Development of Strategies for the Integrated Management of Clubroot of Canola

by

ZhiYu Yu

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Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

Clubroot, caused by the soilborne pathogen *Plasmodiophora brassicae*, is a serious disease threatening the production of canola (*Brassica napus*) in Canada. This project explored various management options for clubroot, including fungicide application, fall and spring liming, and genetic resistance. In the first study, amisulbrom was evaluated for its effectiveness in managing clubroot. *In vitro* treatment of *P. brassicae* resting spores with amisulbrom inhibited spore germination by up to 79% and reduced viable spores by 31% relative to the control. Field and greenhouse experiments demonstrated that both liquid and granular formulations of amisulbrom significantly reduced the clubroot disease severity index (DSI) in both susceptible and moderately resistant canola cultivars ('45H31' and 'CS2000', respectively), relative to the untreated controls. In the second study, fall and spring applications of Zero Grind (ZG) limestone and spring applications of hydrated lime (HL) were assessed for their efficacy in clubroot management on '45H31' in the field, and both '45H31' and 'CS2000' in the greenhouse. Both field and greenhouse results showed that the most significant reductions in DSI were obtained with fall ZG and spring HL applications at a rate of 10 t ha^{-1}, regardless of the cultivar, while most lime treatments reduced resting spore load in the field soil. In the third study, an F_2 population derived from a *B. napus* ssp. *napobrassica* (rutabaga) donor parent FGRA106 and a susceptible spring-type *B. napus* cv. 'Sedo' was evaluated for resistance to *P. brassicae* pathotypes 3A, 3D and 3H. Chi-square (χ^2) goodness of fit tests indicated that the F_2 plants inherited two major clubroot resistance genes from FGRA106. Total RNA from plants resistant (R) or susceptible (S) to each pathotype were pooled and subjected to bulked segregant RNA-sequencing (BSR-Seq). Analysis of gene expression profiles identified 431, 67, and 98 differentially expressed genes (DEGs) between the R and S bulks. Collectively, the application

of amisulbrom and fall limestone demonstrated promising results for clubroot management. Additionally, the identification of resistance in FGRA106 and its associated genetic markers could contribute to the development of clubroot-resistant *B. napus* cultivars for deployment in western Canada.

Preface

This thesis is an original work by me, Mr. Zhiyu Yu, who conducted all of the experiments and wrote the first drafts of each chapter. My Supervisor, Dr. Stephen Strelkov, reviewed and revised all chapters of this thesis. My Co-Supervisor, Dr. Sheau-Fang Hwang, also approved the final version of each chapter prior to inclusion in my thesis. In addition, my Supervisory Committee member Dr. Rudolph Fredua-Agyeman also contributed to the revision of Chapter 4. Once the draft thesis was complete, my Supervisory Committee approved the dissertation to go to defense.

The *Brassica napus* germplasm and *Plasmodiophora brassicae* isolates included in this project were obtained from the collections maintained by the Plant Pathology and Applied Plant Pathology Labs of the University of Alberta. Mr. George Turnbull (University of Alberta) helped to seed, harvest and maintain the field experiments presented in Chapters 2 and 3. Ms. Yixiao Wang (University of Alberta) provided assistance with treatment applications, planting, inoculation and data collection for Chapters 2 – 4. Dr. Fredua-Agyeman provided guidance and suggestions on the experimental design employed in Chapter 4, while Dr. Longfei Wu (University of Alberta) assisted with RNA sampling and validation for this chapter. Other staff and summer students from the Plant Pathology and Applied Plant Pathology Labs provided technical assistance (e.g., weeding, liming and sampling) on occasion for all experiments described in this thesis.

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Chapter 1 – Introduction and literature review

1.1 Canola

The *Brassica* genus comprises 39 species and holds significant importance in agriculture (Al-Shehbaz 2012; Warwick 2011). The genomic relationships among the six cultivated *Brassica* species are described by the "Triangle of U" (Figure 1.1). These species share three core genomes, which include the three diploids *Brassica rapa* L. (2*n* = 20, AA), *Brassica nigra* L. (2*n* = 16, genome BB), and *Brassica oleracea* L. (2*n* = 18, genome CC). Additionally, three amphidiploids, namely *B. juncea* (2*n* = 36, AABB), *B. napus* (2*n* = 38, AACC) and *B. carinata* (2*n* = 34, BBCC), have emerged from hybridization and polyploidization of the diploids (U 1935; Warwick 2011). Each species within the *Brassica* genus hosts numerous important crops. For instance, *B. rapa* includes pak choy, Chinese cabbage, and turnip, while *B. oleracea* encompasses broccoli, cauliflower, cabbage, kale, and Brussels sprouts. *B. napus* is known for canola (syn. oilseed rape) and rutabaga, while *B. nigra* and *B. carinata* include black mustard and Abyssinian mustard, respectively. Additionally, *B. juncea* contains brown and leaf mustards (Warwick 2011). These crops are cultivated for various purposes, serving as vegetables, fodder, oil, condiments, and biofuel.

Rapeseed has a long history of cultivation, with historical records dating back to 2000 B.C.E. in India and approximately 35 B.C.E. in China and Japan (Kole 2007). The use of rapeseed oil as a lamp oil is documented from at least the 13th century in Europe. The exceptional quality of rapeseed oil as a lubricant prompted large-scale cultivation during the industrial revolution, and its cultivation was subsequently introduced to Canada (Iniguez-Luy and Federico 2011; Kole 2007). During the Second World War, supplies of rapeseed oil were cut off, leading to increased rapeseed production in Canada (Casséus 2009). However, rapeseed oil

did not gain favor in the food market until the introduction of varieties with low erucic acid (<2% in the oil) and low glucosinolates (<30 mg/g in the meal). These "double-low" cultivars were first developed in Canada in the 1970s, with the term "canola" derived from "Canadian Oil Low Acid" (Casséus 2009; Kole 2007). The rapid expansion of canola acreage has led to the widespread adoption of "canola" as a generic term for edible rapeseed oil in North America and Australia.

Canola stands as the second most significant oilseed crop globally and is a highly profitable crop for Canada. In 2022, global production of canola/rapeseed reached 87 million tonnes, with Canada emerging as the largest canola producer, accounting for over one-fifth of world production (FAOSTAT 2024). Approximately 90% of the canola grown in Canada is destined for export markets in over 50 countries, led by the United States, China and Japan, with a total export value of CAD $14.4 billion (Canola Council of Canada 2024). The canola industry contributes significantly to the Canadian economy, generating annual economic benefits of CAD $29.9 billion and supporting 207,000 jobs (Canola Council of Canada 2024).

1.2 Clubroot of Canola

1.2.1 Background

Clubroot, caused by the soilborne pathogen *Plasmodiophora brassicae* Woronin, is an important disease of cruciferous crops. Disease progression is characterized by the formation of galls on the host roots, which interfere with normal root function, most notably water and nutrient uptake, and consequently result in wilting, yellowing, stunting, dehydration and even plant death ((Javed et al. 2022; Schwelm et al. 2015). Termed a 'disease of cultivation,' clubroot has historically been associated with the widespread cultivation of cruciferous crops (Feng et al. 2014; Howard et al. 2010). The earliest documented instances of clubroot-like symptoms can be traced back to

Europe, where Pallidus described "spongy roots" on vegetables in 4th century Italy. In 1539, Roy Diaz de Isla noted the occurrence of a "syphilitic condition" of cabbage roots in Spain, which was likely clubroot (Watson and Baker 1969). Afterwards, the disease rapidly expanded across Europe during the 17th and 18th centuries, being reported in England, Scotland, Ireland, France, Spain, Germany, Norway, Finland, Sweden, and Denmark. As cruciferous crops were grown widely during the industrial revolution, the occurrence of clubroot increased in the 19th century and brought more attention to this disease (Howard et al. 2010; Watson and Baker 1969). In the late 1860s, a severe clubroot outbreak occurred in St. Petersburg, Russia, where the causal organism was identified as *P. brassicae* by a Russian biologist, Mikhail Stepanovich Woronin (Watson and Baker 1969; Woronin 1878). Clubroot was introduced to the eastern United States and Japan in the 1850s and 1890s, respectively, with subsequent records of the disease in Canada and China by the 1910s (Chai et al. 2014; Howard et al. 2010; Ikegami et al. 1981; Watson and Baker 1969). At present, clubroot is known to affect cruciferous crops across regions with diverse climates and temperatures, and its presence has been recorded in over 80 countries (Dixon 2009; Saharan et al. 2021).

1.2.2 Yield losses on canola

Clubroot poses a global threat to the production of canola/rapeseed. In Sweden, a field trial showed up to 50% yield losses on rapeseed when the disease incidence was 91% (Wallenhammar et al. 1999). In China, the impact on rapeseed yields ranged from 23.5% to 56.4% with clubroot incidence ranging from 61.2% to 86.2% (Ren et al. 2012). In India, yield losses on rapeseed varied from 32.5% to 54.0% across regions (Bhattacharya et al. 2014). In Canada, yield losses of up to 30% to 100% were observed on susceptible canola cultivars grown in heavily infested fields in Alberta (Strelkov and Hwang 2014; Tewari et al. 2005), and losses of

80% to 91% were reported in Quebec (Pageau et al. 2006). In addition, infection was found to adversely affect canola seed quality, leading to reductions in 1000-seed weights and oil content by 26% and 6.1%, respectively (Pageau et al. 2006).

The extent of yield losses caused by clubroot is influenced by the level of host resistance. Strehlow et al. (2015) examined the risk of clubroot on winter oilseed rape under field conditions, and found that at inoculum densities of 10^6, 10^7, and 10^8 spores per gram of soil, yield losses in a susceptible cultivar reached up to 65%, 93%, and 100%, respectively. In contrast, yield losses in a resistant cultivar were significantly lower at only 2%, 3%, and 17%, highlighting the effectiveness of resistance in reducing the impact of the disease. Furthermore, Botero-Ramírez et al. (2022) developed regression models to explain the effect of clubroot on canola yield under field and greenhouse conditions. In field experiments carried out in central Alberta, Canada, it was found that yield decreased by 0.26% for every 1% increase in disease severity index (DSI). In the greenhouse, this percentage increased to 0.49%. Although the rate of yield loss was the same across cultivars, resistant hosts experienced lower overall yield reductions due to their reduced disease development (Botero-Ramírez et al. 2022).

1.2.3 Taxonomy

Taxonomically, *P. brassicae* is classified in the class Phytomyxea along with various other parasites of agricultural importance, such as *Spongospora subterranea* causing potato powdery scab, as well as *Polymyxa graminis* and *Polymyxa betae*, which serve as vectors of viruses to different crops (Adl et al. 2019; Karling 1968; Neuhauser et al. 2014; Ruggiero et al. 2015). The accurate taxonomic classification of Phytomyxea, a group of biotrophic parasites, has been a subject of debate (Adl et al. 2019; Burki et al. 2010). It was traditionally categorized as a class within the eukaryotic kingdom Protozoa, phylum Cercozoa, and subphylum Endomyxa.

However, advancements in sequencing technologies have led to the differentiation of Phytomyxea from other Cercozoans through phylogenomic approaches (Castlebury and Domier 1998; Neuhauser et al. 2014). While the classification proposed by Ruggiero et al. (2015) maintained Phytomyxea in its established position, a more recent nomenclature suggested by Adl et al. (2019) questions whether the kingdom Protozoa should be retained or replaced by more precise kingdoms and supergroups under different domains in a novel hierarchical system. The updated classification places Phytomyxea as an order within the eukaryotic domain Amorphea, supergroup Rhizaria, and phylum Endomyxa (Adl et al. 2019). This reclassification reflects the ongoing reassessment of *P. brassicae* and its closely related species to refine taxonomic frameworks to reflect advancing understanding of biological relationships.

1.2.4 Life cycle and epidemiology

As a biotroph, *P. brassicae* relies on a plant host to complete its three-stage life cycle: survival in soil as a resting spore, primary root hair infection, and secondary cortical infection (Kageyama and Asano 2009). The pathogen persists in the soil as subspherical or spherical resting spores, originating from decaying root galls. These resting spores can survive for over 17 years in the absence of a host (Wallenhammar 1996). Serving as primary inoculum, the resting spores germinate under suitable temperature, soil moisture, and pH conditions, facilitated by the presence of host root exudates (Gossen et al. 2014; Rashid et al. 2013). Concurrently, the root exudates alter the soil bacterial microbiome, thereby stimulating the germination of resting spores (Wang et al. 2023b). The process releases pyriform and biflagellate primary zoospores that swim to and infect root hairs by penetrating the cell wall and forming primary plasmodia inside the root hair (Kageyama and Asano 2009). Recent studies have shown that this primary infection can also occur in root epidermal cells (Liu et al. 2020).

The uninucleate plasmodia undergo nuclear division and form zoosporangia, each of which can release 4–16 secondary zoospores into the soil. These secondary zoospores penetrate the cortical tissue of the main root system, leading to secondary or cortical infection (Kageyama and Asano 2009). The pathogen then develops into intracellular secondary plasmodia within the host tissues, which are associated with altered host meristematic activities and hormonal regulation, such as auxin and cytokinin metabolism. This results in hyperplasia and hypertrophy of the affected tissues, leading to root gall formation (Liu et al. 2020; Ludwig-Müller 2009). The secondary plasmodium undergoes cleavage, ultimately developing into a new generation of resting spores within each root gall (Kageyama and Asano 2009; Liu et al. 2020). As the root galls decay, the resting spores are released back into the soil to serve as inoculum in future infections.

Clubroot primarily spreads through the movement of resting spores in the soil or via infected plant material (Howard et al. 2010), although dissemination of the pathogen through wind or water erosion (Gossen et al. 2014; Rennie et al. 2015), and potentially as an external contaminant on seeds and tubers (Rennie et al. 2011), has also been suggested. The transmission of *P. brassicae* resting spores in animal manure has been demonstrated (Chai et al. 2016; Watson and Baker 1969). Field equipment carrying infested soil has been the main vector for *P. brassicae* spread across fields in Alberta (Cao et al. 2009). Additionally, the prolific reproduction of the pathogen facilitates its establishment in previously non-infested fields. Observations in controlled environments indicate that primary root hair infection leads to the formation of plasmodia as early as one day post-inoculation, followed by the release of secondary zoospores at 3-5 days (McDonald et al. 2014). While infection can occur with a resting spore concentration

as low as 10^3 spores per gram of soil (Murakami et al. 2002b), up to 1×10^{10} resting spores can be produced from one gram of galls of a susceptible cultivar (Hwang et al. 2013).

