived population. Falk (2010) also suggested that limited backcrossing would be an effective strategy for introgressing genetic

diversity from unadapted to adapted germplasm. However, both populations produced families well within canola-quality standards with acceptable agronomic traits.

Yield performance in the families derived from intraspecific crosses using spring-type *B. napus* canola germplasm has never been a major concern in breeding of this crop; however, the use of winter type in breeding imposes challenges associated with vernalization, days to flowering and seed quality traits (Rahman et al. 2011). In regards to the use of rutabaga in breeding, high glucosinolate and erucic acid content was an additional constraint in the early generations following the initial cross (Rahman et al. 2014); however, by the fourth or fifth generation ($F_{4:5}$,$F_{5:6}$, three-way $F_{5:6}$), i.e., the populations used in this experiment, the majority of families derived from the three-way cross and F_2 were of canola seed quality.

Seed yield in *B. napus* is a complex trait to which several other traits contribute, each controlled by several gene loci (Shi et al. 2009, Cai et al. 2014). Rutabaga is physiologically distinct from spring-type *B. napus*. As rutabaga is a fodder plant rather than primarily a seed-producing plant, improvement for seed yield had never been a focus in rutabaga breeding programs. Therefore, it is unlikely that all families directly derived from a *B. napus* × rutabaga cross would produce high seed yields. However, some of the alleles derived from rutabaga may contribute to seed yield in *B. napus* canola or to heterosis in hybrid canola. Both the three-way cross and F_2-derived populations contained families that exceeded the yield of the checks. On average, the yields of the three-way cross-derived families were higher, most likely due to the increased amount of spring *B. napus* canola background, which resulted from less disruption of favorable allele combinations of canola cultivars achieved through several cycles of breeding (Rahman 2013).

Several traits in *B. napus* influence seed yield, such as number of branches, length of main inflorescence, silique length and density, number of seeds per silique and seed weight. All of these traits are controlled by multiple gene loci (Chen et al. 2007, Shi et al. 2009, Cai et al. 2014, Rahman and Kebede 2014) and can be improved progressively. The use of exotic germplasm in the breeding of spring *B. napus* canola has been demonstrated by several researchers, such as improvement of earliness through the use of alleles from *B. oleracea* (Rahman et al. 2011) and increased seed yield in hybrids through the use of winter canola (Quijada et al. 2004). Although the positive correlation between silique length and number seeds per silique observed in the present study is in agreement with previous findings (Chay and Thurling 1989, Ivanovska et al. 2007), the results obtained for these two traits in terms of correlation with seed yield in this study were inconclusive.

Oil is the main product of canola; therefore, efforts should be made to increase seed oil content. Both three-way cross and F_2-derived populations included families that exceeded the checks for seed oil content. To date, at least 46 quantitative trait loci (QTL) have been identified on 16 of the 19 linkage groups of *B. napus* (Jiang et al. 2014), where positive alleles for high oil content can be found in different germplasm (reviewed in Rahman et al. 2013). Some of the three-way cross and F_2-derived families had higher oil content than the checks, suggesting that rutabaga may carry alleles for increased seed oil content, which have been introgressed into the three-way cross and F_2-derived families. This finding is in contrast to the use of winter *B. napus* canola in breeding, which often results in lines with lower oil content than the checks (Rahman et al. 2011).

Seed protein content remained on par or slightly higher than that of the spring-type *B. napus* checks. This trait varied greatly with site, indicating that there is a strong genotype × site

effect (Pritchard et al. 2000, Si et al. 2003). The negative correlation between seed oil and seed protein contents was confirmed in the current study. However, QTL have been identified that control seed oil content independently of protein content (Zhao et al. 2006). Further research will be needed to examine if protein content in the spring-type *B. napus* canola lines derived from *B. napus* × rutabaga crosses can be increased along with seed oil content.

Genotype × site interactions for different traits in *B. napus* have been well documented, with seed yield highly influenced by this interaction (Zhang et al. 2011, Marjanovic-Jeromela et al. 2011). However, according to Shi et al. (2003), seed quality does not share this genotype × site effect. Seed oil and protein content, although negatively correlated, did not show a distinct genotype × environment interaction across multiple sites; there was no genotype × site effect for seed oil or seed protein content. The results from the three-way cross-derived population are in agreement with those from the literature, although not for the F_2-derived *B. napus* × rutabaga cross population. Information about the effect of genotype × environment on glucosinolate content is limited, although the few reports available suggest that this interaction is minimal (Xu et al. 2015); however; both the three-way cross and F_2-derived populations showed significant genotype × environment effects.

Rutabaga requires vernalization to flower. Days to flowering and the vernalization requirement are under the control of several QTL (Osborn et al. 1997, Raman et al. 2012). In a spring canola breeding program involving the use of rutabaga in crosses, the vernalization requirement is introduced by rutabaga; therefore, this trait has to be selected against in the early generation segregating progeny. However, this was not an issue for the materials used in the present study.

Both the three-way cross and F_2-derived populations generated multiple families that met agronomic and canola-quality standards for the Canadian growing environment. Erucic acid and glucosinolate contents and delayed days to flowering and maturity all proved to be of no consequence in the advanced generation families, which was achieved through selection over several generations. The heterotic potential of these families to be used in hybrid breeding, including both general and specific combining ability, should be investigated to find potential families that could contribute to the development of superior hybrid cultivars.

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Chapter 3:

Screening of Three-way Cross and F_2-derived Families of Canola × Rutabaga Crosses for Resistance to Clubroot Caused by *Plasmodiophora brassicae*

3.0 Introduction

Clubroot disease, caused by the obligate parasite *Plasmodiophora brassicae,* commonly occurs worldwide in all *Brassica* species (Encyclopedia of Life 2014). This disease causes significant yield losses in all *Brassica* crops, including vegetable, fodder, forage and oil-producing types.

Anecdotal occurrences of clubroot were recorded as early as the Fourth century AD in Rome and more recently in 16th century Spain (Buczacki et al. 1985). From there, this disease spread to Great Britain and to all agricultural areas worldwide. The first reports in Canada were specific to vegetable production areas in British Columbia, Ontario and Quebec in the middle of the 20th century (DeWolfe 1962). *P. brassicae* was first confirmed in an agricultural field near St. Albert in 2002 (Howard et al. 2010). This disease has since spread to fields across Alberta and recently, into Saskatchewan (Tewari et al. 2005, Dokken-Bouchard 2011). Confirmed cases in both Manitoba (Canola Council of Canada 2011) and North Dakota have followed in recent years (Chittem et al. 2014). The lack of wild-type *B. napus* coupled with intensive breeding have led to a decrease in the genetic diversity of this already genetically narrow species (reviewed in Rahman 2013). By contrast, *P. brassicae* is genetically diverse. Pathotypes found in isolated cropping areas were found to be genetically distinct from each other, with no single genotype found to be common in two different growing areas (Strehlow et al. 2013). Resistance to all *P.*

brassicae pathotypes, notably 2, 5, 6 and 8, can be found in rutabaga, which can be used to breed clubroot-resistant *B. napus* canola cultivars (Rahman et al. 2014).

Durable resistance must be supplemented with various approaches as part of an integrated disease management strategy (Diederichsen et al. 2009; Donald & Porter 2009). All 'resistant' categorized canola cultivars can still exhibit minor galling under high disease pressure (Leboldus et al. 2012).

The objective of this study was to evaluate two advanced generation populations derived from a three-way cross and an F_2 population of spring canola × rutabaga crosses for resistance to *P. brassicae* pathotype 3, which is common to western Canadian agriculture. In addition, these populations were also tested against four newly emerged virulent *P. brassicae* pathotypes that were discovered in Alberta in 2014 (Dr. S. Strelkov, personal communication). Both three-way cross and F_2-derived populations were expected to produce families showing resistance to *P. brassicae* pathotype 3, as well as the newly emerging pathotypes found in Alberta.

3.1 Materials and Methods

3.1.1 *Brassica napus* Populations

Both the three-way cross and F_2-derived populations used in this experiment were obtained courtesy of the University of Alberta Canola Breeding Program. Initial crosses and selections for spring-type and canola-quality traits were made prior to the experiment, with advanced generation lines handed over for evaluation in this study.

In the fall of 2012, three-way $F_{5:6S}$ families derived from the three-way-cross of [A07-45 NR × Rutabaga-BF] × A07-26NR, and $F_{4:5S}$ and $F_{5:6S}$ families derived from the F_2 of Rutabaga-BF ×A07-26NR, were planted in a greenhouse supplied by the Crop Diversification Centre North (CDC-N), a Government of Alberta, Agriculture and Rural Development facility. Self-

pollinated seeds were harvested and retained for evaluation as separate families. These families were then planted in both field nursery plots in 2013 for advancement of superior families and in a clubroot-infested field in Leduc, AB in 2013 for *P. brassicae* resistance screening. The families originally planted at CDC-N were also screened for *P. brassicae* pathotype 3 in the greenhouse in winter 2012–2013. Self-pollinated seeds (e.g., three-way $F_{5:6S}$, $F_{4:5S}$, $F_{5:6S}$ seeds) from 2013 nursery plots were harvested and used for a greenhouse trial in winter 2013–14 and for a field trial in Leduc in 2014. For further information about the materials used in this study, please see Tables 2.1 and 2.2 in Chapter 2. An overview of clubroot resistance tests and the materials used in all trials is provided in Table 3.1.

3.1.2 *Plasmodiophora brassicae* Population

A single spore isolate of *P. brassicae* pathotype 3, provided by the Plant Pathology Laboratory of the Department of Agricultural, Food and Nutritional Science at the University of Alberta, was used for greenhouse screening. During the 2014 field growing seasons in Western Canada, four new pathotypes, similar to but distinct from pathotype 5, were identified in fields across northern Alberta. These pathotypes were preliminarily designated as LG-1, LG-2, LG-3 and DG-3 by the University of Alberta Plant Pathology Laboratory. These four variants of *P. brassicae* show virulence to the majority of resistant-classified *B. napus* cultivars to date, with no viable sources of resistance to these four pathotypes yet identified (Dr. S. Strelkov, personal communication). Therefore, the spring canola lines derived from *B. napus* × rutabaga crosses were tested against these new pathotypes in the greenhouse as well.

Field tests in both the 2013 and 2014 growing seasons were performed in a clubroot-infested field near Leduc, Alberta. This field is known to contain a mixture of different *P. brassicae* pathotypes, including pathotype 3.