1.2.5 Host range

All species in the family Brassicaceae are potential hosts of *P. brassicae* (Dixon 2009). Karling (1968) confirmed the susceptibility of 89 species across eight genera in this family, including *Brassica*, *Raphanus* and *Arabidopsis*. Similarly, in a recent study by Zamani-Noor et al. (2022) that tested 86 plant species from 19 families as potential hosts, only species in the Brassicaceae were found to be susceptible to clubroot. Nonetheless, Ren et al. (2016) and Ludwig-Müller et al. (1999) found that some species within other families of the order Brassicales showed reduced root development due to *P. brassicae* infection, while there was one case of nasturtium (*Tropaeolum majus* L.) that exhibited very mild clubroot symptoms only observable under the microscope (Ludwig-Müller et al. 1999). Notably, several common cruciferous weeds, including wild mustard (*Sinapis arvensis* L.), shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) and stinkweed (*Thlaspi arvense* L.), are also susceptible to clubroot (Karling 1968; Ludwig-Müller et al. 1999; Ren et al. 2016; Zamani-Noor et al. 2022). These weeds could serve as a reservoir for *P. brassicae,* enabling the maintenance and expansion of the pathogen population even in the absence of cruciferous crops (Howard et al. 2010; Karling 1968).

1.2.6 Detection and quantification of *P. brassicae* in soil

The accurate detection and quantification of *P. brassicae* resting spores in soil is crucial for assessing disease epidemics and formulating effective clubroot management strategies. The traditional method for detecting the presence of *P. brassicae* in soil involves a bioassay, where susceptible hosts are grown in the soil to be tested, and symptoms on plant roots are assessed after six to eight weeks (Faggian and Strelkov 2009). However, this approach is time- and labor-

intensive, and a low concentration of resting spores may not guarantee infection in host plants. An alternative method is to examine fluorescing resting spores in soil suspensions through microscopy (Takahashi and Yamaguchi 1988). Nonetheless, this method is subject to interference from fluorescing soil particles, and depends heavily on operator expertise (Faggian and Strelkov 2009). With the advancement of molecular techniques, more accurate diagnostic methods, based on the polymerase chain reaction (PCR) technique, have been adopted. For example, Cao et al. (2007) developed PCR primers targeting the ribosomal DNA region of the *P. brassicae* genome, offering high specificity and sensitivity in detecting the pathogen in soil or plant tissues. The subsequent development of quantitative PCR (qPCR) assays has enabled the measurement of the amount of *P. brassicae* DNA or resting spores in soil or plant samples (Rennie et al. 2011; Wallenhammar et al. 2012).

1.2.7 Physiologic specialization

Populations or field isolates of *P. brassicae*, collected from infested soil or plant material, exhibit differential virulence on different host genotypes. Therefore, testing of these isolates for their virulence patterns on a specific group of hosts, known as a differential set, can enable their classification into distinct pathotypes based on virulence phenotypes (Buczacki et al. 1975). In recent years, the advent of molecular techniques and high-throughput genotyping has increased interest in the development of molecular markers for the identification of *P. brassicae* pathotypes (Tso et al. 2022). However, most pathotyping is still carried out based on traditional phenotypic bioassays (Javed et al. 2022). While very useful in determining the virulence structure of pathogen populations, the inoculation of host differential sets is a time-consuming and labor-intensive process, requiring a minimum of six weeks from inoculation to disease rating,

substantial space in plant growth facilities, and considerable labor (Javed et al. 2022; Tso et al. 2022).

Multiple host differential sets have been proposed to identify pathotypes of *P. brassicae*, including the systems of Williams (1966), Somé et al. (1996), and the European Clubroot Differential (ECD) (Buczacki et al. 1975). The differential set of Williams (1966), which consists of two rutabagas (*B. napus* ssp. *napobrassica*) 'Laurentian' and 'Wilhelmsburger', and two cabbages (*B. oleracea* var. *capitata*) 'Badger Shipper' and 'Jersey Queen', can distinguish a theoretical maximum of 16 pathotypes, and has been widely accepted worldwide due to its simplicity (Pang et al. 2020; Strelkov et al. 2018). However, these differentials lack differentiating capacity for the complex virulence of *P. brassicae* strains (Buczacki et al. 1975; Pang et al. 2020; Strelkov et al. 2018). Therefore, in recent years the Canadian Clubroot Differential (CCD) and the Sinitic clubroot differential (SCD) have been proposed in response to local clubroot outbreaks in Canada and China, respectively (Pang et al. 2020; Strelkov et al. 2018). The CCD set has been rapidly and widely adopted in Canada since its introduction. It consists of 13 host genotypes, including the hosts of Williams, Somé et al., and selected ECD differentials, as well as the canola/rapeseed cultivars 'Mendel', 'Westar' and '45H29' (Strelkov et al. 2018). At present, each CCD pathotype is denoted by a number corresponding to its Williams classification, followed by a letter indicating its designation on the CCD hosts (e.g., pathotype 3A) (Askarian et al. 2021; Strelkov et al. 2018).

1.3 Management of Clubroot

1.3.1 Background

In Canada, clubroot was mainly a problem for the growers of cruciferous vegetables in British Columbia and from Ontario to the Maritimes during the 20th century (Howard et al. 2010). The

disease gained prominence in western Canada following its initial identification on a dozen canola crops in central Alberta in 2003 (Tewari et al. 2005). Since then, clubroot has spread rapidly across the province, with 3894 individual fields reported as infested in 2022, solidifying its status as one of the most important canola disease issues (Strelkov et al. 2023; Strelkov and Hwang 2014). Presently, clubroot has been reported in 10 provinces across the country, with notable pressure from pathotype shifts (McDonald et al. 2021). The adoption of clubroot-resistant (CR) canola cultivars has proven to be one of the most successful approaches for clubroot management (Hwang et al. 2014b). However, the repeated cultivation of cultivars with similar resistance imposes selection pressure on pathogen populations, leading to the development of pathotypes capable of overcoming this resistance (Strelkov et al. 2016, 2018). This concern is substantiated by the detection of shifts in *P. brassicae* pathotypes across the country, with an increasing number of "resistance-breaking" pathotypes reported (Hollman et al. 2023; McDonald et al. 2021). On the Prairies, as of 2020, 43 pathotypes of *P. brassicae* had been identified based on their virulence on the CCD set, with pathotypes 3A, 3D and 3H being predominant (Hollman et al. 2023). Given the shifts in the virulence of pathogen populations, there is a critical need to develop additional strategies, beyond genetic resistance, for the effective management of clubroot on canola.

1.3.2 Cultural practices

Disease avoidance

The most effective strategy for clubroot management is to maintain fields free of the pathogen. This involves implementing measures that prevent or restrict the transportation of soil or crop residues infested with *P. brassicae* (Hwang et al. 2014b). A crucial aspect of clubroot management schemes is the sanitation of field equipment and machinery, cutting off a primary

means for pathogen spread (Cao et al. 2009). Additionally, avoiding the planting of contaminated seeds or tubers, such as potatoes, can prevent the introduction of the pathogen (Rennie et al. 2011). Good weed management, particularly the removal of cruciferous weeds and volunteer canola, can help to eliminate alternative hosts for *P. brassicae* and contribute to effective clubroot prevention (Hennig et al. 2022).

Roguing, bait and decoy crops

Roguing, bait and decoy crops can be used to enhance the germination of *P. brassicae* resting spores and disrupt the life cycle of the pathogen before its proliferation (Donald and Porter 2009; Friberg et al. 2006; Murakami et al. 2004). Roguing refers to the removal of infected plants to prevent the release of additional inoculum, which was recommended in early studies from the 1940s and 1950s (Donald and Porter 2009). The strategy of planting and early removal of susceptible bait crops follows a similar rationale, which can further reduce soil inoculum levels (Murakami et al. 2000, 2004). Another approach involves the cultivation of non-host crops as decoys, to enhance resting spore germination and primary root hair infection without subsequent secondary cortical infection or the completion of the pathogen life cycle (Friberg et al. 2006). Despite their promise, the efficacy of these management approaches is generally observed only when *P. brassicae* resting spore concentrations are low to moderate; their efficacy under high infestation levels have been found to be very limited (Ahmed et al. 2011; Friberg et al. 2006; Murakami et al. 2004). In addition, these strategies may not be economical from a practical perspective, due to the extra costs and labour involved (Ahmed et al. 2011; Donald and Porter 2009).

Crop rotation

Wallenhammer (1996) reported a half-life of 3.6 years for *P. brassicae* resting spores, determined through soil bioassays, suggesting the necessity of long rotations away from susceptible host crops for adequate control. Dixon (2014) conducted calculations based on this half-life, indicating that in the absence of a susceptible host, it would take 18 years for a pathogen field population to decline to 3% of the original. However, with the advent of qPCR-based methods to measure soil inoculum loads, several studies have found that the decline in resting spore levels is not linear (Peng et al. 2015; Ernst et al. 2019). In field plot studies conducted in Quebec, Peng et al. (2015) observed a 90% reduction in resting spore loads after a ≥ 2-year break from canola, resulting in yield increases of 32% to 76%. Similarly, in an analysis of commercial fields across Alberta, Ernst et al. (2019) found a major decline in *P. brassicae* spore loads following a 2-year break from canola. These and other studies suggest that rotations out of canola for at least 2 years, along with the cultivation only of CR canola cultivars, have the potential to reduce inoculum buildup in the field and decrease the likelihood of shifts towards resistance-breaking pathotypes of *P. brassicae* (Hwang et al. 2019). Nonetheless, in heavily infested fields, the initial levels of inoculum may be sufficiently high to cause significant disease even after 2 years away from canola or longer (Ernst et al. 2019; Hwang et al. 2019). This suggests that while crop rotations contribute to reducing soil inoculum load, they may not be entirely sufficient on their own for effective clubroot management.

Soil amendments

The use of lime as a soil amendment for clubroot control has been a common agricultural practice since the early 19th century (Donald and Porter 2009). Lime products are effective in managing clubroot because the clubroot pathogen, *P. brassicae*, thrives in acidic soils but shows reduced development of plasmodia, zoosporangia, and gall formation in host root tissues when

the pH is 7.2 or higher (Myers and Campbell 1985; MA Webster 1986; Webster and Dixon 1991). The addition of calcium ions in an alkaline environment can further reduce *P. brassicae* resting spore viability and germination, as well as strengthen host root cell walls (Myers and Campbell 1985; Niwa et al. 2008; Webster and Dixon 1991). Both pH and calcium independently and synergistically inhibit zoospore invasion, zoosporangium maturation, and gall development (Niwa et al. 2008; Webster and Dixon 1991).

Several studies have examined the use of lime products for clubroot management, but the effectiveness of formulations, such as limestone (calcium carbonate, $CaCO_3$) and hydrated lime (calcium hydroxide, $Ca(OH)_2$), may vary under field conditions (Hwang et al. 2014b). The particle size of lime plays a significant role in its reactivity, with research showing that a powdery limestone leads to greater reduction in clubroot incidence compared to a coarser fraction (Donald and Porter 2009; Tremblay et al. 2005). Murakami et al. (2002) found significant reductions in clubroot severity on Chinese cabbage, up to 20%, following the application of limestone. Hwang et al. (2011) observed decreased severity with limestone application in canola fields, but results varied across different sites and years. Conversely, hydrated lime was found to be more effective than limestone. Tremblay et al. (2005) reported reductions in clubroot severity on cauliflower from 70% to 20-30% with the use of hydrated lime. In a study conducted by Hennig et al. (2022), a significant reduction in clubroot severity (34% to 36%) and a notable increase in seed yield (70% to 98%) were observed on a clubroot-susceptible canola cultivar when hydrated lime was applied to raise the soil pH from its original range of 5.2-5.5 to 7.2. In a separate study, Fox et al. (2022) compared hydrated lime and limestone, both of which were found to reduce clubroot severity, with hydrated lime demonstrating greater effectiveness.

Calcium cyanamide has been used as a soil amendment for clubroot-infested soil since the early 1900s (Dixon 2017). When it comes into contact with water in the soil, calcium cyanamide undergoes a reaction, creating calcium hydroxide and cyanamide (H_2CN_2). The calcium hydroxide controls clubroot by raising soil pH and introducing calcium ions as described above, while the cyanamide possesses fungitoxic properties, offering an additional layer of control (Cornforth 1971). Calcium cyanamide contains around 20% nitrogen and 50% calcium, making it an effective nitrogenous fertilizer. Multiple studies carried out in Europe, Australia, and North America have shown its efficacy in decreasing clubroot severity in cruciferous vegetables and rapeseed (Dixon et al. 1987; Dixon and Wilson 1983; Donald et al. 2004; McDonald et al. 2004; McGrann et al. 2016). Nonetheless, field experiments conducted in Canada yielded mixed results regarding the effectiveness of calcium cyanamide. While it had minimal impact on clubroot development in canola, it did cause a reduction in disease severity in *B. rapa* and *B. oleracea* (Hwang et al. 2011; McDonald et al. 2004; Tremblay et al. 2005). One significant drawback of calcium cyanamide is its relatively high cost, attributed to limited production, with prices reaching £0.57 (~$0.84 CAD) per kg (Alzchem Group, Trostberg, Germany; personal communication). Given a recommended application rate of 1000 kg per ha, the cost of applying calcium cyanamide would be approximately $840 CAD per ha. Additionally, the current production facilities for calcium cyanamide are located in China, Germany, and Japan (Dixon 2017), which limits its availability in Canada.

1.3.3 Chemical control

Soil fumigation

Soil fumigants are chemicals that form a toxic gas upon application to eliminate pests. However, they come with drawbacks, including potential safety concerns and harm to the entire microbial

community (Donald and Porter 2009; White and Buczacki 1977). As a result, soil fumigants are generally less favored as clubroot management tools and many, such as methyl bromide, have been restricted or prohibited (Donald and Porter 2009). Despite these limitations, there has been a growing interest in using soil fumigation as a strategy to combat localized *P. brassicae* infestations occurring as small patches within fields (Gossen et al. 2013). Two fumigants, metam sodium (Vapam) and dazomet (Basamid), have shown significant success in reducing clubroot on canola during field experiments in western Canada (Hwang et al. 2018; Zuzak et al. 2023). These fumigants may play a role in eliminating isolated infestations, particularly in controlling new introductions where *P. brassicae* is not already present.

Fungicide applications

Fungicides offer another option for managing clubroot, with a variety of options such as pentachloronitrobenzene (PCNB), trichlamide, flusulfamide, fluazinam, and cyazofamid showing effectiveness against the pathogen (Hwang et al. 2011; Mitani et al. 2003; Peng et al. 2014; Suzuki et al. 1995; Tanaka et al. 1999). Fluazinam, sold under brand names such as Allegro® and Omega®, has gained attention in Canada for its registration to control *P. brassicae* on vegetable crops. It works by inhibiting resting spore germination, root-hair infection, and cortical infection (Peng et al. 2014; Suzuki et al. 1995). However, while this fungicide is effective in mildly infested soil, its efficacy is reduced when faced with high inoculum pressure (Hwang et al. 2011; Tanaka et al. 1999).

Two mitochondrial respiration inhibitors, flusulfamide (Nebijin™) and cyazofamid (Ranman™), have garnered interest for their reported ability to inhibit the germination of *P. brassicae* resting spores (Mitani et al. 2003; Peng et al. 2014; Tanaka et al. 1999). These fungicides act through site-specific modes of action, blocking electron flow at one of two

ubiquinone-binding sites (Qo: quinone outside and Qi: quinone inside) in the mitochondrial complex III (Yamaguchi and Fujimura 2005). Both flusulfamide (Qo inhibitor) and cyazofamid (Qi inhibitor) have shown efficacy in reducing clubroot severity in canola field trials conducted in Canada (Hwang et al. 2011; Peng et al. 2014). This efficacy can be influenced by the application method, with soil drenches showing higher control compared to seed treatments (Peng et al. 2014). However, applying liquid fungicide before planting in the large-scale canola farming systems of western Canada may present practical challenges related to equipment, time, and labor constraints (Hwang et al. 2014b; Peng et al. 2014).

1.3.4 Genetic resistance

Sources of clubroot resistance

From an evolutionary perspective, canola, as the amphidiploid *B. napus* carrying an AC-genome, originated from the hybridization of two diploid species: the A-genome *B. rapa* and the C-genome *B. oleracea* (Warwick 2011). Resistance derived from *B. rapa*, *B. oleracea*, and *B. napus* germplasm has been the primary source for improving clubroot resistance in canola (Hasan et al. 2021). In *B. rapa*, certain accessions of turnips (subsp. *rapifera*), Pak Choi (subsp. *chinensis*), and Chinese cabbage (subsp. *pekinensis*) have shown resistance to clubroot (Hasan et al. 2021; Piao et al. 2009). Specifically, European fodder turnip (*B. rapa* subsp. *rapifera*) stands out as a significant source of resistance. Cultivars like ‘Siloga’, ‘Gelria’, ‘Milan White’, and ‘Debra’, as well as the ECD lines 01-04, which are known for their clubroot resistance, have been utilized as key donors in breeding programs (Hirai et al. 2004; Hirani et al. 2018; Kuginuki et al. 1997; Piao et al. 2004; Suwabe et al. 2003). In the case of *B. oleracea*, resistance is less commonly reported, but clubroot-resistant germplasm is primarily found in cabbage (subsp.

capitata), kale (subsp. *acephala*), and Brussels sprouts (subsp. *gemmifera*) (Crisp et al. 1989; Fredua-Agyeman et al. 2019; Hasan et al. 2012).

In *B. napus*, canola or rapeseed (subsp. *napus*) itself has limited clubroot resistance (Fredua-Agyeman et al. 2019). For example, the well-known, clubroot-resistant cultivar 'Mendel' was derived from the highly resistant turnip ECD 04 and a partially resistant cabbage ECD 15 (Diederichsen et al. 2006). The clubroot resistance found in many Canadian canola cultivars appears to have originated from 'Mendel' (Fredua-Agyeman et al. 2018). Conversely, rutabaga (*B. napus* subsp. *napobrassica*) has been frequently reported to exhibit clubroot resistance (Fredua-Agyeman et al. 2019; Hasan et al. 2012). Older rutabaga varieties like 'Laurentian' and 'Wilhelmsburger' are known to possess varying levels of clubroot resistance, with 'Wilhelmsburger' demonstrating resistance to most Canadian pathotypes of *P. brassicae* (Strelkov et al. 2018; Williams 1966). The Canadian rutabaga cultivars 'Brookfield' and 'Polycross' have been identified as possessing broad-spectrum resistance to multiple Canadian pathotypes, and have been utilized as resistance donors in canola breeding programs (Hasan and Rahman 2016; Wang et al. 2022). A study by Fredua-Agyeman et al. (2020) found that 88% of 124 Nordic rutabaga accessions showed resistance to at least one of the 16 pathotypes of *P. brassicae* tested from Canada. This suggests that rutabaga could serve as a valuable source of clubroot resistance to combat newly identified *P. brassicae* pathotypes.

Mapping of CR genes

Numerous clubroot resistance (CR) gene loci have been identified on seven out of 10 A-genome chromosomes in *B. rapa* and *B. napus*. The majority of these loci are mapped on chromosomes A03 and A08. On chromosome A03, these loci include *CRa*, *CRb, CRb^{kato}, CRd, CRq, CRk, Crr3, CRA3.7, Rcr1, Rcr2, Rcr4,* and *Rcr5* (Chu et al. 2014; Hatakeyama et al. 2017; Hirai et al.

2004; Hirani et al. 2018; Huang et al. 2017; Kato et al. 2013; Pang et al. 2018, 2022; Piao et al. 2004; Saito et al. 2006; Sakamoto et al. 2008; Ueno et al. 2012; Yu et al. 2016, 2017). Loci identified on chromosome A08 include *Crr1*, *CRs*, *PbBa8.1*, *Rcr9*, , *Rcr9*^{ECD01} and *qBrCR38-2* (Chen et al. 2013; Karim et al. 2020; Laila et al. 2019; Suwabe et al. 2003; Yu et al. 2017, 2022; Zhu et al. 2019). However, these CR loci were identified in various studies using different host germplasm and pathotypes of *P. brassicae*, and some of these loci may actually represent the same locus designated differently by different researchers (Hasan et al. 2021). Genomic hotspots are located on the top and bottom segments of the A03 chromosome, containing three reported loci (*CRd, CRq,* and *Crr3*) and five reported loci (*CRa*, *CRb, CRb*^{kato}*, Rcr1,* and *BraA.CR.a*), respectively. These loci could confer independent or similar resistance (Fredua-Agyeman et al. 2020b). Crute et al. (1983) suggested that clubroot resistance in *B. rapa* and *B. napus* appears to be controlled by dominant genes, while resistance in *B. oleracea* is more likely to be quantitative. Other studies have also shown that clubroot resistance in *B. oleracea* is determined by multiple loci, with quantitative trait loci (QTLs) for clubroot resistance present on all nine C-genome chromosomes (Voorrips et al. 1997; Rocherieux et al. 2004; Ce et al. 2021; Hasan et al. 2021).

Mechanisms of clubroot resistance

Plants have developed a dual-layered immune system to protect against pathogen attack (Dodds and Rathjen 2010). The first line of defense involves the recognition of evolutionarily conserved Pathogen-Associated Molecular Patterns (PAMPs) by Pattern Recognition Receptors (PRRs) on the host cell's outer surface, activating Pattern-Triggered Immunity (PTI) (Dodds and Rathjen 2010; Zipfel 2014). Subsequently, when pathogens overcome or evolve to suppress PTI by deploying specific effectors to enhance virulence, intracellular receptors associated with

resistance (R) genes detect these effectors, triggering a "gene-for-gene" interaction and initiating the second layer of defense known as Effector-Triggered Immunity (ETI) (Dodds and Rathjen 2010).

Most R genes encode proteins that contain a nucleotide-binding site (NBS) and leucine-rich repeats (LRRs), belonging to the NLR protein family (Eitas and Dangl 2010). The LRR domain recognizes specific pathogen effectors, called Avr proteins, which enhance virulence. Upon recognition, NBS-LRR triggers the exchange of AD*P* for AT*P* to modify the NBS domain, thus affecting the structure of NBS-LRR domains. NLR proteins are classified into subclasses based on different N-terminal domains, such as toll-interleukin receptor (TIR) and coiled-coil (CC) domains, which typically participate in the activation of defense responses (Eitas and Dangl 2010; Hasan et al. 2021). Multiple genes encoding NLR proteins have been reported to be involved in clubroot resistance (Chang et al. 2019; Hatakeyama et al. 2013; Huang et al. 2017; Ueno et al. 2012; Wang et al. 2023a; Yu et al. 2017).

To date, three R genes belonging to two types of NLR proteins that confer clubroot resistance have been identified, cloned, and characterized (Hatakeyama et al. 2013; Ueno et al. 2012; Wang et al. 2023a). Two of these R genes, *CRa* and *Crr1*, were cloned from *B. rapa*, and they encode TIR-NBS-LRR (TNL) proteins (Hatakeyama et al. 2013; Ueno et al. 2012). The specific mechanisms through which these proteins confer resistance to *P. brassicae* are not yet fully understood. Another recently cloned R gene, *WeiTsing* from Arabidopsis (*Arabidopsis thaliana* L.), encodes a CC-NBS-LRR (CNL) protein located in the endoplasmic reticulum. This protein releases cytosolic calcium ions through a cation-selective channel, triggering immune responses (Wang et al. 2023a). The efficacy of this gene in inducing clubroot resistance was validated in transgenic *B. napus* plants. Additionally, both TNL and CNL genes were found to be

highly expressed in the stele and cortical tissues in response to *P. brassicae* infection (Hatakeyama et al. 2013; Wang et al. 2023a).

1.3.5 Biological control

Biological control has garnered significant attention in clubroot research due to its potential as an effective and environmentally friendly tool for disease management (Javed et al. 2022). Various species of biological control agents, such as *Heteroconium chaetospira* (Lahlali et al. 2014; Narisawa et al. 2000), *Lysobacter* spp. (Fu et al. 2018; Zhou et al. 2014), *Trichoderma* spp. (Zhao et al. 2022), and *Bacillus* spp. (Lahlali et al. 2011; Zhu et al. 2020), have shown efficacy in controlling clubroot. In Canada, Peng et al. (2011) conducted experiments using registered biological control agents, including *B. subtilis* (Serenade), *Gliocladium catenulatum* (Prestop), *Streptomyces griseoviridis* (Mycostop), *S. lydicus* (Actinovate), and *T. harzianum* (Root Shield), to assess their efficacy against clubroot. Results showed that under controlled conditions, *B. subtilis*, *G. catenulatum*, and *S. griseoviridis* significantly reduced clubroot severity by 91% to 61%, respectively, under low to moderate inoculum pressure. However, these biocontrol agents did not demonstrate significant effects in field trials. Furthermore, additional field evaluations of Serenade and Prestop showed inconsistent efficacy in clubroot control across various crops and locations (Gossen et al. 2013). The adoption of biological control for clubroot management in Canada is hindered by challenges associated with the short growing season, winter survival, and the mass production and large-scale application of biocontrol agents. Currently, biological control is not widely used as a tool for clubroot management in Canada. Nonetheless, the potential of this approach for the long-term management of this disease is promising, and further research in this area should be encouraged.

1.4 Research objectives and hypotheses

In response to the ongoing spread of *P. brassicae* across the Prairies, the adoption of diverse clubroot management strategies is becoming increasingly important (Hwang et al. 2014b). Based on previous studies conducted with cruciferous vegetables (Kawasaki et al. 2014; Murakami et al. 2005) amisulbrom, a relatively novel fungicide with a mode of action similar to the Qi inhibitor cyazofamid, may hold promise for control of this disease on canola. However, further work is needed to evaluate its efficacy on this crop, particularly under western Canadian conditions, and to identify the optimal formulations of the fungicide.

Similarly, earlier reports have found that hydrated lime provides superior clubroot control compared to the less expensive limestone (Fox et al. 2022; Tremblay et al. 2005). However, these studies were based on spring application of both products. Given that limestone requires a longer period to increase soil pH, a fall application could potentially achieve enhanced clubroot control relative to spring limestone, and at a lower cost than hydrated lime (Havlin et al. 2005; Hwang et al. 2014b).

Ultimately, genetic resistance remains the preferred method for clubroot management, given its effectiveness and ease of use (Peng et al. 2014). However, with the emergence of resistance-breaking pathotypes of *P. brassicae* (Hollman et al. 2023; Strelkov et al. 2018), there is a pressing need for identification and incorporation of novel clubroot resistance sources (Hasan et al. 2021). Six rutabaga accessions identified by Fredua-Agyeman et al. (2020) exhibited broad-spectrum resistance to a large number of Canadian pathotypes, suggesting that the may be particularly helpful for the development clubroot-resistant canola.