Table 3.1. Spring *Brassica napus* families derived from spring *B. napus* canola × rutabaga crosses tested for resistance to *Plasmodiophora brassicae*

Cross	Generation	No. of families	Seeding time	Location
Three-way cross-derived families:				
[A07-45NR × Rutabaga-BF] × A07-26NR	Three-way $F_{5:6S}$	80	Winter 2012–13	Greenhouse
[A07-45NR × Rutabaga-BF] × A07-26NR	Three-way $F_{5:7S}$	207	June 2013	Field
[A07-45NR × Rutabaga-BF] × A07-26NR	Three-way $F_{7:8S}$	146	Winter 2013–14	Greenhouse
[A07-45NR × Rutabaga-BF] × A07-26NR	Three-way $F_{7:8S}$	136	June 2014	Field
[A07-45NR × Rutabaga-BF] × A07-26NR	Three-way $F_{7:8S}$	48	October 2014	Greenhouse
F_2-derived families:				
Rutabaga-BF × A07-26NR	$F_{4:5S}$	3	Winter 2012–13	Greenhouse
	$F_{5:6S}$	22	Winter 2012–13	Greenhouse
Rutabaga-BF × A07-26NR	$F_{4:6S}$	9	June 2013	Field
	$F_{5:7S}$	41	June 2013	Field
Rutabaga-BF × A07-26NR	$F_{6:7S}$	6	Winter 2013–14	Greenhouse
	$F_{7:8S}$	44	Winter 2013–14	Greenhouse
Rutabaga-BF × A07-26NR	$F_{6:7S}$	5	June 2014	Field
	$F_{7:8S}$	41	June 2014	Field
Rutabaga-BF × A07-26NR	$F_{6:7S}$	0	October 2014	Greenhouse
	$F_{7:8S}$	9	October 2014	Greenhouse

3.1.3 Greenhouse Experiments

All trials were conducted in a greenhouse located atop the Agriculture-Forestry building at the University of Alberta main campus. This greenhouse is specifically used for *P. brassicae* testing, and all biosafety regulations for handling this pathogen were followed. All experiments were conducted under a 16 hr photoperiod and 20°C/15°C ± 3ºC (day/night) temperatures. Self-pollinated seeds of each family (Table 3.1) were planted together with the susceptible *B. napus* checks. The checks used were cv. 'Hi-Q' or 'Q2'. Both cultivars were developed at the University of Alberta, and both are highly susceptible to all known *P. brassicae* pathotypes common to Western Canada *B. napus* canola production areas.

The experiments were conducted in 72-cell trays (ITML Horticultural Products, Brantford, Ont Canada). The trays were filled with Sunshine Professional Growing Mix (Seba Beach, AB, Canada), and eight seedlings per family were grown in eight cells around a susceptible check seedling (Figure 3.1). This layout allowed eight families to be evaluated per tray and provided reliable comparisons of a family against the susceptible check.

3.1.4 Preparation of Inoculum

Inoculum was prepared using the method reported by Strelkov et al. (2006), with some modifications. Pathotype 3 spore isolates were obtained from the Plant Pathology Laboratory of the University of Alberta in the form of galls from infected plant specimens. Spores were extracted from dried root tissue via grinding using an electric blender and mixed with sterile, distilled water at a rate of 8 g dry root material to 100 mL of distilled water to achieve a desired concentration of ~1.0 x 10^7 spores/mL. According to Voorips and Visser (1993), this spore concentration is ideal for successful inoculation. To obtain the spore suspension, the water/gall

homogenate was filtered through eight layers of cheesecloth (American Fiber and Finishing Inc., Albemarle, NC, USA) into an Erlenmeyer flask via a funnel. The resulting suspension was stored for no more than 2 days at 4°C before inoculations were carried out on plant material.

3.1.5 Inoculation

Two inoculation techniques were followed: direct inoculation and root-dip inoculation. For experiments conducted in winter 2012–13 and winter 2013–14 with pathotype 3, the direct inoculation technique was used. Direct inoculation of seedlings was performed by adding 1 mL of the spore isolate suspension into each cell containing a seedling using an Eppendorf pipette. Inoculation was performed one week after seeding, when the majority of seedlings were in the advanced cotyledon stage. After inoculation, the trays were kept partially submerged in water to ensure optimal conditions for successful infection. After one week of complete soil saturation, the trays were drained and the seedlings were watered as required.

Figure 3.1. Experimental design used to evaluate the advanced generation families derived from a three-way cross and an F_2 of spring canola × rutabaga crosses. P1 to P8 are the eight seedlings of a family; Check is seedling of the susceptible cv. Hi-Q or Q2

P1	P2	P3
P8	Check	P4
P7	P6	P5

The plants were supplemented with liquid fertilizer (20:20:20 of N:P:K) as required. At 45 days after seeding, the plants were removed from the trays and scored for severity of infection.

The greenhouse trial in 2014 was conducted to examine the three-way $F_{7:8S}$, $F_{6:7S}$ and $F_{7:8S}$ families for resistance to the new pathotypes LG-1, LG-2, LG-3 and DG-3. For this trial, instead of direct inoculation as described above, the root-dip method was followed. As described

by Strelkov et al. (2008), the seedlings were germinated on Whatman filter paper in 90 × 15 mm^2 size Petri plates for 7 days, inoculated by dipping the roots in spore suspension for 5 seconds and planted immediately in 72-cell trays as described above (Figure 3.1). To ensure successful inoculation, an additional 1 mL of spore suspension was added to each cell. After inoculation, the plants were fertilized and watered as described for the previous greenhouse experiments. Plants were scored at 45 days post seeding for resistance to these pathotypes.

3.1.6 Scoring

Forty-five days post seeding, plants were removed from the cells, and the roots were carefully washed with water and examined for galling. The check plant for each family was examined first to confirm successful inoculation. If the check plant exhibited moderate to severe galling, infection could be confirmed for the eight plants of the family surrounding it. Scoring was done on a 0–3 scale, where: 0 = no galling, 1 = a few small galls, 2 = moderate galling and 3 = severe galling (Kuginuki et al. 1999).

Clubroot resistance for each family was calculated based on the Disease Severity Index (DSI), which was developed by Horiuchi and Hori (1980) and modified by Strelkov et al. (2006):

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

In this equation, *n* is the total number of plants per class (0–3) and 0, 1, 2 and 3 are the symptom severity classes (Hasan et al. 2012). For the purposes of this experiment, 0% DSI is considered completely resistant, 0–10% moderately resistant, 10–40% moderately susceptible and 40–100% completely susceptible (Strelkov et al. 2007).

3.1.7 Field Evaluation

Over two successive seasons, both three-way cross and F_2-derived families were screened in a field confirmed positive for pathotype 3. Screenings took place at the same field southwest of Leduc, Alberta, which also served as a clubroot screening site for several private companies in the 2013 and 2014 growing seasons.

Each family was seeded in a single row two meters long, with checks seeded every 10th plot. Checks consisted of susceptible cultivars 'Hi-Q' and 'Q2', as well resistant cultivars '45H29' and '9568GC'. After flowering was completed, 25 plants per row were pulled and the roots examined. Individual plants in each row were scored following the scale described for the greenhouse experiments, and DSI for each family calculated.

3.1.8 Statistical Analysis Methods

Using the Welch's *t*-test function of SAS software, three-way cross and F_2-derived populations were analyzed to examine the variance between the two populations and to determine if the populations were statistically distinct. The formula for this test is as follows:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{{s_1}^2}{N_1} + \frac{{S_2}^2}{N_2}}}$$

, where $\bar{X}_1$is the population mean, ${s_1}^2$ is the sample variance and N_1 refers to the sample size. The Satterthwaite method (unequal variance) was used to determine if the two populations were distinct from each other. The threshold value to accept/reject the null hypothesis was set at p = 0.05. A p-value at or below 0.05 indicated that there was distinct variation between the three-way cross and F_2-derived populations for resistance to *P. brassicae*.

Examining the families for resistance to the four new pathotypes of *P. brassicae* (LG-1, LG-2, LG-3 and DG-3) required different statistical measures. LS means were calculated to

determine if populations from the various screenings were similar to each other using the Proc GLM procedure in SAS.

A Pearson correlation between DSI values for resistance to pathotype 3 and the four new pathotypes was calculated to study the relationship between the genetic control of resistance to these pathotypes. The Pearson correlation formula is as follows:

$$r = \frac{\sum(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum(X_i - \bar{X})^2 \sum(Y_i - \bar{Y})^2}}$$

where X refers to Pathotype 3 DSI and Y refers to each new pathotype DSI.

3.2 Results

3.2.1 Evaluation of Three-way Cross and F_2-derived Populations

Two experiments were conducted in the greenhouse in winter 2012–13 and in the field in 2013 with the three-way $F_{5:6S}$, $F_{4:5S}$ and $F_{5:6S}$ families. Of the 28 three-way $F_{5:7S}$ families, 82% were resistant to clubroot disease, while of the 25 $F_{4:5}/F_{5:6}$ families, 48% were resistant. Thus, a greater proportion of three-way cross-derived families showed resistance to pathotype 3. However, a lower proportion of families showed phenotypic resistance in the field compared to the greenhouse (Table 3.3). Amongst the three-way cross-derived population, 82% of families were resistant in the greenhouse experiment compared to only 33% in the field, and amongst the F_2-derived population, 48% of the families were resistant in the greenhouse experiment compared to 37% in the field. These results can be attributed to the variation in the pathogen in the field compared to the pathotypes used in the greenhouse experiment. Field populations of *P. brassicae* are often composed of a mixture of different pathotypes with different levels of pathogenicity to break down different resistance genes (Kuginuki et al. 1999), which may explain the lower resistance under field conditions. The checks were completely susceptible to

pathotype 3 in the greenhouse experiment, whereas only 84% of the check plots showed susceptibility in the field. This difference might have resulted from a lack of uniform disease infestation throughout the field.

Both three-way cross (three-way $F_{7:8S}$) and F_2-derived ($F_{6:7}$, $F_{7:8}$) populations were again evaluated in the greenhouse in winter 2013–14 and in the field in 2014. Resistance in the three-way cross-derived population appeared to have stabilized relative to the previous growing seasons, with 31% of families in the greenhouse completely resistant compared to 34% of families in the field trial (Table 3.2). In the F_2-derived population, 52% of families were completely susceptible in the greenhouse experiment compared to 63% in the field trial. The remaining F_2-derived families were segregating for resistance in both the greenhouse experiment and field trials, and none were completely resistant (Table 3.2).

The difference in the extent of resistance present in the three-way cross and F_2-derived populations is also evident from the estimates of mean DSI (Table 3.3). A *t*-test was used to compare the three-way cross and F_2-derived populations in each of the four experiments. The results show that the three-way cross and F_2-derived advanced generation populations were distinct for DSI (Table 3.3). LS means values of both three-way cross and F_2-derived populations tested in the four experiments were compared. The results from the three-way $F_{7:8S}$ population tested in both the greenhouse and field in 2014 were statistically similar. This population was derived from the three-way $F_{5:7S}$ population through selection for agronomic and seed quality traits. The non-significant difference between the three-way $F_{5:7S}$ and three-way $F_{7:8S}$ populations (Table 3.4) indicates that selection for agronomic and seed quality traits did not have a significant effect on resistance to *P. brassicae*. On the other hand, the F_2-derived population showed significant variation between generations. The $F_{6:7S}$ and $F_{7:8S}$ populations tested in the

greenhouse in winter 2013–14 and in the field in 2014 exhibited significantly higher DSI compared to the earlier generation populations (Table 3.4), which partly resulted from differences in selection pressure. As shown in Chapter 2 (Tables 2.3, 2.4), the variation for agronomic and seed quality traits was much higher in the F_2-derived population than in the three-way cross-derived population; therefore, more intense selection was applied to the F_2-derived population. Over the course of selection, families with resistance to *P. brassicae* could well have been selected against, leading to more distinct variation between the generations.