In this context, the research presented in thesis aimed to achieve three main objectives:

1. Investigate the efficacy of granular fertilizer formulations containing amisulbrom against clubroot and compare their effectiveness with a liquid formulation
2. Assess the effectiveness of fall and spring applications of limestone, as well as spring applications of hydrated lime, for clubroot management
3. Evaluate an F_2 population derived from a rutabaga accession, FGRA106, which exhibited resistance to 16 *P. brassicae* pathotypes. Specifically, assess its resistance to pathotypes 3A, 3D, and 3H, and identify QTLs conferring clubroot resistance using bulk segregant RNA sequencing (BSR-seq)

The hypotheses corresponding to these objectives were that:

1. Both the liquid and granular fertilizer formulations containing amisulbrom can significantly reduce clubroot severity compared to untreated controls, and there is no difference in the efficacy of the formulations
2. Applications of limestone and hydrated lime can significantly reduce clubroot severity compared to untreated controls. Fall applications of limestone demonstrate a significantly greater reduction in clubroot severity compared to spring applications. The effects of fall limestone and spring hydrated lime applications at the same rate do not differ significantly
3. Phenotypic segregation of clubroot resistance will be observed in the F_2 population derived from FGRA106, and the resistance of this population to pathotypes 3A, 3D, and 3H is conferred by different QTLs

1.5 Figures

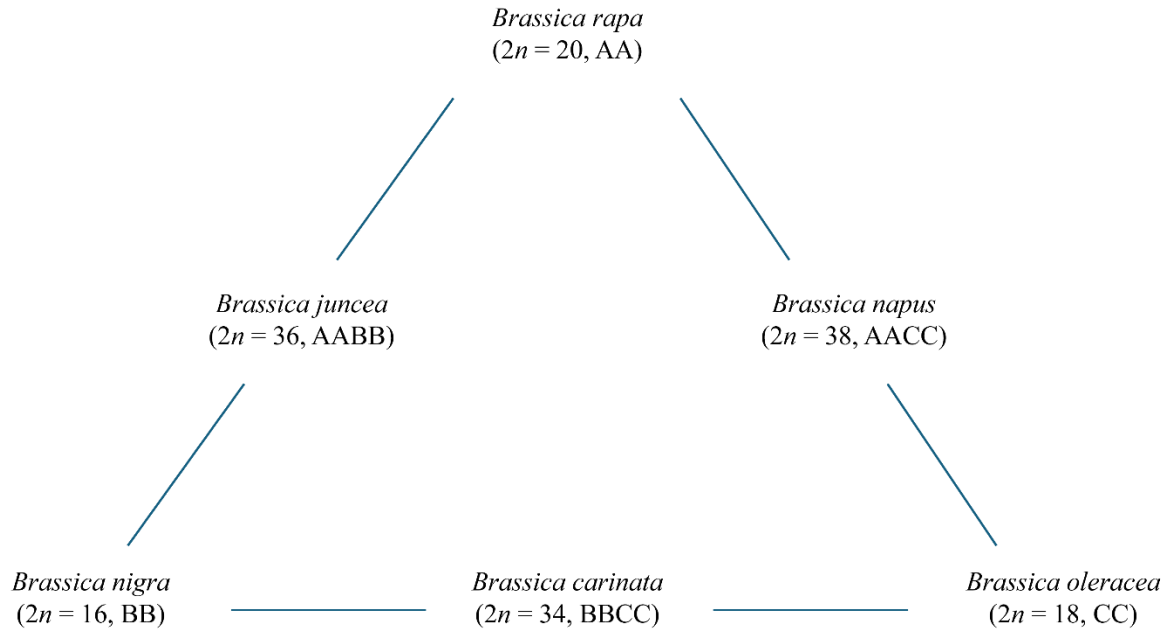


Figure 1.1. The "Triangle of U", showing the genetic relationships between the six *Brassica* species *B. rapa, B. juncea, B. napus, B. nigra, B. carinata,* and *B. olereacea*. The letters A, B, C indicate each haploid genome type; *n* indicates the number of chromosome sets (Based on U 1935).

Chapter 2 – Evaluation of amisulbrom products for the management of clubroot of canola (*Brassica napus*)

2.1 Introduction

Canola (oilseed rape, *Brassica napus* L.) is an important crop, accounting for approximately 12% of the total oilseed production worldwide (Zheng and Liu 2022). While Canada is a major producer of canola, in recent years, the emergence of clubroot disease, caused by *Plasmodiophora brassicae* Woronin, has posed a significant threat to yields (Cornelsen et al. 2023). The disease is characterized by the development of galls or clubs on the roots of infected plants, which can severely limit growth and yield. The pathogen can produce large numbers of resting spores in infected host tissues, which are released back into the soil as the galls decay, persisting for many years and serving as inoculum for future infections (Hwang et al. 2013; Wallenhammar 1996).

Sustainable clubroot management requires an integrated approach (Cornelsen et al. 2023; Donald and Porter 2009). Currently, clubroot control in western Canada relies heavily on resistant cultivars. Unfortunately, the emergence of resistance-breaking pathotypes of *P. brassicae* suggests that additional strategies are needed to complement host resistance (Hollman 2021; Strelkov et al. 2016, 2018). The soil fumigant metam sodium (Vapam) has proven to be an effective chemical for clubroot control (Buczacki and White 1977; Hwang et al. 2014a; Zuzak et al. 2023). Multiple jurisdictions, however, have banned this product due to public health concerns (Donald and Porter 2009); moreover, its cost makes the application of metam sodium feasible only for eradicating isolated infection foci in canola cropping systems (Hwang et al. 2014a; Zuzak et al. 2023). Fungicides represent another potential tool for the management of clubroot. Products such as pentachloronitrobenzene (PCNB), trichlamide, flusulfamide,

fluazinam, and cyazofamid have been reported to show efficacy against clubroot (Donald and Porter 2009; Mitani et al. 2003; Naiki and Dixon 1987; Tanaka et al. 1999).

Mitochondrial respiration inhibitors are preferred for clubroot control, as they have a shorter half-life and act on specific molecular sites of certain orders of microorganisms (Donald and Porter 2009; Yamaguchi and Fujimura 2005). Protectant fungicides that act on the mitochondrial complex III block electron flow at either of two ubiquinone-binding sites (Qo—quinone outside and Qi—quinone inside) and are therefore classified as Qo and Qi inhibitors (QoI and QiI) (Yamaguchi and Fujimura 2005). Flusulfamide (QoI) and cyazofamid (QiI) have been reported to inhibit *P. brassicae* resting spore germination (Mitani et al. 2003; Tanaka et al. 1999), and field experiments with these products in Canada indicated some efficacy in reducing clubroot severity on canola (Hwang et al. 2011, 2012). The QiI cyazofamid is preferred for clubroot management, since it required a lower dosage than the QoI flusulfamide and showed a high specificity on oomycetes and plasmodiophorids (Mitani et al. 2003).

Amisulbrom is a relatively novel sulfonamide QiI fungicide used to control various plant diseases, including downy mildews and Phytophthora blights, caused by oomycetes (Fontaine et al. 2019; Honda et al. 2009, 2007). While classified in the Rhizaria, *P. brassicae* shares many characteristics as a pathogen with the oomycetes. In oomycetes, amisulbrom restricts the development of zoosporangia and eliminates the mobile zoospores (Fontaine et al. 2019; Honda et al. 2009). This mode of action suggests that amisulbrom may also be effective against the zoospores and sporangia of *P. brassicae*, thereby helping to prevent both primary and secondary infection. Kawasaki et al. (Kawasaki et al. 2014) reported that a broadcast application of 0.1% amisulbrom solution at seeding significantly reduced clubroot incidence and severity on Osaka-shirona (*B. rapa* L. ssp. *pekinensis*). Another study by Hollman (Hollman 2021) showed that

seed-row applications of an amisulbrom formulation prior to seeding reduced clubroot severity under field and greenhouse conditions. The objectives of this study were to examine the efficacy of granular fertilizer formulations of amisulbrom against clubroot and to compare their effectiveness with a liquid formulation.

2.2 Materials and Methods

Amisulbrom

All products were provided by Gowan Canada (Winnipeg, MB), including a 20% (w/v) amisulbrom stock solution, the granular amisulbrom/fertilizer formulations AF700, AF1000 and AF1500 (700, 1000 and 1500 g ai ha^{-1} of amisulbrom, respectively, plus monoammonium phosphate (MAP, 11-52-0 N:P:K)), and fertilizer without amisulbrom. The amisulbrom stock solution was diluted from 10% to 0.01% to investigate the effect of the fungicide on *P. brassicae* resting spores and to a 0.1% solution for field and greenhouse applications at 1000 g ai ha^{-1} as the liquid formulation AL1000.

Effect of amisulbrom on resting spore germination

The effect of amisulbrom on *P. brassicae* resting spore germination was examined using spores suspended in a host root exudates solution. The root exudates were prepared following Macfarlane (1970), Suzuki et al. (1992) and Lahlali et al. (2011) with some modifications. Briefly, 100 seeds of the clubroot-susceptible *B. napus* cv. 'Westar' were surface-disinfected with 70% ethanol, rinsed twice in sterile distilled H_2O and placed on cheesecloth just immersed near the surface of 100 mL Hoagland's solution in a 500 mL glass beaker. The top of the beaker was covered with aluminum foil and the beaker was kept at ~25°C under a 16 h/8 h (light/dark) photoperiod for 14 days. At that time, the cheesecloth and germinated seedlings were discarded,

and the solution was adjusted to pH 6.0 and passed through a 0.2-µm syringe filter (VWR International, Mississauga, ON) for use in the spore germination assays.

Resting spores of a single-spore isolate representing pathotype 3H of *P. brassicae*, as defined on the Canadian Clubroot Differential (CCD) set (Strelkov et al. 2018), were extracted from infected roots as described in Strelkov et al. (Strelkov et al. 2006). The spores were then suspended at 5×10^7 spores mL^{-1} in 10 mL of root exudate solution amended with 0, 0.01, 0.1, 1, and 10% amisulbrom in 15 mL Falcon tubes (VWR International, Mississauga, ON). The tubes were incubated in darkness at 25 °C and assessed for germination every 48 h over 10 days. In brief, 25 µL aliquots of the spore suspensions were collected with a micropipette, stained with equal volumes (25 µL) of 2% acetic orcein (Fisher Scientific, Markham, ON) (Lahlali et al. 2011; Naiki et al. 1987), and examined on glass slides under a light microscope (Nikon Instruments Inc., Melville, NY, USA). Stained spores were regarded as ungerminated, while nonstained (empty) spores were considered to have germinated. Each treatment was replicated five times (one Falcon tube per replicate), with the percentage germination estimated by evaluating at least 100 spores in three different fields of view (Lahlali et al. 2011; Naiki et al. 1987) for each replicate. The experiment was repeated.

Effect of amisulbrom on resting spore viability

The effect of amisulbrom on spore viability was assessed in a manner similar to the spore germination assay, except that a suspension of 5×10^7 spores mL^{-1} was generated in 10 mL sterile distilled H_2O (instead of root exudates) and amended with 0, 0.01, 0.1, 1, and 10% (*w*/*v*) amisulbrom. The resting spores were stained with Evan's blue (Harding et al. 2019; Newcombe and Thomas 1990; Tanaka et al. 1999) at 2-day intervals over 10 days. Spores with stained

cytoplasm were considered dead, while unstained spores were considered viable. This experiment was also repeated.

Field trials

Field trials were conducted in 2019 (one site) and 2020 (two sites, Site 1 and Site 2) at the Crop Diversification Centre North (53 38′48″ N, 113 22′33″ W), Alberta Agriculture and Irrigation, Edmonton, AB, in dedicated clubroot nurseries. These are biosecure facilities infested with an average 1×10^5 resting spores g^{-1} soil. The three sites were located at a minimum 100 m distance from each other. Treatments were arranged in a split-plot design with four replicates. Two canola hybrids '45H31' (clubroot-susceptible) and 'CS2000' (moderately resistant) were seeded on 12 June 2019, and 4 June 2020. The treatments included an untreated control (UTC, fertilizer only), AF700, AF1000, and AF1500, and the liquid formulation AL1000 (Table 2.1). Fertilizer treatments (with or without amisulbrom) were applied to four 6 m rows per plot at seeding, with approximately 0.7 g of seed planted per row with a push-seeder. The liquid formulation AL1000 was applied to the rows prior to seeding. Since AL1000 did not include any fertilizer, MA*P* was also included in treatments with this formulation. Untreated control (UTC) treatments received fertilizer alone. Fifteen plants were dug from each plot at eight weeks after seeding and evaluated for clubroot symptom severity as described below. Plant height and aboveground weight were also measured. After plant sampling, soil samples from the top 10 cm layer of each plot were collected for spore density measurement. The trials were harvested on 24 October 2019 and 8 October 2020, and yields were calculated based on grain weight per plot area and recorded.

Greenhouse trials

Pathogen-free field soil was mixed with Sunshine® Mix #4 Aggregate Plus (Sun Gro, Agawam, MA, USA) at a 1:1 ratio (*v*:*v*) and inoculated with a *P. brassicae* resting spore suspension to

produce final spore concentrations of 1×10^5 and 1×10^7 resting spores g^{-1} soil mix. The resting spores were extracted, following Strelkov et al. (Strelkov et al. 2006), from clubbed canola roots collected from the nurseries; pathotyping on the CCD set (Strelkov et al. 2018) confirmed a pathotype 3H designation. Plastic tubs (43 cm × 28 cm × 17.8 cm) were filled with 4 kg (~8 L) soil mix, and two (30 cm-long) seed rows with 10 cm row spacing were prepared per tub for product application and seeding at a rate of 12 seeds per row. Treatments included the untreated control (UTC), AF700, AF1000, AF1500, and the liquid formulation AL1000 (Table 2.1). As in the field trials, the canola cultivars '45H31' and 'CS2000' were used in the greenhouse tests. The greenhouse was maintained at 20–25 °C (day) and 15–18 °C (night) under natural light supplemented by artificial lighting (16 h light/8 h dark). Two experiments (representing the two different spore concentrations) were set up on different benches in the greenhouse, with four replicates (tubs) of each treatment arranged in a split-plot design. Ten plants were sampled from each tub 8 weeks after seeding and evaluated for disease severity, root gall weight, plant height, and total biomass. The greenhouse trials were independently repeated.

Assessment of clubroot severity

Canola roots were rated for clubroot symptom severity on a 0 to 3 scale, where 0 = no galling, 1 = a few galls on lateral roots, 2 = moderate galling on main and lateral roots, and 3 = severe galling on all roots (Kuginuki et al. 1999). A disease severity index (DSI) was calculated from the individual plant ratings based on the formula of Horiuchi and Hori (1980) as modified by Strelkov et al. (2006):

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

Where n = number of plants in each rating category and N = total number of plants in an experimental unit.