The greater proportion of families showing resistance to *P. brassicae* in the 2013 field trial may also have resulted from a lack of uniform infection throughout the field, as evidenced by the fact that some plants of the susceptible check cvs. 'Hi-Q' and 'Q2' showed resistant phenotypes.

3.2.2 Evaluation of Three-way Cross and F_2 Populations for Resistance to New Virulent CR Pathotypes

Representative samples of both three-way cross and F_2-derived families were evaluated for resistance to the newly discovered *P. brassicae* pathotypes, which are highly virulent to the available resistant canola cultivars of spring-type *B. napus* (Dr. S. Strelkov, University of Alberta, personal communication). Since none of the F_2-derived $F_{6:7S}$ and $F_{7:8S}$ families had complete resistance to pathotype 3, a representative sample of these families that were either susceptible or segregating for resistance to pathotype 3 was examined. A total of 57 three-way $F_{7:8S}$, $F_{6:7S}$, and $F_{7:8S}$ families were tested for resistance to the four new pathotypes. Seed germination in Petri dishes was performed, followed by root dip inoculation to ensure successful infection. Significant plant death occurred, particularly in the experiment with the DG-3 pathotype; therefore, all families could not be screened against all four pathotypes.

F_2-derived families were found to be completely susceptible LG-1, with a few families segregating for resistance to pathotypes LG-2, LG-3 and DG-3 (Table 3.5) Of the three-way cross-derived families (three-way $F_{7:8S}$), 27–42% of families showed resistance to at least one of the four pathotypes (Table 3.5); however, only 8% of the three-way cross-derived families were completely resistant to all four new pathotypes.

The LS means values for DSI (%) were calculated for the three-way cross and F_2-derived families (Table 3.6). The three-way cross-derived population showed greater resistance to all four new pathotypes compared to the F_2-derived population.

Table 3.2. Evaluation of three-way cross and F_2-derived advanced generation families of spring *B. napus* canola derived from spring *B. napus* canola × rutabaga crosses. Evaluation took place over two growing seasons, both in the greenhouses at the University of Alberta and at a clubroot-infested field near Leduc, Alberta. Susceptible cultivars Hi-Q and Q2 and cultivars 45-H29 and 9562GC, which are resistant to *P. brassicae* pathotype 3, were used as check cultivars.

	Families[a]				Number of Plants				
Experiments and Families	Total	No. R	No. Seg	No. S	Total	No. R	% R	No.S	% S
Greenhouse 2012-13									
Three-way $F_{5:6S}$	28	23 (82)	4 (14)	1 (4)	174	161	93	13	7
$F_{4:5S}$, $F_{5:6S}$	25	12 (48)	4 (16)	9 (36)	138	66	48	72	52
Hi-Q, Q2	2	0	0	2 (100)	53	0	0	53	100
Leduc 2013									
Three-way $F_{5:7S}$	207	68 (33)	28 (13)	111 (54)	4955	1944	39	3011	61
$F_{4:6S}$, $F_{5:7S}$	49	18 (37)	12 (24)	19 (39)	1161	530	46	631	54
Hi-Q, Q2	31[b]	0	5 (16)[c]	26 (84)	727	55[c]	8	672	92
Greenhouse 2013-14									
Three-way $F_{7:8S}$	146	45 (31)	72 (49)	29 (19)	1152	434	38	718	62
$F_{6:7S}$, $F_{7:8S}$	50	0	24 (48)	26 (52)	391	32	8	359	92
Hi-Q, Q2	2	0	0	2 (100)	196	0	0	196	100
Leduc 2014									
Three-way $F_{7:8S}$	74	25 (34)	9 (12)	40 (54)	1718	622	36	1096	64
$F_{6:7S}$, $F_{7:8S}$	32	0	12 (37)	20 (63)	785	35	4	750	96
Hi-Q, Q2	7[b]	0	0	2 (100)	175	0	0	175	100
45-H29, 9562GC	7[b]	2 (100)	0	0	175	175	100	0	0

[a]Percentage of the total number of families is shown in brackets.
[b]Indicates number of plots.
[c]Resistant phenotype due to lack of disease infection.

Families with variable levels of resistance to pathotypes 3, such as DSI (%) of 0%, 1–10%, 10–40% and >40%, were compared with families with resistance to the newly discovered pathotypes. The frequency distributions of the three-way cross-derived populations for resistance to pathotype 3 and to LG-1, LG-2, LG-3 and DG-3 are presented in Figures 3.2, 3.3, 3.4 and 3.5. A representative sample of the F_2 cross-derived families was scored for resistance to all four new pathotypes. Most of these families were completely susceptible to the new pathotypes similar to pathotype 3 and therefore, the data were not presented.

Approximately 50% of the three-way cross-derived families with a DSI of 0% for resistance to pathotype 3 had a DSI of 0% for resistance to LG-1, LG-2, LG-3 and DG-3 (Figures 3.2, 3.3, 3.4, 3.5). Families with more than 40% DSI for resistance to pathotype 3 tended to also be susceptible to most of the newly discovered pathotypes. Resistance to newly identified pathotypes LG-1, LG-2, LG-3 and DG-3 exists in these canola lines derived from spring canola × rutabaga crosses; however, further tests will be required to confirm if the families show stable horizontal resistance to all four new pathotypes of *P. brassicae.*

Table 3.3. Range and mean values of disease severity index (DSI, %) for resistance to *P. brassicae* in three-way cross and F_2-derived populations of spring *B. napus* canola × rutabaga crosses

	Three-way cross-derived families				F_2-derived families				*t*-test: three-way vs. F_2
Experiment	Generations	No. families	Range (DSI)	Mean ± SD	Generation	No. families	Range (DSI)	Mean ± SD	(p-value)
Greenhouse 2012–13	Three-way $F_{5:6S}$	28	0–100	66.1 ± 44.27a	$F_{4:5S}$, $F_{5:6S}$	25	0–100	46.1 ± 47.63a	0.05
Field 2013	Three-way $F_{5:7S}$	207	0–100	58.6 ± 47.02ab	$F_{4:6S}$, $F_{5:7S}$	49	0–100	44.0 ± 46.64a	0.05
Greenhouse 2013–14	Three-way $F_{7:8S}$	146	0–100	51.1 ± 40.42ab	$F_{6:7S}$, $F_{7:8S}$	50	8–100	85.2 ± 22.02b	0.0002
Field 2014	Three-way $F_{7:8S}$	74	0–100	53.9 ± 45.75b	$F_{6:7S}$, $F_{7:8S}$	32	5–100	81.5 ± 29.50b	0.0002

Within this column, values followed by same letters are not significantly different at $P < 0.05$.

Table 3.4. Evaluation of three-way $F_{7:8S}$, $F_{6:7S}$ and $F_{7:8S}$ families of spring *B. napus* canola derived from spring canola × rutabaga crosses for resistance to four new *P. brassicae* pathotypes. The percentage of total families is shown in brackets.

	Three-way Cross Derived					F_2 Derived				
Pathotype	Total Families	No. R	No. Seg	No. S	N/A*	Total Families	No. R	No. Seg	No. S	N/A*
LG-1	48	16 (33)	22 (46)	7 (15)	3 (6)	9	0	0	9 (100)	0
LG-2	48	13 (27)	15 (31)	9 (19)	11 (23)	9	0	1 (11)	8 (89)	0
LG-3	48	20 (42)	14 (29)	4 (8)	10 (21)	9	0	4 (44)	5 (56)	0
DG-3	48	15 (31)	12 (25)	0	21 (44)	9	0	5 (56)	4 (44)	0

R = Resistant; non-segregating Seg = segregating for resistance; S = susceptible
* All plants of the family died, so data are unavailable.

Table 3.5. LS means of disease severity index (DSI, %) for three-way cross and F_2-derived families of spring canola *B. napus* × rutabaga crosses for resistance to new pathotypes of *P. brassicae* discovered during the 2014 growing season

Pathotype	Population	LS mean ± SE
LG-1	Three-way cross derived	34.3 ± 2.89
LG-1	F_2 derived	91.3 ± 5.92
LG-2	Three-way cross derived	47.9 ± 6.08
LG-2	F_2 derived	100 ± 12.5
LG-3	Three-way cross derived	23.6 ± 5.27
LG-3	F_2 derived	85.0 ± 10.8
DG-3	Three-way cross derived	22.2 ± 5.21
DG-3	F_2 derived	80.1 ± 9.20

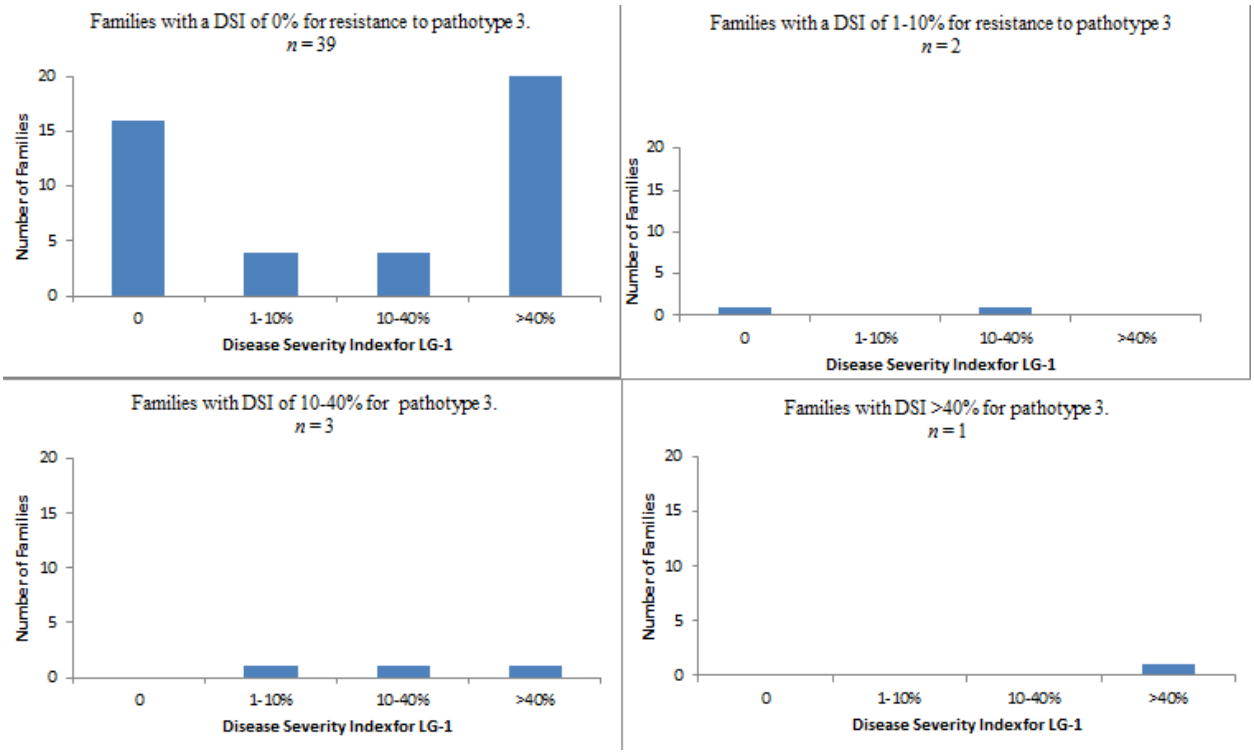

Figure 3.2. Distribution of three-way $F_{7:8S}$ families carrying different levels of resistance to *P. brassicae* pathotype 3 for resistance to newly discovered pathotype LG-1.

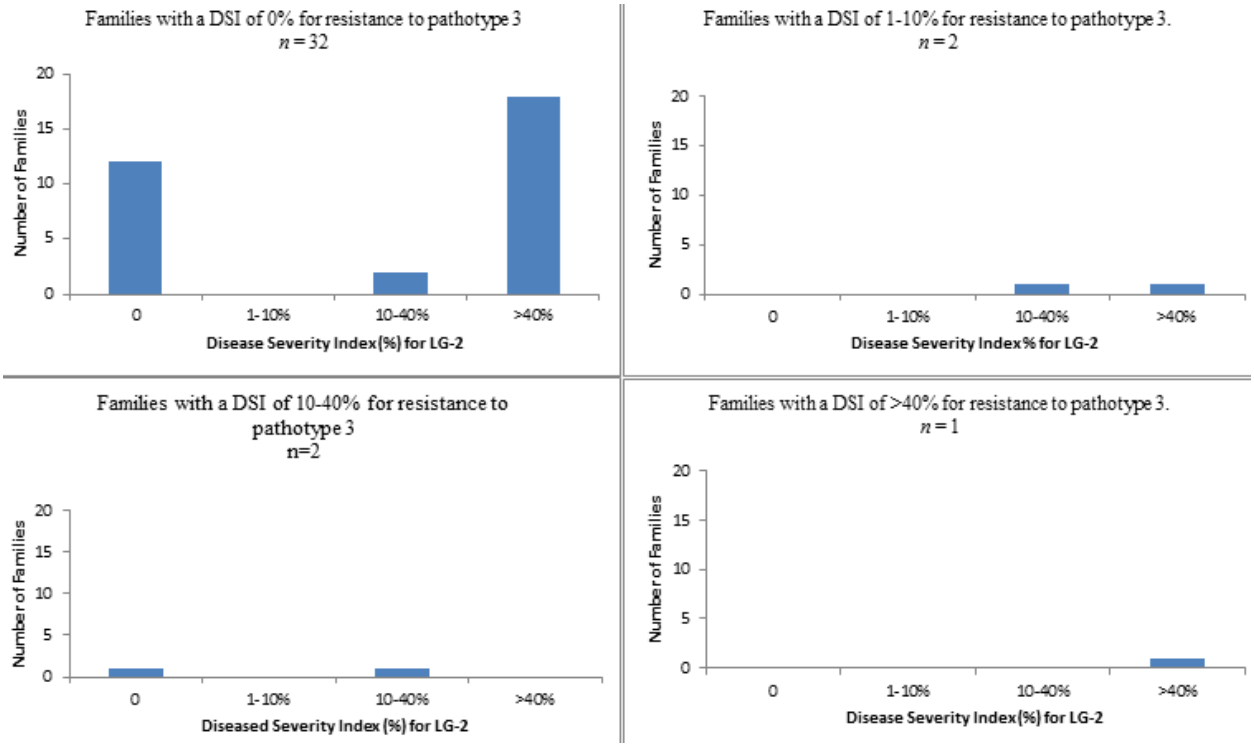

Figure 3.3. Distribution of three-way $F_{7:8S}$ families carrying different levels of resistance to *P. brassicae* pathotype 3 for resistance to newly discovered pathotype LG-2.

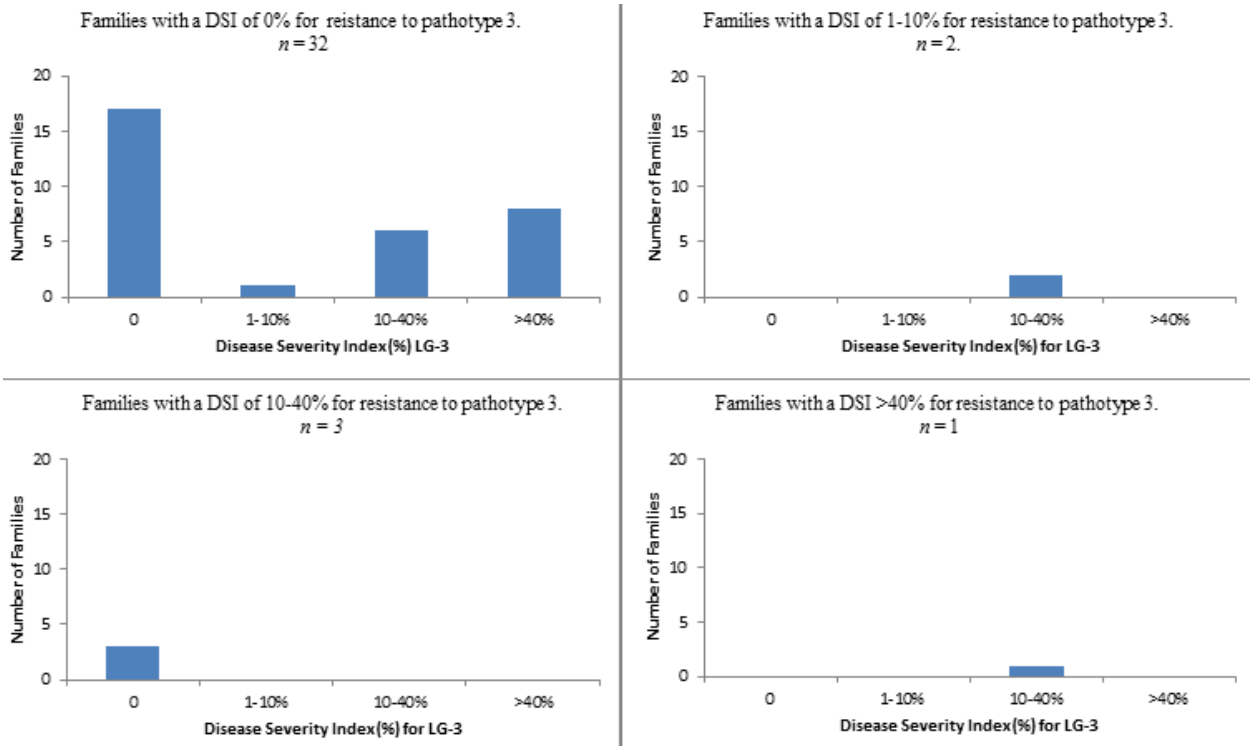

Figure 3.4. Distribution of three-way $F_{7:8S}$ families carrying different levels of resistance to *P. brassicae* pathotype 3 for resistance to newly discovered pathotype LG-3.

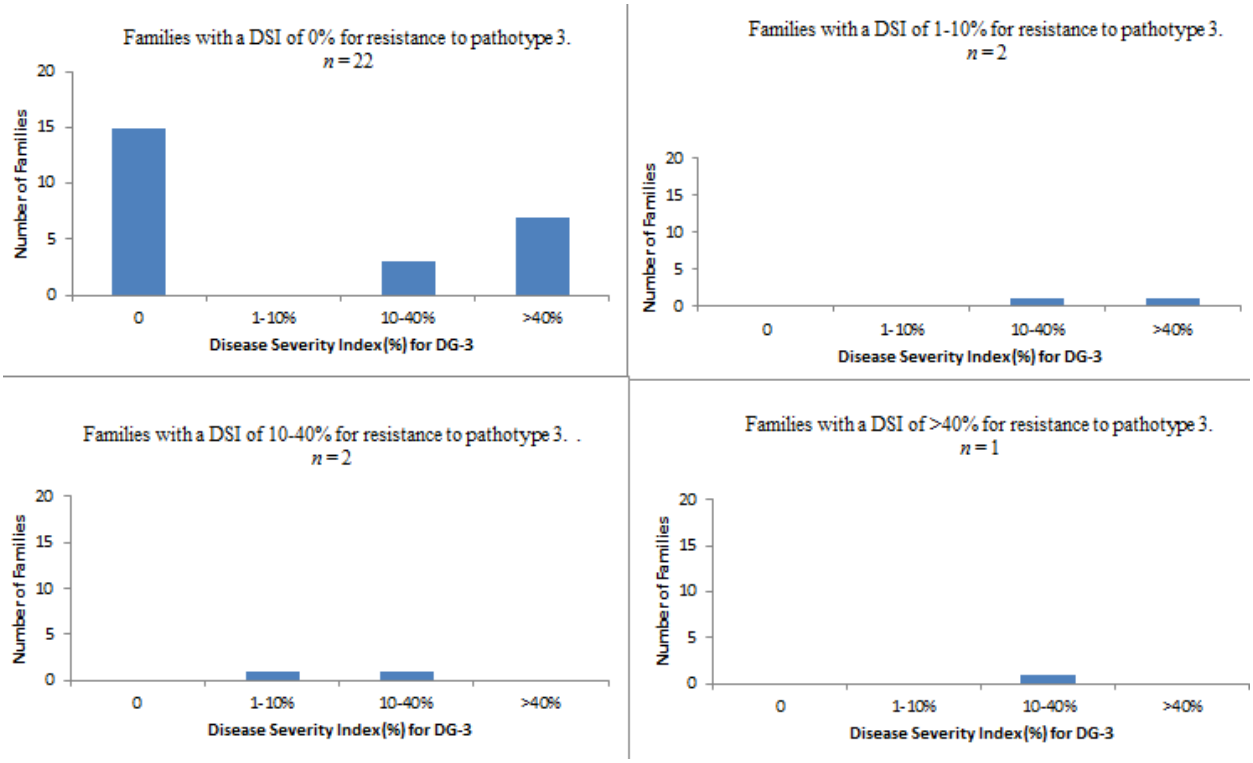

Figure 3.5. Distribution of three-way $F_{7:8S}$ families carrying different levels of resistance to *P. brassicae* pathotype 3 for resistance to newly discovered pathotype DG-3

3.3 Discussion

Resistance in a cultivar can break down in a short period of time if exposures to *P. brassicae* pathotypes are continuous (Leboldus et al. 2012). The mode of resistance is likely a dynamic process, as resistant plants showed no signs of infection at low levels. When pathogen pressure is high, resistant plants begin to show root hair infection, although the rate of infection is slower in resistant plants than in susceptible plants (Deora et al. 2013).

Many QTL for resistance to *P. brassicae* are found in several linkage groups of the *B. napus* genome (Werner et al. 2008, Manganeres-Dauleaux et al. 2000). Simple Mendelian genetic control of resistance has been identified in European turnip (*B. rapa*) and has been used extensively in the breeding of vegetable *B. rapa* cultivars (reviewed in Hirai 2006, Piao et al.