PCR and qPCR analysis

Soil samples collected from the field plots were air-dried at room temperature and ground to homogeneity in a mortar with a pestle. Total genomic DNA was extracted from 0.25 g of each sample with a DNeasy PowerSoil Kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. The concentration and quality of the DNA samples were determined with a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The samples were evaluated for the presence of *P. brassicae* DNA by a conventional PCR analysis following the protocol of Cao et al. (2007). Briefly, 10 ng of DNA was added to a 20 µL PCR reaction with the primers TC1F and TC1R (Cao et al. 2007). The amplified products were visualized on a 2% agarose gel. Samples with a band of the expected size (548 bp) were confirmed as positive for *P. brassicae* DNA and subjected to a quantitative PCR (qPCR) analysis according to Rennie et al. (Rennie et al. 2011) with the primers DR1F and DR1R. Briefly, the DNA samples were diluted 10-fold with nuclease-free water and 2.5 µL of the solution was added to a 12.5 µL reaction mixture. Previously quantified *P. brassicae* DNA standards representing five spore densities (1×10^2, 1×10^3, 1×10^4, 1×10^5, and 1×10^6 resting spores g^{-1} soil) were also included in the analysis to relate DNA levels to resting spore densities in soil samples. The reactions were conducted with a StepOnePlus Real Time PCR System (Applied Biosystems, Foster City, CA, USA).

Data analysis

Statistical analyses were performed with R 3.6.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). An analysis of variance (ANOVA) was conducted to assess the

impact of amisulbrom on resting spore germination or viability, as well as on clubroot development in both field and greenhouse experiments. The amisulbrom treatments were considered as fixed effects, while field sites, greenhouse blocks, and cultivars were included as random effects to account for statistical variations. In cases where the effects of different field sites, greenhouse blocks, and cultivars were significant, their data were analyzed separately. The Shapiro–Wilk and Bartlett tests were used to validate the normality of the data and homogeneity of the variances, respectively. Data were then compared with Fisher's LSD test using the 'Agricolae' package (Mendiburu and Simon 2015) in R 3.6.2. Differences were considered statistically significant if $P < 0.05$.

2.3 Results

Effect of amisulbrom on resting spore germination

A reduction in *P. brassicae* resting spore germination relative to the control was observed in all of the amisulbrom treatments at all of the sampling times (Figure 2.1a). This reduction was more pronounced as the concentration of amisulbrom increased, with germination rates at 10 days ranging from 63.6% ± 8.5% in the presence of 0.01% (*w*:*v*) amisulbrom to 7.4% ± 2.1% in 10% (*w*:*v*) amisulbrom. When amisulbrom was applied at a rate of 0.1% (*w:v*), which was equivalent to the liquid formulation used in the field and greenhouse trials (1000 g active ingredient (ai) ha^{-1}), the percentage of germinated spores after 10 days declined to 34.4% ± 4.0% (SD) compared with 86.7% ± 3.9% in the control treatment.

Effect of Amisulbrom on resting spore viability

The inclusion of amisulbrom in the resting spore suspensions reduced their viability at all of the concentrations of the fungicide evaluated (Figure 2.1b). While spore viability in the untreated

control was 70.0% ± 3.8% after 10 days incubation, it was only 42.6% ± 1.7% and 39.1% ± 3.1%, respectively, in the 1% and 10% (*w*:*v*) amisulbrom treatments. At a rate of 0.1% (*w*:*v*) amisulbrom, 51.4% ± 2.3% of the resting spores were viable.

Field trials

As expected, clubroot severity was lower on the moderately resistant canola 'CS2000′ compared with the susceptible '45H31' in both 2019 and 2020 (Figure 2.2). Nonetheless, all treatments with granular or liquid formulations of amisulbrom significantly ($P < 0.05$) reduced clubroot DSI relative to the untreated control (UTC) in both cultivars at all three sites over two years (Figure 2.2).

In 2019, the average DSI on the UTCs of '45H31' and 'CS2000' was 37.8% and 25.9%, respectively. However, the application of amisulbrom resulted in significant reductions in DSI, with clubroot severity dropping to a range of 6.9% to 13.7% on '45H31' and 5.4% to 9.3% on 'CS2000' (Figure 2.2a). The reductions in DSI obtained with the different rates or formulations of amisulbrom (granular: AF700, 700 g ai ha^{-1}; AF1000, 1000 g ai ha^{-1}; and liquid: AL1000, 1000 g ai ha^{-1}) were not significantly different.

In 2020, clubroot development was more severe than in 2019, with DSIs of 67.1% and 64.5% on the UTC of '45H31' at Sites 1 and 2, respectively, and 37.0% and 38.6% on the 'CS2000' controls (Figure 2.2b,c). As in 2019, the application of amisulbrom significantly reduced DSI relative to the UTCs. However, in addition to significant reductions in DSI between the UTCs and the amisulbrom-treated plots, significant differences were also observed among the different rates and formulations of the fungicide (Figure 2.2b,c). The greatest reductions in DSI were generally obtained with AF1500 and AL1000. Treatment with AF1500 lowered the DSI on '45H31' to 25.7% and 21.0% at Sites 1 and 2, while AL1000 reduced it to 8.8% and

13.0%, respectively. On ‘CS2000’, treatment with AF1500 reduced the DSI to 4.7% and 8.7% at Sites 1 and 2, respectively, while AL1000 demonstrated a similar effectiveness, reducing it to values ranging from 4.3% to 7.6% (Figure 2.2b,c).

Amisulbrom-treated plots exhibited a higher individual plant height, aboveground biomass, and yield compared with the UTCs. In 2019, AF1500-treated plants displayed an approximate 8 cm increase in height and produced 18.2% to 21.2% more aboveground biomass than the UTCs for both cultivars (Table 2.2, 2.3). Significant yield improvements were observed only in ‘CS2000’, where the AF700 application resulted in a 50.1% increase in yield relative to the UTC (Table 2.2, 2.3). While a numerical increase in yield of up to 52.2% was seen in ‘45H31’, this was not significant. In 2020, plant height in the amisulbrom-treated plots did not differ significantly from the UTCs, except for AL1000 on ‘45H31’ at Site 1 (Table 2.2, 2.3). However, AF1500 and AL1000 significantly increased the total biomass for ‘45H31’ at both sites, by up to 89.2% and 96.5%, respectively (Table 2.2). For ‘CS2000’, these treatments led to a higher numerical biomass at both sites, but significantly increased biomass only at Site 1 (Table 2.3). Additionally, in 2020, the AF1500 and AL1000 treatments resulted in yield increases of 30.8% to 79.6% for ‘45H31’ and 18.4% to 101.8% for ‘CS2000’, respectively (Table 2.2, 2.3).

Greenhouse trials

The two runs of the greenhouse experiment showed no significant differences, so the data were pooled for all further analysis. Significant treatment effects were observed for both cultivars at both resting spore concentrations evaluated (Figure 2.3). At the low spore concentration (1×10^5 resting spores g^{-1} soil mix), all amisulbrom rates or products significantly reduced DSI on the susceptible cultivar ‘45H31’ relative to the UTC. The most substantial reductions, however,

were achieved with AF1500 and AL1000, which reduced DSI by 42.6% and 46.5%, respectively (Figure 2.3a). Similarly, at the high spore concentration (1×10^7 resting spores g^{-1} soil mix), all treatments also resulted in significant reductions in DSI on the susceptible cultivar, with the greatest reduction (63.1% compared with the UTC) obtained with the application of AL1000 (Figure 2.3a). On the moderately resistant 'CS2000', significant reductions in DSI were also observed with all treatments at both inoculum concentrations (Figure 2.3b). Notably, AL1000 showed the most effective results, with reductions in DSIs of 37.7% and 47.0% on 'CS2000' at the lower and higher spore concentrations, respectively (Figure 2.3b).

Significant differences between the UTC and amisulbrom treatments were also observed with respect to plant height and biomass under greenhouse conditions. At the lower spore concentration, plants of '45H31' treated with AF1000 and AF1500 were 17.1–17.5 cm taller than in the UTC and had a 52.8–62.2% greater biomass (Table 2.4). Plants of 'CS2000' were 11.1–16.6 cm taller in the AF1500 and AF1000 treatments, relative to the UTC, with increases in biomass of 45.8–46.9% (Table 2.5). At the higher spore concentration, '45H31' plants in the AL1000 treatments were 10.9 cm taller than in the UTC, while AL1000 and AF1500 significantly increased biomass by 33.9% and 43.0%, respectively (Table 2.4). In the case of 'CS2000', treatment with AF1000, AF1500 or AL1000 had similar effects on plant height and biomass; plants were 8.5–9.8 cm taller in these treatments, relative to the UTC, with significant increases in biomass of 26.1–36.2% (Table 2.5).

Resting spore densities

Amisulbrom treatments did not have a significant effect on *P. brassicae* resting spore density in 2019 nor in 2020 at either field site (Figure 2.4), although numerical decreases were consistently observed in 2019. The resting spore concentration ranged from 6.6×10^4 to 9.5×10^4 resting

spores g^{-1} soil across the field plots in 2019, and from approximately 7.9×10^5 to 8.0×10^5 and 8.0×10^5 to 8.8×10^5 resting spores g^{-1} soil at Sites 1 and 2, respectively, in 2020.

2.4 Discussion

While the management of clubroot on canola relies heavily on the deployment of resistant cultivars, the recent emergence of pathotypes able to overcome this resistance highlights the need for a more integrated disease management approach (Hollman et al. 2021, 2023; Strelkov et al. 2018). In this study, various amisulbrom treatments were compared for their efficacy in reducing clubroot severity and improving plant growth and yields. In general, amisulbrom showed promise for clubroot control under both field and greenhouse conditions.

In the field, all of the amisulbrom treatments significantly reduced clubroot severity relative to the UTCs, although no significant differences were observed among the different rates or formulations of the fungicide applied in 2019. In contrast, in 2020, disease severity generally declined further as the rate of amisulbrom increased. This likely reflected more severe clubroot development in 2020 vs. 2019. In 2019, the DSI in the susceptible untreated control remained below 40%, while it exceeded 60% in 2020, allowing for a greater range of disease severities with the different treatments. Ultimately, the DSI in the most effective treatments in both 2019 and 2020 was similar (<10%). It is worth noting that the *P. brassicae* resting spore density was about one order of magnitude greater at the field sites selected in 2020 vs. 2019, which may have contributed to the more severe disease development in the control treatments in 2020 (Hwang et al. 2013; Wallenhammar 1996). As in the field, all treatments also significantly reduced DSI at the two spore concentrations evaluated in the greenhouse, relative to the UTCs.

The liquid formulation, AL1000, was one of the most effective treatments in both the field and greenhouse, exhibiting a comparable and sometimes superior efficacy to the granular

formulation AF1500, despite the former containing 1000 g ai ha^{-1} vs. the latter's 1500 g ai ha^{-1}. This could reflect a slower release of the active ingredient from the granular form, resulting in lower levels of amisulbrom earlier in the growing season, and therefore reduced protection from early infection by *P. brassicae*; early infection is associated with more severe damage on susceptible hosts (Kim et al. 2000). Soil drenches with liquid amisulbrom are frequently used in the production of cruciferous vegetables in small acreage farms in China and Japan (Chai et al. 2014; Donald and Porter 2009). However, the in-furrow application of liquid fungicide formulations in the broad-acre canola cropping systems of western Canada may be more challenging, given demands on equipment, time, and labour (Hwang et al. 2014b). In this context, the application of granular amisulbrom is perhaps more practical, since most seeders used by western Canadian growers are capable of applying fertilizer granules into the seed rows when seeding.

The granular formulations of amisulbrom evaluated in this study included monoammonium phosphate, which was also was applied with the UTC and the liquid formulation at seeding. Monoammonium phosphate releases ammonium (NH_4^+) and phosphate ($H_2PO_4^-$), which could result in a reduction in soil pH (Lombi et al. 2004). Although there does not appear to be any evidence that this fertilizer or its end-products affect the effectiveness of amisulbrom, a lower soil pH can be more conducive to clubroot development (Donald and Porter 2009). Kawasaki et al. (2014) found that the application of liquid amisulbrom to limed soil provided better clubroot control than its application to soil that had not been treated with lime and almost eliminated disease incidence. Similarly, Nakanishi and Mori (2017) also reported that applying a mix of amisulbrom powder and hydrated lime suppressed clubroot development, maintaining the healthy growth of broccoli. These studies, combined with reports of the

effectiveness of lime as a clubroot management tool (Donald and Porter 2009; Fox et al. 2022), indicate that combinations of lime and amisulbrom may hold promise for the improved control of clubroot on canola.

The consistent reduction in clubroot severity observed on susceptible and moderately resistant canola following amisulbrom treatment under both field and greenhouse conditions suggests the potential of this fungicide to enhance the efficacy and durability of genetic resistance to this disease. Even the most highly resistant canola cultivars may still develop mild symptoms of clubroot, particularly under high inoculum pressure (Hwang et al. 2013, 2017). By combining amisulbrom applications with the planting of resistant cultivars, the incidence and severity of clubroot could be diminished further, thereby reducing the amount of inoculum (resting spores) produced in the host and returned to the soil. Since the galls produced in resistant hosts are likely enriched for pathogen genotypes able to overcome that resistance (Ernst et al. 2019), this could slow the emergence of resistance-breaking pathotypes of *P. brassicae*.

The declines in clubroot severity that were observed with amisulbrom treatment were reflected in an increased plant height and biomass in both the greenhouse and field experiments, and ultimately, in increased yields in the field (as yield was not monitored in the greenhouse). Studies have shown that increased clubroot severity results in a reduction in vegetative growth and yield of canola/oilseed rape (Wallenhammar et al. 1999; Strehlow et al. 2015; Botero-Ramírez et al. 2022). In Sweden (Wallenhammar et al. 1999) and Germany (Strehlow et al. 2015), yield losses in oilseed rape approached 100% under very high clubroot severity. In Canada, yield of canola was reduced between 0.26% and 0.49% for each 1% increment in DSI, regardless of host genetics (Botero-Ramírez et al. 2022). Therefore, by reducing clubroot severity, the application of amisulbrom could protect canola yields in clubroot-infested fields.

It has been suggested that amisulbrom and another QiI fungicide, cyazofamid, reduce *P. brassicae* infection by inhibiting the pathogen zoospores and restricting the growth of sporangia (Mitani et al. 2003; Honda et al. 2007, 2009; Fontaine et al. 2019). A previous report (Lahlali et al. 2011) that used in vitro methodologies similar to this study indicated that *P. brassicae* resting spore germination in a root-exudate solution could exceed 90%, while the average resting spore viability was 84% in sterilized water after 10 days, values consistent with the 87% germination and 70% viability found here in the UTCs. The reductions in resting spore viability and germination with amisulbrom treatments observed in this study indicate that amisulbrom may also reduce clubroot via a direct effect on the resting spores. This hypothesis needs to be further tested, particularly with experiments looking at the effect of amisulbrom on resting spores in the soil, since no significant declines in resting spore density in the soil were found after fungicide treatment. Nonetheless, it is interesting to note that cyazofamid also inhibited *P. brassicae* resting spore germination and reduced spore viability (Mitani et al. 2003).

In conclusion, amisulbrom appeared to be effective at reducing clubroot severity and preserving yields in canola, suggesting that this fungicide could have a role in the integrated management of this disease, likely in conjunction with genetic resistance and other control strategies. Before specific recommendations can be made to growers, however, more research will be required to optimize and validate application methods in broad-acre canola crops.

2.5 Tables

Table 2.1. List of amisulbrom treatments evaluated in greenhouse and field trials in Edmonton, Alberta, in 2019 and 2020.

Treatment	Amisulbrom rate	Formulation
UTC	0	N.A.
AF700	700g ai/ha	Granular
AF1000	1000g ai/ha	Granular
AF1500	1500g ai/ha	Granular
AL1000	1000g ai/ha	Liquid

Note: AF700, AF1000 and AF1500 refer to 700, 1000 and 1500 g ai ha^{-1} of a granular formulation of amisulbrom, respectively, which includes monoammonium phosphate (MAP; 11-52-0 N:P:K); UTC refers to the untreated control (no amisulbrom, only MAP); AL1000 refers to a liquid formulation of amisulbrom at 1000 g ai/ha, with MA*P* applied.

Table 2.2. Average individual plant height, biomass, and yield of the canola hybrid '45H31' in field trials conducted in clubroot-infested soil at Edmonton, Alberta, in 2019 and 2020.

Treatment	Plant height (cm)			Plant Biomass (g)			Yield (t/ha)		
	2019	2020S1	2020S2	2019	2020S1	2020S2	2019	2020S1	2020S2
UTC	87.81 c	127.48 b	96.68 a	100.89 b	95.65 b	67.23 c	0.95a	2.48d	1.10b
AF700	94.51 b	134.78 ab	99.81 a	110.44 ab	139.13 a	94.36 bc	1.44a	2.64cd	1.53ab
AF1000	94.79 b	133.03 ab	101.81 a	111.71 ab	125.12 ab	85.27 c	1.41a	2.95bc	1.55ab
AF1500	98.47 a	132.91 ab	103.93 a	119.22 a	165.13 a	127.21 ab	1.36a	3.24ab	1.88a
AL1000	96.62 ab	142.82 a	103.15 a	111.17 ab	143.97 a	131.51 a	0.99a	3.54a	1.98a

Note: UTC, untreated control; AF700, granular amisulbrom at 700 g ai ha^{-1}; AF1000, granular amisulbrom at 1000 g ai ha^{-1}; AF1500, granular amisulbrom at 1500 g ai ha^{-1}; AL1000, liquid amisulbrom at 1000 g ai ha^{-1}; 2020S1, Site 1 in 2020; 2020S2, Site 2 in 2020. Treatments followed by the same letter are not significantly different at $P < 0.05$.

Table 2.3. Average individual plant height, biomass, and yield of the canola hybrid 'CS2000' in field trials conducted in clubroot-infested soil at Edmonton, Alberta, in 2019 and 2020.

Treatment	Plant height (cm)			Plant Biomass (g)			Yield (t/ha)		
	2019	2020S1	2020S2	2019	2020S1	2020S2	2019	2020S1	2020S2
UTC	100.08 c	145.10 a	101.47 a	129.84 b	117.88 b	103.75 a	1.51b	2.83b	1.48b
AF700	105.65 b	137.20 a	101.18 a	135.44 b	150.83 ab	114.66 a	2.27a	3.17a	1.82ab
AF1000	105.70 b	140.48 a	100.24 a	142.61 ab	139.56 ab	132.32 a	2.06ab	3.20a	1.78ab
AF1500	107.92 a	144.31 a	105.82 a	157.42 a	175.80 a	148.64 a	1.99ab	3.35a	2.23a
AL1000	105.96 ab	142.33 a	99.91 a	136.19 b	155.83 a	108.75 a	2.18ab	3.24a	2.12ab

Note: UTC, untreated control; AF700, granular amisulbrom at 700 g ai ha^{-1}; AF1000, granular amisulbrom at 1000 g ai ha^{-1}; AF1500, granular amisulbrom at 1500 g ai ha^{-1}; AL1000, liquid amisulbrom at 1000 g ai ha^{-1}; 2020S1, Site 1 in 2020; 2020S2, Site 2 in 2020. Treatments followed by the same letter are not significantly different at $P < 0.05$.

Table 2.4. Average individual plant height and biomass in the canola hybrid '45H31' under greenhouse conditions in a soil mix inoculated with 1×10^5 or 1×10^7 resting spores of *Plasmodiophora brassicae* g^{-1} soil mix.

Treatment	Plant height (cm)		Plant Biomass (g)	
	10^5	10^7	10^5	10^7
UTC	83.64 c	74.54 b	14.59 b	9.60 b
AF700	92.94 b	81.68 ab	17.07 b	12.60 ab
AF1000	100.71 a	81.92 ab	23.66 a	11.90 ab
AF1500	101.11 a	82.38 ab	22.30 a	13.73 a
AL1000	86.97 bc	85.47 a	16.01 b	12.85 a

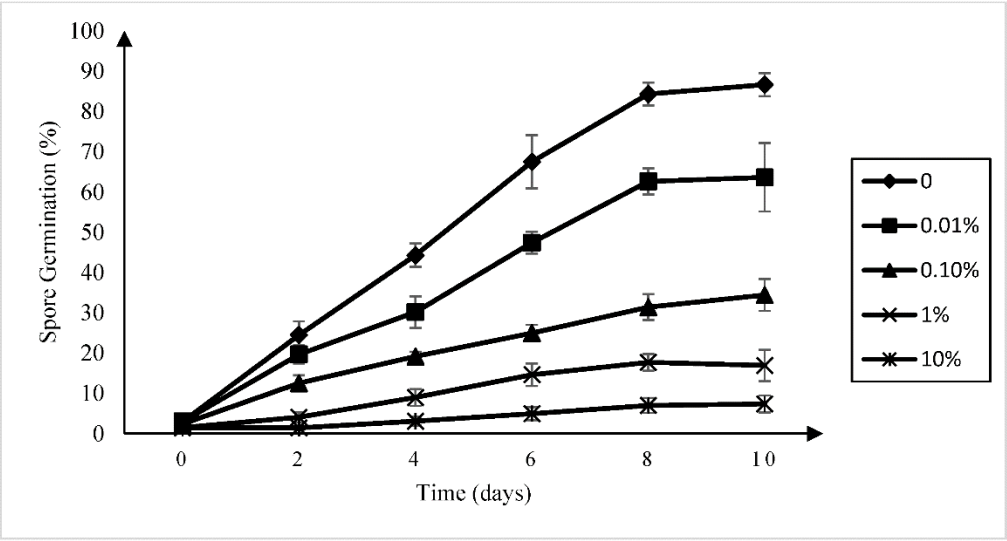
Note: UTC, untreated control; AF700, granular amisulbrom at 700 g ai ha^{-1}; AF1000, granular amisulbrom at 1000 g ai ha^{-1}; AF1500, granular amisulbrom at 1500 g ai ha^{-1}; AL1000, liquid amisulbrom at 1000 g ai ha^{-1}; 2020S1, Site 1 in 2020; 2020S2, Site 2 in 2020. Treatments followed by the same letter are not significantly different at $P < 0.05$.

Table 2.5. Average individual plant height and biomass in the canola hybrid 'CS2000' under greenhouse conditions in a soil mix inoculated with 1×10^5 or 1×10^7 resting spores of *Plasmodiophora brassicae* g^{-1} soil mix.

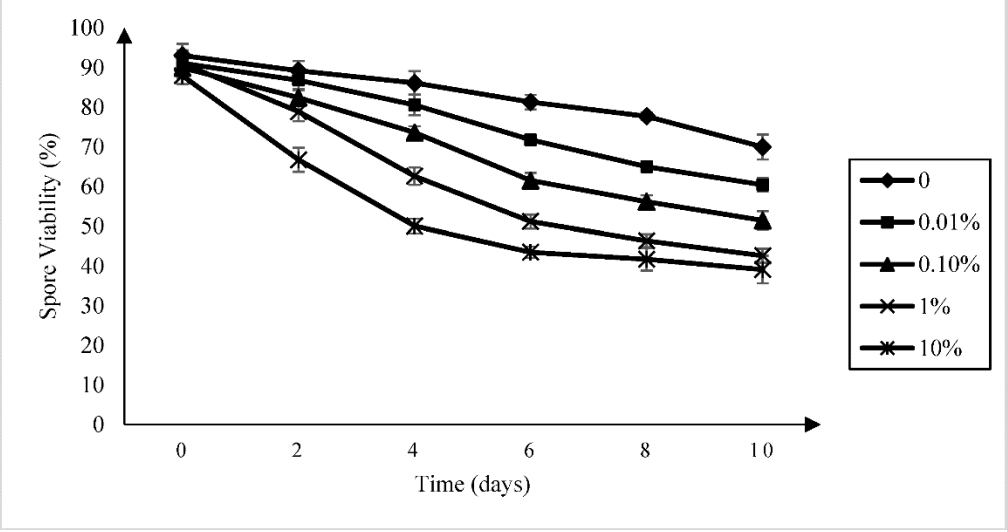
Treatment	Plant height (cm)		Plant Biomass (g)	
	10^5	10^7	10^5	10^7
UTC	89.49 d	79.24 b	15.12 b	12.32 c
AF700	96.32 bc	85.46 ab	21.08 a	14.92 b
AF1000	106.07 a	88.99 a	22.05 a	15.98 ab
AF1500	100.61 b	88.78 a	22.21 a	16.78 a
AL1000	93.15 cd	87.75 a	19.64 a	15.53 ab

Note: UTC, untreated control; AF700, granular amisulbrom at 700 g ai ha^{-1}; AF1000, granular amisulbrom at 1000 g ai ha^{-1}; AF1500, granular amisulbrom at 1500 g ai ha^{-1}; AL1000, liquid amisulbrom at 1000 g ai ha^{-1}; 2020S1, Site 1 in 2020; 2020S2, Site 2 in 2020. Treatments followed by the same letter are not significantly different at $P< 0.05$.

2.6 Figures



(**a**)



(**b**)

Figure 2.1. Germination (**a**) and viability (**b**) of *Plasmodiophora brassicae* resting spores in a canola root exudates solution and sterilized water, respectively, amended with amisulbrom at 0, 0.01, 0.1, 1, and 10% (*w*:*v*) over a 10-day period. Each point indicates the mean ± standard deviation.

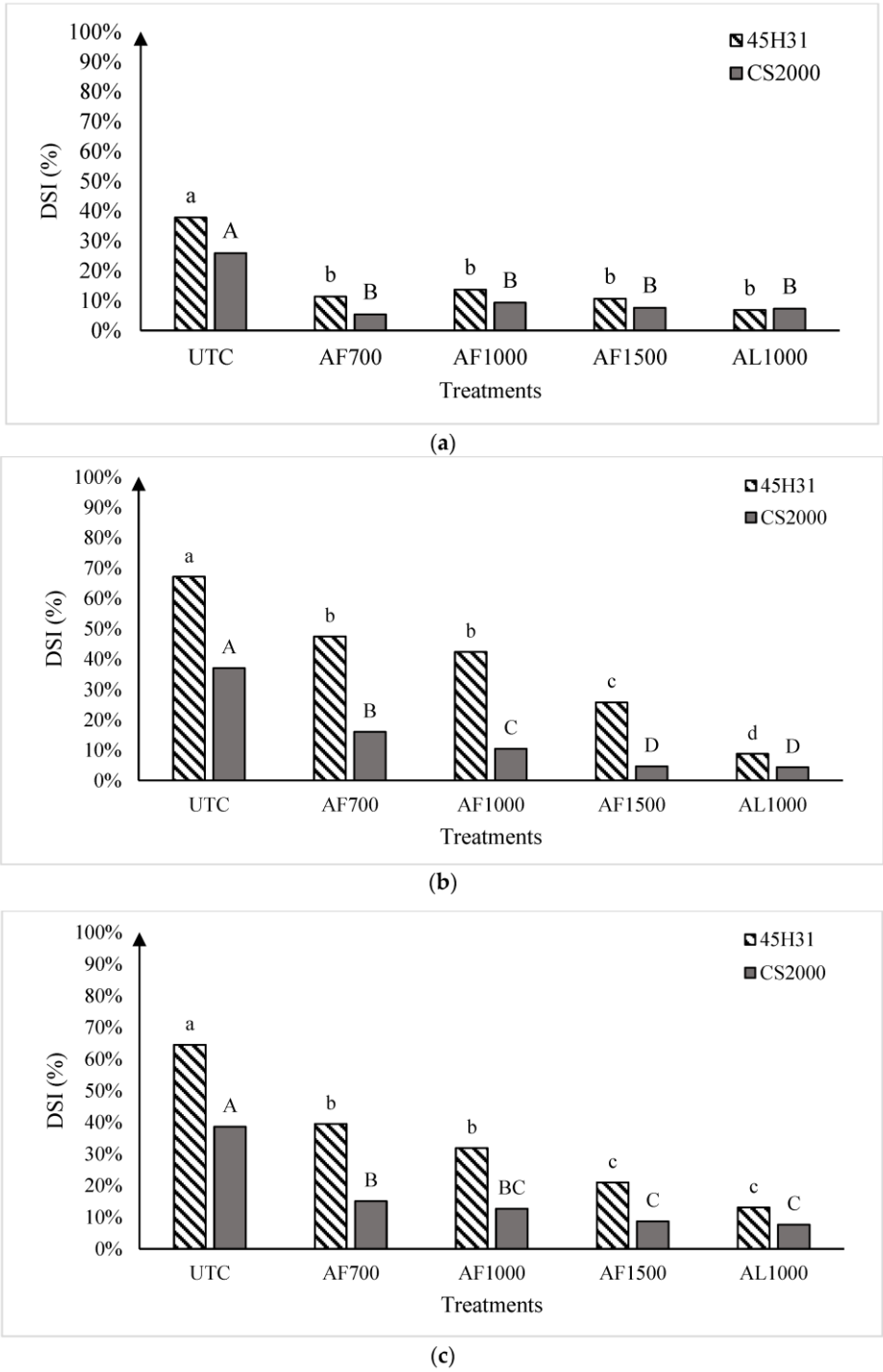
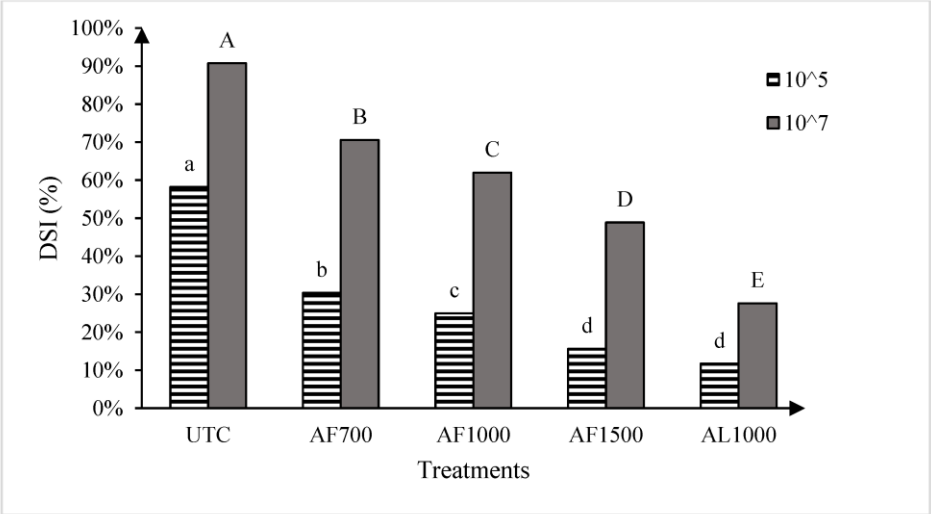
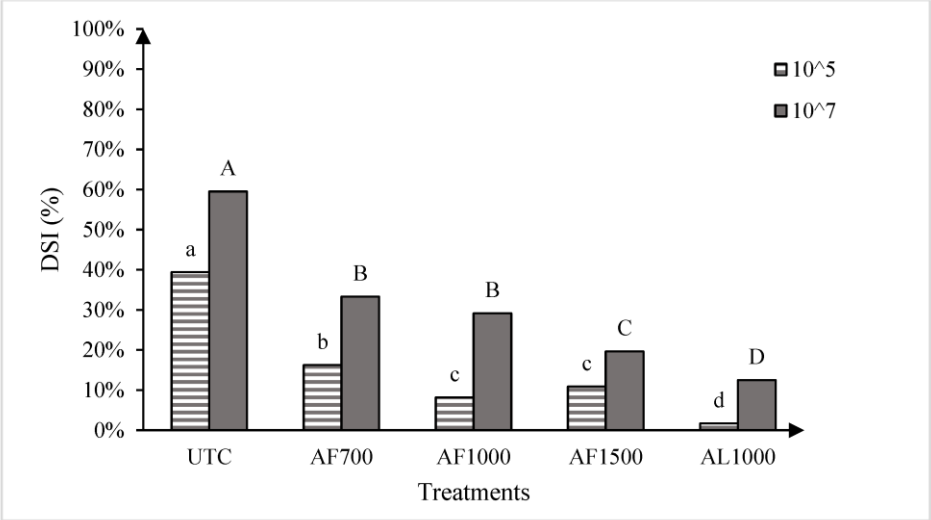