2009). Resistance in winter-type *B. napus* cv. Mendel appears to be controlled by a single Mendelian dominant gene derived from turnip (Diederichsen et al. 2009, Rahman et al. 2011). There are several sources of resistance in the *Brassica* A (*B. rapa*), B, *(B. nigra*) and C (*B. oleracea*) genomes (Hasan et al. 2011, Peng et al. 2014), although to date, the resistance genes of *B. rapa* confer strong resistance to the majority of *P. brassicae* pathotypes and resistance is often controlled by single Mendelian dominant gene (reviewed in Piao et al. 2009, Dixon 2014, Rahman et al. 2014). Many *B. rapa* resistance genes have been extensively studied and mapped (Zheng et al. 2014, Hatakeyama et al. 2013; reviewed in Piao et al. 2009). This species contains more major resistance genes than *B. oleracea* (Peng et al. 2014). In *B. oleracea,* both dominant and recessive control of resistance can be found (reviewed in Rahman et al. 2014). Breakdown of resistance controlled by a single gene has been reported in Japan (reviewed in Hirai 2006); therefore, introgressing multiple resistance genes is required for durable resistance in Canadian canola.

Introgression of multiple resistance genes into spring-type *B. napus* can be achieved through resynthesis of *B. napus* (Rahman 2004) from clubroot-resistant *B. oleracea* and *B. rapa*. Some accessions of *B. oleracea* and *B. rapa* (Hasan et al. 2011, Goa et al. 2014) carry resistance to *P. brassicae*. Creation of a synthetic *B. napus* would allow for introgression of as many *P. brassicae* resistance genes as are present in the two parental species of *B. napus*. The approach of using resynthesized *B. napus* to introgress multiple *P. brassicae* resistance genes into elite canola lines would require intensive breeding efforts, as resynthesized *B. napus* often carries poor agronomic and seed quality traits introduced from the parental species (reviewed in Rahman 2014). Marker assisted selection would increase the breeding efficiency of this process.

Resistance to *P. brassicae* is predominantly pathotype specific (Diederichsen et al. 2009). Continuous planting and the use of bait crops have not had a positive effect on limiting virulence (Ahmed et al. 2011). With the spread of *P. brassicae* over the majority of *B. napus* growing areas in Canada and worldwide, a more focused approach to breeding for resistance to clubroot is required. Some studies have focused on introducing the resistance gene in *B. napus* canola (Rahman et al. 2011, 2014); however, little research has been done on the *P. brassicae* pathogen itself. There are many virulent *P. brassicae* pathotypes that vary within different regions (Cao 2009); however, to date, no specific function of these virulence or avirulence genes has been identified (for further review, see Wallenhammar et al. 2014).

P. brassicae's position amongst other protists is still ambiguous; it is grouped most closely to *Spongospora subterranea* (Burki et al. 2010), a pathogen of the potato plant. Both pathogens are similar in that resistance can be identified within the plant genome and allied species, yet the mechanism of virulence of the pathogen remains unknown (Paget et al. 2014).

A study at the University of Alberta has demonstrated that some rutabaga genotypes show complete resistance to pathotypes 3 and 5, which are prevalent in Canada (Hasan et al. 2012). The *P. brassicae* resistance trait in rutabaga has yet to be mapped, although several QTL involved in the control of resistance to the pathotypes prevalent in Europe have been identified in winter oilseed *B. napus* (Manzanares-Dauleux et al. 2000). Control of resistance by a single, major gene (Ayers and Lelacheur 1972) and by a combination of dominant and recessive genes (Lammerink 1966) has also been reported in rutabaga.

Breeding efforts by the Canola Group at the University of Alberta have led to the production of spring-type families with canola-quality traits and complete resistance to *P. brassicae* pathotypes common to the Canadian Prairies (Rahman et al. 2014). The current study

identified several elite families amongst the three-way cross-derived populations of *B. napus* × rutabaga cross that met the agronomic and seed quality standards for growth on the Canadian Prairies (See Chapter 2). These families also showed phenotypic resistance not only to pathotype 3, but also to the newly emerging virulent pathotypes.

Future efforts should focus on fine mapping the resistance genes and identifying molecular markers tightly linked to resistance genes as they are discovered for use in marker assisted selection. Different accessions of rutabaga should be examined for resistance to *P. brassicae*, which can be used as potential parents for crossing with Canadian spring canola. Conscientious efforts must be made to limit opportunities for *P. brassicae* to develop virulence to the resistant sources derived from rutabaga and to allied species of *Brassica napus*.

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Chapter 4:

Examining Genetic Diversity in Both Three-way Cross and F_2-Derived Populations of Spring-type *B. napus* × Rutabaga Crosses

4.0 Introduction

Among the *Brassica* species, spring-type *B. napus* is the least genetically diverse (Hasan et al. 2006). *B. napus* (AACC, 2n = 38) was most likely derived from multiple interspecific crosses between *B. oleracea* (CC, 2n = 18) and *B. rapa* (AA, 2n = 20) in multiple growing areas (Rakow 2004). The lack of wide genetic diversity in spring canola is partly due to intensive breeding efforts carried out within a restricted gene pool (Fu and Gugel 2009, reviewed in Rahman 2013).

Although *B. napus* populations from the worldwide collection share some common alleles, there are also alleles specific to *B. napus* populations from Australia, EU/Canada, China and India (Chen et al. 2007, Ahmad et al. 2013). Rutabaga is genetically distinct from spring-type *B. napus* (Diers and Osborn 1994, Bus et al. 2011) and represents a viable source of germplasm for increasing the genetic diversity of spring-type *B. napus*. Crosses between rutabaga and spring-type *B. napus* are possible; however, the use of this germplasm in crossings introduces high erucic acid and glucosinolate contents in breeding populations (Rahman et al. 2014). In addition to these non-canola-quality traits, the use of this of exotic germplasm in breeding also introduces several other negative traits, such as delayed flowering and maturity

(Butruille et al. 1999, Rahman and Kebede 2013). Repeated cycles of breeding are required to achieve canola-quality standards combined with acceptable agronomic traits.

Molecular marker analysis is an excellent tool for examining genetic diversity in *B. napus* populations. Different types of markers can be used to detect polymorphisms in a *B. napus* population. Simple sequence repeat (SSR) markers have been used to genotype *B. napus* populations with great success (Rahman et al. 2015, Hasan et al. 2006). SSR markers are highly polymorphic and are thus suitable for detecting genetic variation over an entire population (Piquemal et al. 2005).

The objective of this study was to examine a set of advanced generation families derived from spring-type *B. napus* × rutabaga crosses for allelic diversity using SSR markers. Some families are expected to be similar to *B. napus* parents A07-26NR, as well as the rutabaga parent, with the majority of families interspersed somewhere in between the parents.

4.1 Materials and Methods

A set of three-way $F_{7:8S}$, $F_{6:7S}$ and $F_{7:8S}$ families was used for molecular marker analysis. All of these families meet canola-quality standards and possess spring growth habit. A list of these materials is presented in Tables 4.2 and 4.3 and can be found in Appendix 1. All families were previously phenotyped for resistance to clubroot disease. A total of 45 SSR markers from 19 *B. napus* linkage groups were used. SSR markers used in this study are listed in Table 4.1.

DNA was extracted as follows: Approximately 100 g of leaf tissue was collected from three-week-old seedlings grown in the greenhouse in winter 2013–14. Samples were collected and stored at -80ºC prior to DNA extraction. For DNA extraction, the tissue was ground in liquid N_2 using a pestle and suspended in 400 µL of DNA lysis solution (200 nM Tris-HCl pH 7.5, 250

nM NaCl, 250 mM EDTA pH 8.0, 0.5% SDS). Samples were incubated at 65°C for 15 minutes, combined with 130 µL of DNA precipitation solution, mixed by repeated inversion and incubated on ice for 5 minutes. After adding 400 µL of chloroform, each sample was centrifuged at 5,000 rpm for 10 minutes to pellet out unwanted plant debris. The aqueous layer was mixed with 700 µL of -20°C isopropanol and centrifuged at 8,000 rpm for 10 minutes. The supernatant was extracted, leaving only the pellet in the tube. The pellet was washed with 300 µL of -20°C ethanol, centrifuged at 5,000 rpm for 2 minutes, air dried for 15 minutes and resuspended in 200 µL of TE buffer. Samples were stored at 4°C until use.

PCR amplification was carried out in 15 µL reaction mixtures containing 3 µL of 10 ng/µL genomic DNA and 12 µL of PCR master mix consisting of 7.4 µL ddH_20, 2.5 µL 5x buffer, 1.0 µL 25 mM MgCl2, 0.25 µL of 10 mM ddNTP, 0.25 µL of 10 mM concentration of both forward and reverse primers and 0.125 µl 5 U/µL Taq DNA Polymerase (Promega Corporation, Madison, Wisconsin, USA). PCR was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, USA) under the following conditions: 1 cycle at 95ºC for 5 minutes, 35 cycles at 95ºC for 1 min, 56ºC for 1 min and 72ºC for 90 s. After 35 cycles, there was a final extension cycle at 72ºC for 30 min. Products were run through a 3% UltraPure™ agarose gel (Life Technologies, Burlington, Ont, Canada) for a minimum of two hours to allow for separation of the DNA fragments to be read. Gels were imaged and scored using a Typhoon FLA 9500 laser scanner (General Electric, Baie d'Urfe, Que, Canada).

4.2 Statistical Analysis Methods

Molecular marker data were arranged in an Excel file in a matrix. When a marker produced the expected size band in one of the three parents or in the progeny, it was recorded as '1' in the matrix, and if the expected band was not present, it was recorded as '0'. If a sample was lost due to pipetting error or other experimental error, it was marked as '9' so as not to be included in the analysis. The matrix was analyzed using Numerical Taxonomy System (NTSYS-PC) software, version 2.21 (Rohlf 1998). The Dice similarity coefficient was computed using the formula 2a/(2a+b+c), where a is the number of SSR bands shared by the genotypes in each pairwise comparison and b and c are the number of SSR bands present in one genotype but not the other (Soengas et al. 2006). Cluster analysis using the NTSYS program (Rohlf 1998) was completed using the unweighted pair group method with arithmetic averages (UPGMA). The sequential, agglomerative, hierarchic and non-overlapping (SAHN) cluster method was then used to test for goodness-of-fit between the similarity matrix obtained from the cluster analysis and the original similarity matrix inputted into NTSYS (Rohlf 1998). Data for the entire population were analyzed as a whole and separated to perform cluster analysis for the three-way cross and F_2-derived populations separately as well.

4.3 Results

A total of 45 markers from 19 *B. napus* linkage groups were tested for polymorphism between the three parents, of which 41 (91%) from 17 linkage groups (missing markers from A7 and A10) were found to be polymorphic. These markers were previously identified to be polymorphic between the rutabaga parent used in this study and a different *B. napus* line. These

markers showed high levels of polymorphism between the parents. The number of markers per linkage group ranged from 1 to 8, with a mean of 2.6 per linkage group.

Among the three parents, the Rutabaga-BF and spring canola line A07-26NR showed the greatest diversity, with a Dice similarity coefficient of approximately 0.55, while Rutabaga-BF and A07-45NR showed a similarity coefficient of approximately 0.74. A07-45NR was not a parent of the F_2-derived population; however, it was included in the dendrogram for the populations derived from F_2 for comparison. A07-45NR showed greater similarity to Rutabaga-BF than to the spring canola line A07-26NR (A07-26NR vs. A07-45NR similarity coefficient = 0.74), perhaps because A07-45NR was developed from a winter × spring canola cross and because the rutabaga parent used in this study might be genetically closer to winter canola than to spring canola.

Significant genetic diversity was detected among both the three-way cross and F_2-derived families. When comparing the three-way cross and F_2-derived families, the F_2-derived families showed less genetic variation compared to the families derived from the three-way-cross (Figures 4.1, 4.2). This might have resulted from intensive selection for agronomic and canola-quality traits in earlier generations of these populations, as many undesired traits associated with rutabaga would have been selected against and thus, significant diversity would have been lost. This is also evident from pedigree information about these two populations. The F_2-derived population generated from only seven F_5 or F_6 families (Table 4.3), while the three-way cross-derived population generated from 27 three-way F_6 families (Table 4.2).

The families derived from both the three-way cross and F_2 formed several groups: several families falling close to Rutabaga-BF, A07-45NR and A07-26NR could be found. Several families, such as 3wy-17, 3wy-18, 3wy-19, 3wy-20, 3wy-63 and 3wy-64, which were previously

found in this study to be resistant to *P. brassicae* pathotype 3, showed greater similarity to the rutabaga parent than to either of the spring-type *B. napus* parents.

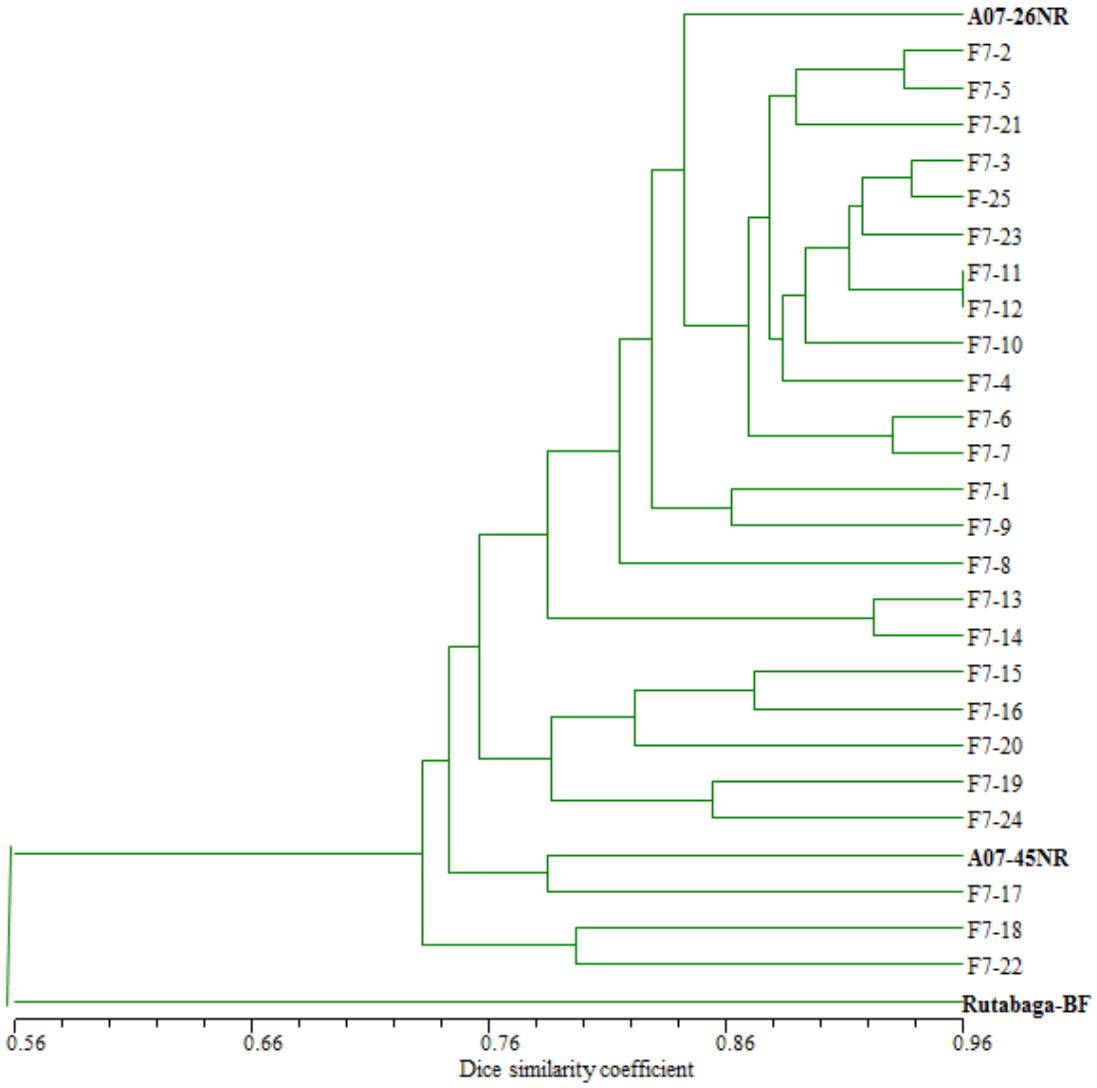

Figure 4.1. Genetic diversity, estimated using SSR markers, among the $F_{7:8S}$ and $F_{6:7S}$ families derived from spring *B. napus* canola × rutabaga crosses.

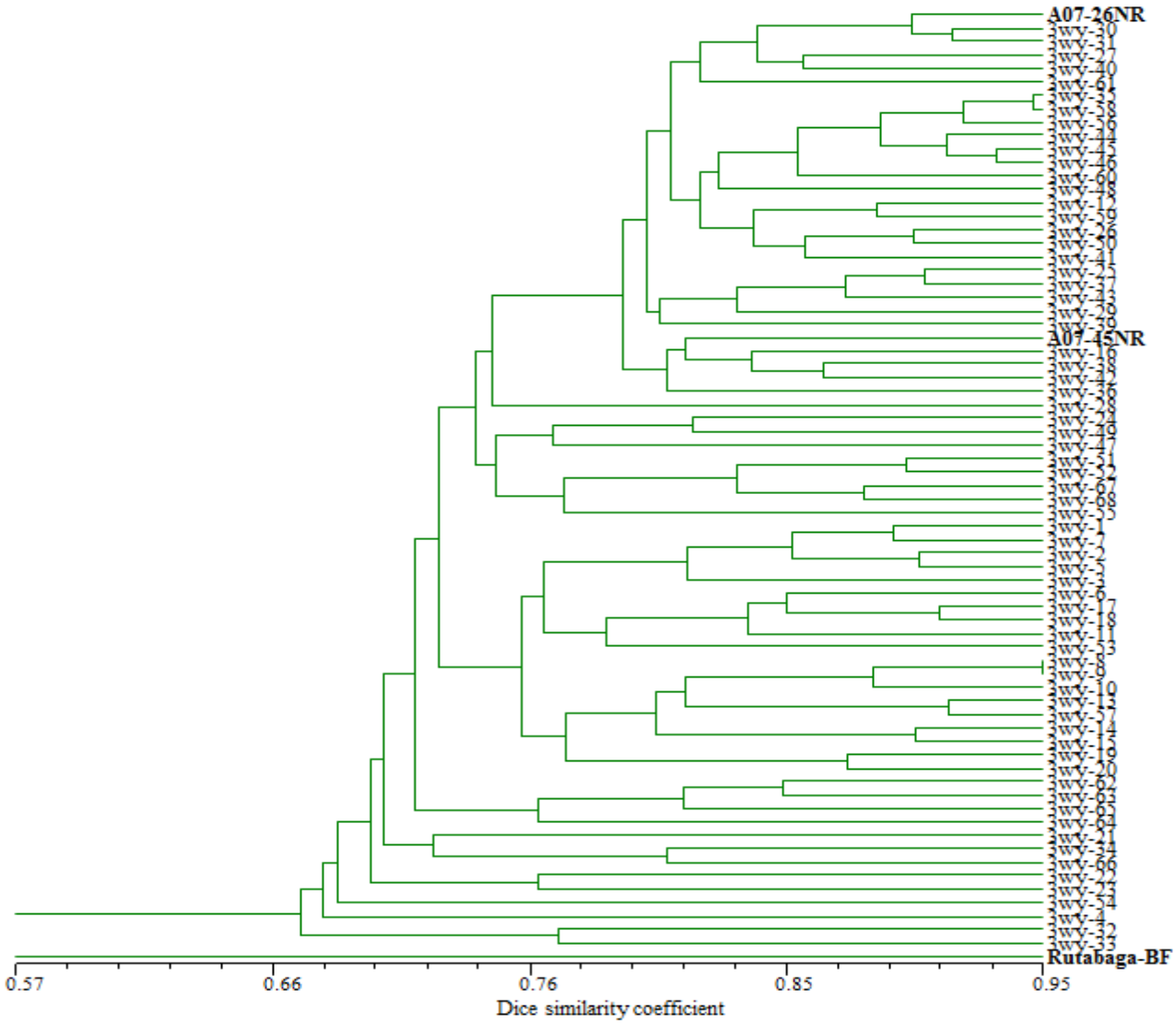

Figure 4.2. Genetic diversity, estimated using SSR markers, among the three-way $F_{7:8S}$ families derived from spring *B. napus* canola × rutabaga crosses

The families derived from both the three-way cross and F_2 formed several groups; several families falling close to Rutabaga-BF and A07-26NR could also be found. Several families, such as 3wy-17, 3wy-18, 3wy-19, 3wy-20, 3wy-63 and 3wy-64, which were found in this study to be resistant to *P. brassicae* pathotype 3, showed greater similarity to the rutabaga parent than to either of the spring-type *B. napus* parents

4.4 Discussion

Broadening genetic diversity of spring-type *B. napus* via interspecific crosses with different plant species within the *Brassica* genus has been studied extensively. Amongst *B. napus* germplasm, there is distinct variation for agronomic and seed quality characteristics (Hu et al. 2007). *B. napus* germplasm in each growing area is generally distinct worldwide (Ahmad et al. 2014), with spring-type *B. napus* possessing the least genetic diversity (Hasan et al. 2006). Introgression of allelic diversity from winter-type *B. napus* into spring type has demonstrated the potential for improving the seed yield of this crop in both hybrid (Quijada et al. 2006) and open-pollinated cultivars (Kebede et al. 2010). However, introgression of allelic diversity from winter-type into spring-type *B. napus* also introduces some unfavorable traits, such as lateness of flowering and delayed maturity (Kebede et al. 2010, Rahman and Kebede 2012); repeated cycles of breeding are often needed to improve these traits (Rahman et al. 2014).

Little research has been done on improving spring-type *B. napus* through introgressing allelic diversity from rutabaga, with such research only becoming a focus due to the strong resistance to *P. brassicae* present in rutabaga (Rahman et al. 2014). Rutabaga is genetically distinct from spring-type *B. napus* (Bus et al. 2011) and was most likely derived from a turnip-type *B. rapa* × *B. oleracea* cross (Bonnema 2012). Rutabaga is distinct from North American

populations of spring-type *B. napus*, as well as winter-type *B. napus* (Diers & Osborn 1994, Song & Osborn 1992).