Figure 2.2. Clubroot disease severity index in the canola hybrids '45H31' and 'CS2000' under field conditions in Edmonton in 2019 (panel (**a**)), Edmonton Site 1 in 2020 (panel (**b**)), and Edmonton Site 2 in 2020 (panel (**c**)), following treatment with various amisulbrom rates and formulations. UTC, untreated control; AF700, granular amisulbrom at 700 g active ingredient

(ai) ha^{-1}; AF1000, granular amisulbrom at 1000 g ai ha^{-1}; AF1500, granular amisulbrom at 1500 g ai ha^{-1}; AL1000, liquid amisulbrom at 1000 g ai ha^{-1}. Bars topped by the same letter are not significantly different at $P < 0.05$; lowercase letters compare differences among treatments for the canola '45H31', while uppercase letters compare differences among treatments for the canola 'CS2000'.



(**a**)



(**b**)

Figure 2.3. Clubroot disease severity index (DSI) in the canola hybrids '45H31' (**a**) and 'CS2000' (**b**) under greenhouse conditions. Treatments were evaluated at two *Plasmodiophora brassicae* resting spore concentrations, 1×10^5 and 1×10^7 resting spores g^{-1} soil mix. UTC, untreated control; AF700, granular amisulbrom at 700 g active ingredient (ai) ha^{-1}; AF1000, granular amisulbrom at 1000 g ai ha^{-1}; AF1500, granular amisulbrom at 1500 g ai ha^{-1}; AL1000, liquid amisulbrom at 1000 g ai ha^{-1}. Bars topped by the same letter are not significantly different at $P < 0.05$; lowercase letters compare differences among treatments at 1×10^5 resting spores/g

soil mix, while uppercase letters compare differences among treatments at 1×10^7 resting spores/g soil mix.

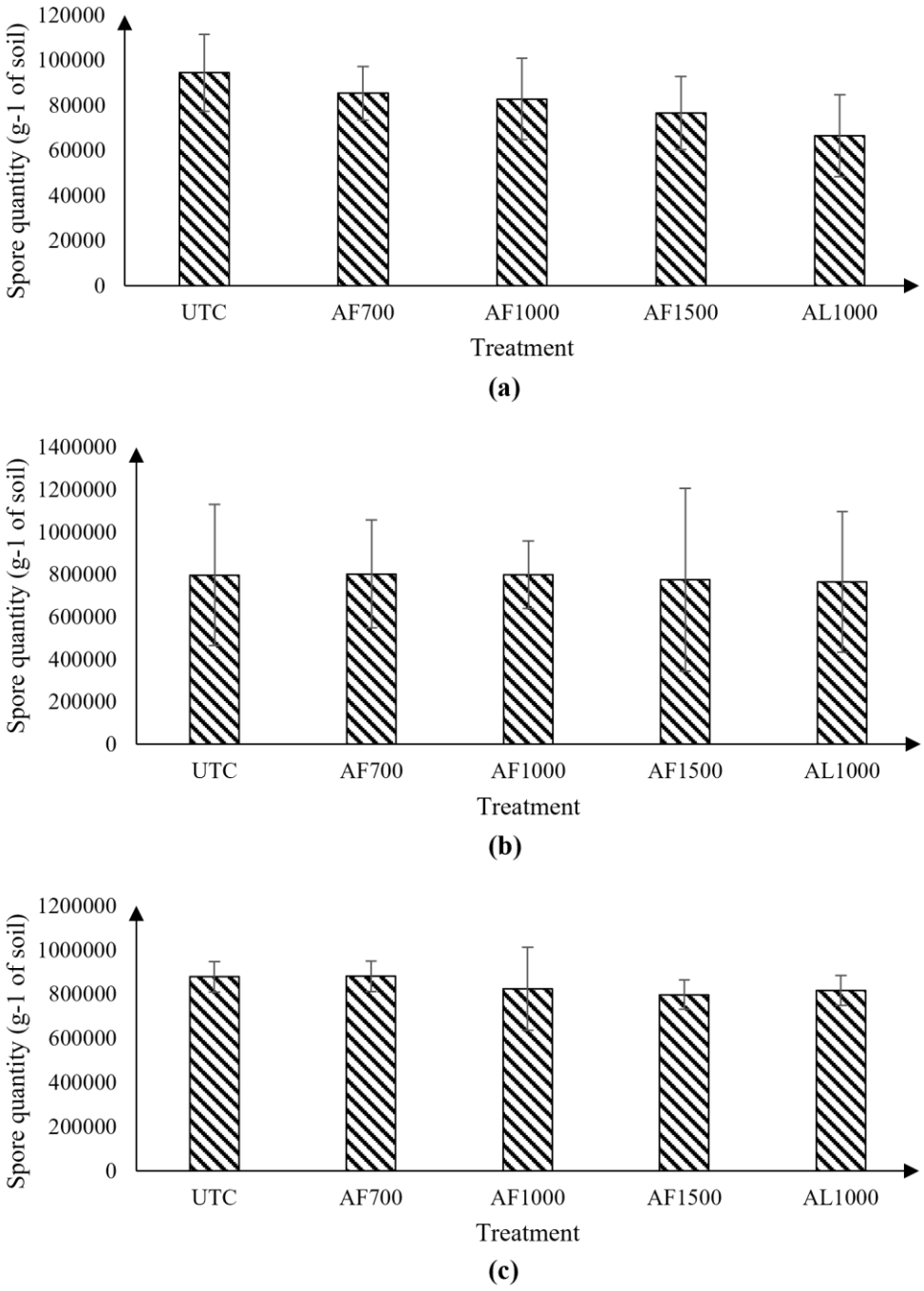


Figure 2.4. Effect of amisulbrom treatments on *Plasmodiophora brassicae* resting spore quantity in the soil under field conditions at Edmonton in 2019 **(a)**, and at Edmonton Site 1 **(b)** and Site 2 **(c)** in 2020. UTC, untreated control; AF700, granular amisulbrom at 700 g active ingredient (ai) ha^{-1}; AF1000, granular amisulbrom at 1000 g ai ha^{-1}; AF1500, granular amisulbrom at 1500 g ai

ha^{-1}; AL1000, liquid amisulbrom at 1000 g ai ha^{-1}. Error bars indicate the standard deviation. No significant differences ($P < 0.05$) were detected for any of the treatments.

Chapter 3 – Evaluation of fall and spring lime applications for the management of clubroot of canola

3.1 Introduction

Clubroot, caused by the soilborne pathogen *Plasmodiophora brassicae* Wor., poses a significant threat to canola (oilseed rape, *Brassica napus* L.) production in western Canada (Strelkov and Hwang 2014). Infected plants develop distorted root systems, forming large galls or 'clubs' that interrupt water and nutrient absorption, leading to wilting, stunting, and premature ripening. In severe cases, *P. brassicae* infection can result in total loss of a crop and a 5–6% reduction in canola seed oil content (Pageau et al. 2006; Strelkov and Hwang 2014). The clubroot pathogen survives as resting spores in the soil that can persist for many years, making management of the disease difficult (Wallenhammar 1996). While long established in Canada as a disease of cruciferous vegetables, clubroot was not identified on the canola crop until 2003, when 12 infested fields were found in central Alberta (Tewari et al., 2005). The disease has continued to spread over the past two decades, and by 2022 there were 3894 confirmed field infestations across the province (Strelkov et al., 2023), as well as dozens of documented cases in Saskatchewan (Ziesman et al., 2019) and Manitoba (Froese et al., 2019).

While clubroot management relies mainly on the deployment of resistant canola cultivars, the selection pressure imposed by the widespread cultivation of these varieties has resulted in the emergence of 'resistance breaking' pathotypes of *P. brassicae*, which can cause significant levels of disease on previously resistant hosts (Strelkov et al. 2018; Hollman et al. 2023). This highlights the need for an integrated approach for clubroot control, where multiple practices are employed to mitigate its impact (Strelkov and Hwang 2014). Such practices may include sanitization of tools and field equipment to reduce pathogen spread (Howard et al. 2010), crop

rotation out of susceptible hosts (Peng et al. 2014), and the application of soil fumigants such as metam sodium to eradicate localized outbreaks of the disease (Donald and Porter 2009; Hwang et al. 2014a). However, some of these practices may be impractical, require the cultivation of other, less lucrative crops, or, in the case of soil fumigation, raise serious environmental concerns.

The use of lime as a soil amendment has been recognized and used for the control of clubroot on vegetable *Brassicas* for many decades (Myers and Campbell 1985; Donald and Porter 2009). Clubroot development is favored in acidic soils, with a noticeable reduction in disease severity observed at a pH of 7.2 or higher (Donald and Porter 2009). Increased soil pH restricts the formation of *P. brassicae* plasmodia and zoosporangia, reducing secondary infection and gall development in the host roots (Webster and Dixon 1991). Moreover, liming increases soil calcium content, which diminishes the viability and germination of the pathogen resting spores. Additionally, calcium ions strengthen the cell walls of root hairs, thereby limiting primary infection (Myers and Campbell 1985; Webster and Dixon 1991; Niwa et al. 2008). Both independent and synergistic effects of pH and calcium have been reported on host penetration rates by pathogen zoospores, maturation of zoosporangia, and gall formation (Webster and Dixon 1991).

The various forms of lime, including limestone (calcium carbonate), hydrated lime (calcium hydroxide), dolomitic lime (containing calcium and magnesium carbonate), and quicklime (calcium oxide), can vary in their efficacy for disease reduction under field conditions (Hwang et al., 2014). Tremblay et al. (2005) reported a reduction in clubroot severity from 70% to under 30% in cruciferous vegetables following the application of hydrated lime. Murakami et al. (2002) found reductions of up to 20% in clubroot severity with dolomitic lime and limestone.

Hwang et al. (2011) observed a significant decrease in clubroot severity with the application of limestone at rates of 5.0 and 7.5 t ha^{-1} one week prior to seeding. However, the consistency of results varied across different sites and years. Fox et al. (2022) compared hydrated lime and limestone in a greenhouse study and found that both products reduced clubroot severity on canola, but hydrated lime provided a stronger level of control. Under field conditions, hydrated lime has shown promise for reducing clubroot (Tremblay et al., 2005; Hwang et al., 2011; Fox et al., 2022).

Hydrated lime and quicklime exhibit faster reactivity in the soil compared with limestone and dolomitic lime, which are relatively slow-acting (Hwang et al., 2014). In theory, limestone may require a minimum of three months to increase the pH of the soil fully (Havlin et al., 2005). Consequently, the application of these slow-acting lime products should ideally occur during the autumn months (September to December in western Canada) to allow for dispersion of the amendments in the soil before spring seeding (Hwang et al., 2014). Limited research, however, has been conducted on fall versus spring applications of limestone for clubroot control, particularly under western Canadian conditions. An optimized timing of lime application, particularly resulting in the increased efficacy of slower-acting but less expensive products such as limestone, could facilitate disease control. The objective of this study was to assess the efficacy of fall and spring applications of limestone, as well as spring applications of hydrated lime, for the management of clubroot.