A direct comparison of the two populations (three-way cross and F_2 -derived) examining the effectiveness of increasing genetic diversity in *B. napus* while retaining desirable agronomic and seed quality traits is difficult to make based on data from the current experiment. The three-way cross-derived population, possessing greater diversity, not only originated from the rutabaga parent, but also from winter-type canola through the parent A07-45NR, which was developed from a winter × spring canola cross. The three-way cross-derived population contained multiple families with greater genetic dissimilarity from the spring-type *B. napus* parent. These advanced generation families should be evaluated for heterosis for seed yield in a hybrid breeding program. Heterosis is the superior performance of F_1 hybrids relative to the better parent or midparent in a hybrid. Heterosis for seed yield in *B. napus* is under complex genetic control, with many loci exerting dominance and epistatic effects (Radeov et al. 2008). Genetic distances between parents are somewhat correlated with seed yield heterosis (Girke et al. 2012, Lees and Duncan 2014); however, this has not been proven in all cases (Qian et al. 2007). Spring-type lines derived from winter × spring crosses serve as good parents for high heterosis in spring canola hybrids (Quijada et al. 2006, Butruille et al. 1999).

Chinese semi-winter-type *B. napus* shows promise for use in spring canola breeding for high heterosis. In this case, heterosis for seed yield was found not to be dependent on the genetic diversity of the parents, instead relying on the general combining ability of the parents (Qian et al. 2007). Li et al. (2014) found that *B. napu*s plants derived from interspecific cross *B. napus* × *B. oleracea* showed heterosis for seed yield when crossed with natural *B. napus*. Thus, the

genetically distinct lines derived from spring canola × rutabaga crosses may have heterotic potential in spring canola hybrids; this needs to be investigated in the future.

Table 4.1. List of SSR used to detect polymorphisms in both three-way cross and F_2-derived spring-type *B. napus* × rutabaga crosses

Marker Name	Source	Marker #	Linkage Group
sN12112	AAFC[1]	72	4
sN12508II	AAFC	77	6
sN0412(a)	AAFC	217	4
sN3850(a)	AAFC	242	3
sN11641	AAFC	257	1
sORB29A	AAFC	311	18
sN3761	AAFC	315	2
sR12156	AAFC	318	6
CB10080	Mittasch et al. (2010)	357	5
BRMS-042	Suwabe et al. (2002)	396	17
CB10036A	Tsuda et al. (2012)	435	13
Na12-E02	Fayyaz et al. (2014)	461	3
O111-B05	Rygulla et al. (2008)	462	3
BnGMS291	Wang et al. (2015)	652	3
BnGMS416	Wang et al. (2015)	654	3
CB10064	Wen et al. (2015)	914	19
Na10D11	Geng et al. (2012)	983	15
Na10F06	Jiang et al. (2014)	984	14
KBRB023K01	Jiang et al. (2014)	1,029	9
BoGMS0702	Tomita et al. (2013)	1,084	13
sN37678 (a)	AAFC	2,041	6
sORB56 (aNP)	AAFC	2,079	12
BoGMS0631	Tomita et al. (2013)	2,245	8
sS1725	AAFC	2,307	11
sR042	AAFC	2,362	16
sN11904	AAFC	2,365	16
sN12940	AAFC	2,385	17
sORB17	AAFC	2,445	15
sORF31	AAFC	2,456	15
sN809	AAFC	2,495	4
A08_5021	Assembly v1.5[2]	2,577	8
A08_5024	Assembly v1.5	2,578	8
A02_497	Assembly v1.5	2,712	2
A03_2948	Assembly v1.5	2,719	3
A09_6143	Assembly v1.5	2,756	9
A09_17847	Assembly v1.5	2,759	9
KB59N08	Kato et al. (2013)	2,820	3
B4732	Kato et al. (2013)	2,826	3
A08_4610	Assembly v1.5	2,839	8
A08_4650	Assembly v1.5	2,842	8
A08_4661	Assembly v1.5	2,845	8

[1]Received from Agriculture and Agri-Food Canada (AAFC) through a material transfer agreement
[2]Designed by the Canola Program of the University of Alberta based on *Brassica rapa* genome sequence information

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Chapter 5: Discussion and Conclusion

5.0 Introduction

The narrow genetic base of spring-type *B. napus* (Hasan et al. 2006) is well known. Continued intensive spring-type *B. napus* production across Western Canada coupled with increasing disease pressures necessitate further research into increasing the genetic diversity of spring-type *B. napus* and identifying new sources of resistance to pathogens, including *P. brassicae*. Increasing the pool of germplasm for genetic diversity will increase the possibility of developing cultivars with improved agronomic traits and seed yield, as well as resistance to diseases (Burdon 2001).

The primary gene pool of *B. napus*, such as rutabaga, can be used to increase genetic diversity in spring canola (Rahman 2013). When crossing spring canola and rutabaga for introgression of genetic diversity, it will be important to evaluate these materials for resistance to clubroot disease. However, successful introgression of genetic diversity is moot unless the resulting germplasm are agronomically viable with the potential to be resistant to *P. brassicae* pathotypes.

5.1 Summary and General Discussion

Within both the three-way cross and F_2-derived populations of *B. napus* × rutabaga crosses were families that were on par with the check for DTF and DTM. Yields for both

populations were comparable to those of the checks, with some families exceeding check yields at some locations in both years of yield trials. Seed quality for these populations was equal to or exceeded that of the checks at all locations. Therefore, the agronomic performance of families derived from the *B. napus* × rutabaga crosses is not a concern, with families superior to the checks found in both the three-way cross and F_2-derived populations.

The spread of *P. brassicae* across the prairies has created the need for new sources of resistance to be identified and introgressed into spring-type *B. napus* on an immediate and continuous basis. Since its initial identification in Alberta fields in 2003 (Strelkov et al. 2006), the pathogen has spread across Saskatchewan and Manitoba, as well as North Dakota in the United States (Canola Council of Canada 2015, Markell et al. 2014). The current results indicate that populations derived from a spring-type *B. napus* × rutabaga cross can yield families that are resistant to multiple pathotypes of *P. brassicae* that are virulent to the spring-type *B. napus* sown across the prairies. The need for stable and durable resistance to *P. brassicae* requires spring-type *B. napus* × rutabaga cross populations to be studied to determine the genetic basis of resistance not only to pathotypes 3 and new pathotypes, but also to other pathotypes common to areas of Western Canada and worldwide.

Only the three-way cross-derived population produced families that had stable, non-segregating resistance to *P. brassicae* pathotype 3 and to new P. *brassicae* isolates found in Alberta in 2014. Although the F_2 -derived population contained no families with stable, non-segregating resistance to either pathotype 3 or to any of the new pathotypes, it is possible that resistance was inadvertently selected against in this population in the earlier generations. Early generation (F_2,$F_3$$F_4$, F_5) families were selected solely based on agronomic and seed quality traits and not on *P. brassicae* resistance.

The narrow genetic diversity in spring-type *B. napus* is well documented (Hasan et al. 2006), and *B. napus* var. *napobrassica* is genetically distinct from spring-type *B. napus* (Soengas et al. 2006, Bus et al. 2011). The use of rutabaga in breeding of spring canola is expected to broaden genetic diversity in this crop. The results of the current study, showing a similarity coefficient of ~0.68–0.90 between families within both the three-way cross and F_2-derived populations, confirm that rutabaga is a promising germplasm source for increasing genetic diversity in spring *B. napus*. Additionally, families that were resistant (and non-segregating) to both *P. brassicae* pathotype 3 and to some of the new pathotypes found in northern Alberta in 2014 were found to be genetically distinct from their spring-type *B. napus* parent and more similar to rutabaga. These results mirror the previous finding that spring canola lines with stable introgression of *P. brassicae* resistance to multiple pathotypes can be obtained from spring canola × rutabaga crosses (Rahman et al. 2014).

Families 3wy-17, 3wy-18, 3wy-19, 3wy-20, 3wy-63 and 3wy-64, with complete resistance to *P. brassicae* pathotype 3 and strong resistance to new pathotypes, had strong agronomic performance. The yields of these families were similar, and at separate sites, they outyielded the parental checks. These findings indicate that the progeny of a spring-type *B. napus* × rutabaga can produce families that are agronomically superior, high yielding and exhibit strong resistance to *P. brassicae* populations common to Western Canada. All of these populations were genetically more similar to rutabaga than to either spring-type *B. napus* parent, confirming that rutabaga is a viable source of germplasm for increasing the genetic diversity of spring-type *B. napus*.

5.2 Conclusions

Several conclusions can be drawn from the results of this study:

- Families derived from spring-type *B. napus* × rutabaga crosses can meet the standards of their spring-type *B. napus* parents for major agronomic and seed quality traits. Both three-way cross and F_2 cross families met these standards.
- Spring-type families from *B. napus* × rutabaga crosses, particularly from the three-way cross-derived populations, produced families with strong phenotypic resistance to both *P. brassicae* pathotype 3 and newly emerged pathotypes. Although the advanced-generation families derived from F_2 did not produce a single family with stable resistance to any *P. brassicae* pathotype, intensive selection for disease resistance and agronomic performance from early generations could identify families exhibiting stable resistance.
- Increasing genetic diversity of spring-type *B. napus* via the introgression of allelic diversity from rutabaga is possible, as shown in the genetic diversity analysis of the two populations derived from *B. napus* × rutabaga crosses. These spring-type *B. napus* crosses produced families with acceptable agronomic and seed quality traits that were genetically distinct from their spring-type *B. napus* parents; some of these families were more similar to the rutabaga parent.

5.3 Future Research Focus

The results of this study show that the genetic diversity of spring-type *B. napus* can be increased via the use of rutabaga. There are opportunities to exploit this gene pool to generate canola lines with improved agronomic traits and resistance to disease and to increase the genetic

diversity of Canadian spring canola. Seed oil content, controlled by multiple QTL, could be further improved using different rutabaga genotypes in breeding. Lines with higher oil content than the spring canola checks were found in this study; indeed, Delourme et al. (2006) found that high oil QTL alleles can be found in different *B. napus* populations. Selecting for genetically distinct rutabaga to cross with spring *B. napus* canola would increase the possibility of selecting high oil progeny from the resulting population.

Seed protein content and seed oil content in *B. napus* had a significant Genotype × Site interaction. Although there is evidence for a negative correlation between seed oil and protein content (Si et al. 2003), Zhao et al. (2006) found QTL that control seed protein content independently of seed oil content. Future efforts should focus on examining and exploiting different types of spring-type *B. napus* × rutabaga crosses for potential high oil and high protein QTL alleles. Increasing genetic diversity in *B. napus* via *B. napus* × rutabaga crosses has the potential to generate significant heterosis for seed yield; indeed, increased genetic diversity has a moderate relationship to heterosis for seed yield in *B. napus* (Riaz et al. 2001). The lack of published data regarding the heterotic potential of lines derived from *B. napus* × rutabaga crosses indicates that there is a need to further investigate the potential of using rutabaga in breeding for seed yield heterosis in spring canola.

The increasing occurrence of virulent pathotypes of *P. brassicae* across Western Canada necessitates further research to identify and introgress resistance to *P. brassicae* into spring-type *B. napus*. Although the current study revealed multiple families with resistance to *P. brassicae* pathotype 3 as well as new virulent pathotypes, this resistance was not successfully confirmed via molecular marker analysis. Future efforts should focus on mapping *P. brassicae* resistance genes in populations derived from spring-type *B. napus* × rutabaga crosses. Improving the

complicated and still largely ambiguous use of genetic resistance to different *P. brassicae* pathotypes (Manzanares-Dauleux et al. 2000, Rahman et al. 2014) in breeding will require extensive mapping work and identification of molecular markers (Zhang et al. 2015). Selection of disease resistant families could at least produce parental lines for use in the next cycles of breeding to produce agronomically superior spring-type *B. napus* lines with strong resistance, as reported by Rahman et al. (2014).

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Appendix 1

Table 4.2. List of three-way $F_{7:8S}$ families derived from spring-type *B. napus* × rutabaga cross screened for genetic diversity. ID numbers of these families were used in the dendrogram in this chapter.

ID	Three-way $F_{7:8}$ Families[1]	Three-way $F_{6:7}$ Families[2]	Three-way $F_{5:6}$ Families[3]	Pedigree
3wy-1	1RA1697.003-A1283	1RA1305.717-A1272	1RA1305.359-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-2	1RA1697.004-A1283	1RA1305.717-A1272	1RA1305.359-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-3	1RA1697.004-A1283	1RA1305.717-A1272	1RA1305.359-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-4	1RA1697.007-A1283	1RA1305.718-A1272	1RA1305.360-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-5	1RA1697.009-A1283	1RA1305.719-A1272	1RA1305.360-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-6	1RA1697.009-A1283	1RA1305.719-A1272	1RA1305.360-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-7	1RA1697.012-A1283	1RA1305.720-A1272	1RA1305.360-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-8	1RA1697.014-A1283	1RA1305.721-A1272	1RA1305.361-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-9	1RA1697.014-A1283	1RA1305.721-A1272	1RA1305.361-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-10	1RA1697.021-A1283	1RA1305.724-A1272	1RA1305.362-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-11	1RA1697.024-A1283	1RA1305.725-A1272	1RA1305.362-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-12	1RA1697.027-A1283	1RA1305.756-A1272	1RA1305.362-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-13	1RA1697.027-A1283	1RA1305.756-A1272	1RA1305.362-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-14	1RA1697.030-A1283	1RA1305.727-A1272	1RA1305.363-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-15	1RA1697.030-A1283	1RA1305.727-A1272	1RA1305.363-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-16	1RA1697.033-A1283	1RA1305.728-A1272	1RA1305.363-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-17	1RA1697.037-A1283	1RA1305.729-A1272	1RA1305.365-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-18	1RA1697.037-A1283	1RA1305.729-A1272	1RA1305.365-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-19	1AR1697.039-A1283	1RA1305.730-A1272	1RA1305.365-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-20	1RA1697.042-A1283	1RA1305.731-A1272	1RA1305.365-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-21	1RA1697.046-A1283	1RA1305.733-A1272	1RA1305.366-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-22	1RA1697.048-A1283	1RA1305.733-A1272	1RA1305.366-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-23	1RA1697.053-A1283	1RA1305.735-A1272	1RA1605.372-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-24	1RA1697.075-A1283	1RA1305.743-A1272	1RA1305.379-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-25	1RA1697.090-A1283	1RA1305.748-A1272	1RA1305.380-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-26	1RA1697.091-A1283	1RA1305.748-A1272	1RA1305.380-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-27	1RA1697.095-A1283	1RA1305.749-A1272	1RA1305.399-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-28	1RA1697.096-A1283	1RA1305.750-A1272	1RA1305.399-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-29	1RA1697.100-A1283	1RA1305.751-A1272	1RA1305.400-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-30	1RA1697.101-A1283	1RA1305.751-A1272	1RA1305.400-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-31	1RA1697.102-A1283	1RA1305.752-A1272	1RA1305.400-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-32	1RA1697.115-A1283	1RA1305.757-A1272	1RA1305.426-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR

3wy-33	1RA1697.116-A1283	1RA1305.757-A1272	1RA1305.426-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-34	1RA1697.118-A1283	1RA1305.758-A1272	1RA1305.426-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-35	1RA1697.125-A1283	1RA1305.760-A1272	1RA1305.466-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-36	1RA1697.127-A1283	1RA1305.761-A1272	1RA1305.466-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-37	1RA1697.128-A1283	1RA1305.761-A1272	1RA1305.466-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-38	1RA1697.129-A1283	1RA1305.762-A1272	1RA1305.470-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-39	1RA1697.137-A1283	1RA1305.765-A1272	1RA1305.472-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-40	1RA1697.138-A1283	1RA1305.765-A1272	1RA1305.472-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-41	1RA1697.138-A1283	1RA1305.765-A1272	1RA1305.472-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-42	1RA1697.144-A1283	1RA1305.767-A1272	1RA1305.502-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-43	1RA1697.145-A1283	1RA1305.768-A1272	1RA1305.502-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-44	1RA1697.145-A1283	1RA1305.768-A1272	1RA1305.502-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-45	1RA1697.147-A1283	1RA1305.768-A1272	1RA1305.502-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-46	1RA1697.149-A1283	1RA1305.769-A1272	1RA1305.502-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-47	1RA1697.167-A1283	1RA1305.775-A1272	1RA1305.594-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-48	1RA1697.170-A1283	1RA1305.777-A1272	1RA1305.595-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-49	1RA1697.172-A1283	1RA1305.778-A1272	1RA1305.595-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-50	1RA1697.186-A1283	1RA1305.783-A1272	1RA1305.599-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-51	1RA1697.187-A1283	1RA1305.783-A1272	1RA1305.599-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-52	1RA1697.187-A1283	1RA1305.783-A1272	1RA1305.599-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-53	1RA1697.193-A1283	1RA1305.785-A1272	1RA1305.603-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-54	1RA1697.198-A1283	1RA1305.787-A1272	1RA1305.603-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-55	1RA1697.203-A1283	1RA1305.789.A1272	1RA1305.608-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-56	1RA1697.213-A1283	1RA1305.792-A1272	1RA1305.609-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-57	1RA1697.214-A1283	1RA1305.793-A1272	1RA1305.609-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-58	1RA1697.217-A1283	1RA1305.794-A1272	1RA1305.610-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-59	1RA1697.217-A1283	1RA1305.794-A1272	1RA1305.610-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-60	1RA1697.220-A1283	1RA1305.795-A1272	1RA1305.610-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-61	1RA1697.223-A1283	1RA1305.796-A1272	1RA1305.616-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-62	1RA1697.233-A1283	1RA1305.800-A1272	1RA1305.621-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-63	1RA1697.233-A1283	1RA1305.800-A1272	1RA1305.621-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-64	1RA1697.234-A1283	1RA1305.800-A1272	1RA1305.621-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-65	1RA1697.237-A1283	1RA1305.801-A1272	1RA1305.621-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-66	1RA1697.239.A1283	1RA1305.800-A1272	1RA1305.621-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-67	1RA1697.245-A1283	1RA1305.804-A1272	1RA1305.626-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR
3wy-68	1RA1697.245-A1283	1RA1305.804-A1272	1RA1305.626-A1262	[A07-45 NR (P5) × Brookfield-9005 (P3)] × A07-26NR

[1]Corresponding unique accession for each individual ID grown in the field in 2014
[2]Corresponding unique accession for each individual ID grown in the field in 2013
[3]Corresponding unique accession for each individual ID at the beginning of MSc research in 2012

Table 4.3. List of $F_{6:7S}$ and $F_{7:8S}$ screened for genetic diversity. ID designates individual families used for the genetic diversity study, along with corresponding accession.

ID	$F_{7:8}$, $F_{6:7}$ Families	$F_{5:6}$, $F_{6:7}$ Families[2]	$F_{4:5}$, $F_{5:6}$ Families	Pedigree
F7-1	1RA1199.812-A1283	1RA1199.706-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-2	1RA1199.813-A1283	1RA1199.706-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-3	1RA1199.814-A1283	1RA1199.706-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-4	1RA1199.817-A1283	1RA1199.707-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-5	1RA1199.818-A1283	1RA1199.707-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-6	1RA1199.819-A1283	1RA1199.707-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-7	1RA1199.819-A1283	1RA1199.707-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-8	1RA1199.822-A1283	1RA1199.708-A1272	1RA1199.505-A1262	Brookfield-9005 X A07-26NR
F7-9	1RA1199.824-A1283	1RA1199.709-A1272	1RA1199.498-A1262	Brookfield-9005 X A07-26NR
F7-10	1RA1199.828-A1283	1RA199.710-A1272	1RA1199.498-A1262	Brookfield-9005 X A07-26NR
F7-11	1RA1199.831-A1283	1RA1199.711-A1272	1RA1199.498-A1262	Brookfield-9005 X A07-26NR
F7-12	1RA1199.831-A1283	1RA1199.711-A1272	1RA1199.498-A1262	Brookfield-9005 X A07-26NR
F7-13	1RA1199.834-A1283	1RA1199.712-A1272	1RA1199.502-A1262	Brookfield-9005 X A07-26NR
F7-14	1RA1199.834-A1283	1RA1199.712-A1272	1RA1199.502-A1262	Brookfield-9005 X A07-26NR
F7-15	1RA1199.836-A1283	1RA1199.713-A1272	1RA1199.502-A1262	Brookfield-9005 X A07-26NR
F7-16	1RA1199.836-A1283	1RA1199.713-A1272	1RA1199.502-A1262	Brookfield-9005 X A07-26NR
F7-17	1RA1199.853-A1283	1RA1199.717-A1272	1RA1199.500-A1262	Brookfield-9005 X A07-26NR
F7-18	1RA1199.853-A1283	1RA1199.717-A1272	1RA1199.500-A1262	Brookfield-9005 X A07-26NR
F7-19	1RA1199.896-A1273	1RA1199.729-A1262	1RA1199.639-A1252	Brookfield-9005 X A07-26NR
F7-20	1RA1199.896-A1273	1RA1199.729-A1262	1RA1199.639-A1252	Brookfield-9005 X A07-26NR
F7-21	1RA1199.899-A1273	1RA1199.730-A1262	1RA1199.640-A1252	Brookfield-9005 X A07-26NR
F7-22	1RA1199.899-A1273	1RA1199.730-A1262	1RA1199.640-A1252	Brookfield-9005 X A07-26NR
F7-23	1RA1199.903-A1273	1RA1199.731-A1262	1RA1199.640-A1252	Brookfield-9005 X A07-26NR
F7-24	1RA1199.903-A1273	1RA1199.731-A1262	1RA1199.640-A1252	Brookfield-9005 X A07-26NR
F7-25	1RA1199.832-A1283	1RA1199.711-A1272	1RA1199.498-A1262	Brookfield-9005 X A07-26NR

[1]Corresponding unique accession for each individual ID that was grown in the field in 2014
[2]Corresponding unique accession for each individual ID that was grown in the field in 2013
[3]Corresponding unique accession for each individual ID at the beginning of MSc research in 2012