3.2 Materials and Methods

Lime products and plant materials

A pulverized limestone product (Zero Grind (ZG, $CaCO_3$)) and hydrated lime (HL, $Ca(OH)_2$), provided by Graymont Limited (Richmond, BC, Canada), were applied under both field and

greenhouse conditions to assess their effectiveness in controlling clubroot. In the greenhouse trials, two canola hybrids, '45H31' (susceptible) and 'CS2000' (moderately resistant), were utilized, while only '45H31' was employed in the field trials.

Field experiments

Fields experiments were conducted in naturally infested clubroot nurseries located at the Crop Diversification Centre North, Alberta Agriculture and Irrigation, Edmonton, Alberta, in 2019-2020 and 2021-2022. In each year, two separate sites were selected, approximately 200 m apart. The experiments were arranged in a randomized complete block design (RCBD) with four replicates, where each plot (6 m × 1.5 m) served as one experimental unit. Lime treatments were spread uniformly across the plots by hand and immediately incorporated to a depth of approximately 10 cm using a roll-tiller. The fall lime treatments were applied on October 24th, 2019, and October 16th, 2021, while spring liming was conducted on May 25th, 2020, and May 16th, 2022. The treatments consisted of ZG applied at rates of 5.0 and 10 t ha^{-1} in either fall or spring, spring application of HL at 5.0 and 10 t ha^{-1}, or a combination of fall ZG application with spring HL application at rates of 2.5 or 5.0 t ha^{-1} each. The untreated controls (UTCs) did not receive any lime.

One week following spring liming, the clubroot-susceptible canola ‘45H31’ was seeded in a split-plot design. Approximately 0.7 g of seeds were sown in each of four rows per plot with a push-seeder. Soil samples from the top 10-cm layer of each plot were collected twice, before fall lime application and 10 days after seeding, for soil pH and resting spore density measurements. Eight weeks after seeding, 20 plants from each plot were sampled to measure plant height, weigh aboveground biomass and root tissues, and evaluate clubroot disease severity as described below. The trials were harvested at the end of the growing season (October 5th,

2020, and September 9th, 2022), and yields were calculated based on grain weight per plot area and recorded.

Greenhouse experiments

In October 2019 and 2020, pathogen-free field soil was combined with Sunshine Mix #4 Aggregate Plus (Sun Gro Horticulture, Glory Hills, AB) at a 1:1 ratio (v:v). This mixture was inoculated with a resting spore suspension of *P. brassicae* pathotype 3D or 3H, as designated on the Canadian Clubroot Differential set (Strelkov et al. 2018), to a final concentration of 1×10^6 resting spores per g potting mix. The inoculated mixture and non-inoculated controls were placed in 38-L tubs (61 cm × 41 cm × 22.2 cm; Rubbermaid, Atlanta, GA). Fall treatments of ZG were incorporated one week following inoculation, after which all tubs were placed outdoors for overwintering. Spring applications of ZG and HL were applied in May 2020 and 2021, and the tubs moved into a greenhouse. The lime treatments were as described for the field experiments, and consisted of ZG at rates of 5.0 and 10 t ha^{-1} in either fall or spring, spring application of HL at 5.0 and 10 t ha^{-1}, or a combination of fall ZG application with spring HL application at rates of 2.5 or 5.0 t ha^{-1} each. The UTCs did not receive any lime product. Four replicates (tubs) of each treatment were arranged in a RCBD, with each replicate placed on different benches in the greenhouse.

The canola hybrids '45H31' and 'CS2000' were seeded as four rows with 10 cm row spacing in each tub, at a rate of 12 seeds per row, one week after the tubs had been transferred to the greenhouse. Soil samples were collected from each tub for soil pH measurement before fall lime application and 10 days after seeding. The greenhouse was maintained at 25°C to 30°C during the day and 18°C to 23°C at night, with natural light supplemented by artificial lighting (16 h light/8 h dark). The tubs were kept well-watered, ensuring that the potting mix remained

moist to the touch, and fertilizer (20N-20P-20K) was applied at the 2nd, 4th and 6th week after seeding. Eight weeks after seeding, 10 plants were sampled from each tub, and clubroot severity was assessed as described below. Plant height, aboveground biomass, and root weight were also measured and recorded at this time. After 4 months of growth, all of the remaining plants in each tub were harvested, and yields were calculated based on grain weight per tub area and recorded.

Disease severity

Canola roots were rated for clubroot symptom severity on a 0 to 3 scale based on Kuginuki et al. (1999) with minor modifications, where: 0 = no galling, 1 = small galls on lateral roots, 2 = moderate galling on main and lateral roots, and 3 = severe galling on all roots. The disease severity index (DSI) for each field plot or greenhouse tub was calculated according to Horiuchi and Hori (1980) as modified by Strelkov et al. (2006): DSI (%) = $\frac{\sum (n \times 0) + (n \times 1) + (n \times 2) + (n \times 3)}{N \times 3} \times 100\%$, where n = number of plants in each rating class, and N = total number of plants in an experimental unit.

Soil pH

Soil samples (~500 mL) were collected from each plot or tub in both the field and greenhouse experiments. The pH of each sample was measured using a portable pH meter (Hanna Instruments, Woonsocket, RI), following the manufacturer's instructions. Three measurements were taken per sample, and the average value was recorded.

Conventional and quantitative PCR and qPCR

Conventional and quantitative PCR analyses were conducted as described in Chapter 2. Briefly, soil samples collected from the field or greenhouse were air-dried at room temperature and subsequently ground to homogeneity with a mortar and pestle. Total genomic DNA was

extracted from 0.25 g of each sample and tested for the presence of *P. brassicae* DNA in a conventional PCR assay with the primers TC1F and TC1R (Cao et al. 2007). Samples that tested positive for *P. brassicae* were analyzed further by quantitative PCR (qPCR) using the primers DR1F and DR1R following Rennie et al. (2011). Resting spore concentrations were estimated based on a five-point standard curve generated with standards containing 1×10^2, 1×10^3, 1×10^4, 1×10^5, and 1×10^6 resting spores per g of soil (Rennie et al. 2011).

Data analyses

All statistical analyses were performed with R 4.2.3 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). An analysis of variance (ANOVA) was performed to evaluate the impact of lime treatments on clubroot development in both field and greenhouse experiments. The lime treatments were treated as fixed effects, while field sites, greenhouse blocks (years), and cultivars were regarded as random effects. Data from different field sites, greenhouse blocks, and cultivars were analyzed separately when their effects were found significant. The normality of the data and homogeneity of variances were evaluated using the Shapiro-Wilk and Bartlett tests, respectively. Comparisons of DSI, plant height, yield, and aboveground and root weights were subjected to Fisher's LSD test with the 'Agricolae' package (Mendiburu and Simon 2015) to determine significant differences at $P < 0.05$. Changes in soil pH and spore quantity before and after treatments were assessed through a paired two-sample t-test. The correlation of variables was examined and plotted using the 'PerformanceAnalytics' package.

3.3 Results

Field experiments

Significant ($P < 0.05$) increases in soil pH were observed across all four sites in both 2019-2020 and 2021-2022, before and after lime treatment (Figure 3.1). Prior to lime application, the average soil pH was 5.36 and 5.24 in 2019-2020, and 4.92 and 4.99 in 2021-2022, for sites 1 and 2, respectively. After applying lime treatments, the highest recorded pH values were 7.49 and 7.36 for sites 1 and 2 in 2019-2020, and 7.08 and 7.24 for sites 1 and 2 in 2021-2022, respectively.

Regardless of the type of product or the timing of application, lime treatments at a rate of 5 t ha^{-1} resulted in a soil pH increase between 0.87 and 1.20 (Figure 3.1). The largest pH increases at this rate were associated with spring HL (pH values raised by 1.03 to 1.20), and the combination of fall ZG with spring HL (1.06 to 1.20). Fall ZG application on its own at 5 t ha^{-1} led to pH increases ranging from 0.94 to 1.01 at three sites, with a notable increase of 1.20 observed at site 2 in 2019-2020. When lime products were applied at 10 t ha^{-1}, the pH values exhibited larger increases, ranging from 1.56 to 1.98. Again, spring HL and the combination of fall ZG with spring HL resulted in the greatest increase in pH, ranging from 1.91 to 1.98 and from 1.89 to 1.95, respectively. Fall ZG application on its own at 10 t ha^{-1} resulted in increases in pH ranging from 1.76 to 1.95, with some variability among sites. The smallest increases in soil pH across all treatments were associated with the application of spring ZG at either 5 t ha^{-1} or 10 t ha^{-1}, and ranged from 0.87 to 0.95 and 1.56 to 1.86, respectively.

Significant reductions ($P < 0.05$) in clubroot severity on the clubroot susceptible canola '45H31' were observed on all the lime-treated plots compared with the UTCs, except for the spring ZG treatment (5 t ha^{-1}) at site 2 in 2021-2022 (Figure 3.2). For all four treatments, including fall ZG, spring ZG, spring HL, and the combination of fall ZG and spring HL, applications to a total rate of 10 t ha^{-1} resulted in lower disease severity than applications to a

total rate of 5 t ha^{-1} (Figure 3.2). Fall ZG application and the combination of fall ZG with spring HL at 10 t ha^{-1} showed the most significant reductions in clubroot severity relative to the UTCs, ranging from 54.9% to 69.6% and 54.7% to 69.6%, respectively. Spring HL on its own lowered the DSI by 48.6% to 60.1%. The application of spring ZG resulted in the smallest reductions in clubroot severity, with the 10 t ha^{-1} spring ZG treatment reducing DSI by 33.4% to 52.3%.

Yield increases were observed in all plots treated with lime relative to the UTCs, with all treatments at 10 t ha^{-1} significantly enhancing yields across all four sites in both years (Table 3.1, 3.2). At the two sites in 2019-2020, the greatest yields were observed in plots treated with the combination of fall ZG (5 t ha^{-1}) and spring HL (5 t ha^{-1}), resulting in yield increases of 80.6% and 584% at sites 1 and 2, respectively (Table 3.1). The second-highest yield increase in 2019-2020 was observed with fall ZG at 10 t ha^{-1}, which raised yields by 70.5% and 571% at sites 1 and 2, respectively (Table 3.1). In 2021-2022, the highest yields were obtained in plots treated with fall ZG at 10 t ha^{-1}, with increases of 296% and 220% at sites 1 and 2, respectively. The second-highest yield increases in 2021-2022 were found with the combination of fall ZG (5 t ha^{-1}) and spring HL (5 t ha^{-1}), which increased yields by 275% and 191% at the two sites (Table 3.2). Spring HL at 10 t ha^{-1} increased yields by 57.2% and 383% at sites 1 and 2 in 2019-2020, and by 203% and 154% at sites 1 and 2 in 2021-2022, respectively. These increases were greater than the 42.9%, 360%, 170%, and 132%, respectively, observed with spring ZG applied on its own at 10 t ha^{-1} (Tables 3.1, 3.2).

In both 2019-2020 and 2021-2022, lime-treated plots exhibited greater individual plant height, aboveground biomass, and lower clubbed root weight compared with control plots (Table 3.1, 3.2). At both sites in 2019-2020, plants in all lime-treated plots grew significantly taller than the untreated controls (UTCs), except for the combination of fall ZG and spring HL at 5 t ha^{-1} in

site 2 (Table 3.1). In 2021-2022, however, significantly greater plant height was observed only in plots treated with spring HL at 5 t ha^{-1}, and in the combination of fall ZG and spring HL at 10 t ha^{-1} (Table 3.2). The greatest plant heights at sites 1 and 2 in 2019-2020, and at site 1 in 2021-2022, were observed in the plots treated with a combination of fall ZG and spring HL at 10 t ha^{-1}; these plants were, on average, 25.8 cm, 20.7 cm, and 27.4 cm taller, respectively, than the UTCs (Tables 3.1, 3.2). In 2021-2022 at site 2, plants from the plots treated with fall ZG at 10 t ha^{-1} grew the tallest, with a mean height 16.4 cm greater than the UTCs (Table 3.2).

In addition, plants grown in soil treated fall ZG at 10 t ha^{-1} or the combination of fall ZG and spring HL at 10 t ha^{-1} produced significantly greater aboveground biomass than the UTCs across all four sites (Tables 3.1, 3.2). In 2019-2020 at site 1 and in 2021-2022 at sites 1 and 2, fall ZG at 10 t ha^{-1} resulted in the most biomass, which was 127 %, 81.8%, and 79.0% greater than in the UTCs, respectively. Additionally, the combination of fall ZG and spring HL at 10 t ha^{-1} produced the highest aboveground biomass in 2019-2020 at site 2, showing a 126% increase compared with the UTC (Tables 3.1, 3.2). Furthermore, plants in the UTCs exhibited significantly greater clubbed root weights relative to those in the lime-treated plots across all four sites, whereas gall weights in plots treated with different lime products or timing did not show significant differences (Tables 3.1, 3.2).

Greenhouse trials

Significant increases ($P < 0.05$) in the pH of the soil/potting mix combination were observed in all tubs treated with lime across 2019–2020 and 2020–2021, when comparing the pH levels before and after treatment (Figure 3.3). However, the average pH values before lime application in the greenhouse trials were 6.03 and 5.95 in 2019–2020 and 2020–2021, respectively, which were higher than the soil pH levels before treatment in the field. As a result, the highest observed

pH levels after lime treatment in the greenhouse were 8.00 and 7.96 in 2019-2020 and 2020-2021, respectively. Applications of lime at 5 t ha^{-1} raised pH values by 0.92 to 1.10, while applications at 10 t ha^{-1} resulted in a pH increase of 1.82 to 2.00. The highest increases in the pH of the potting mix in both 2020 and 2021 were observed in tubs treated with spring HL at 10 t ha^{-1}, resulting in pH increases of 1.95 and 2.00, respectively. Following closely was the combination of spring ZG and HL at the same rate, which increased pH by 1.92 and 1.97. Additionally, at a rate of 10 t ha^{-1}, fall ZG increased the pH of the potting mix by 1.87 and 1.92 in 2019-2020 and 2020-2021, respectively, while spring ZG increased the pH by 1.82 and 1.90.

The clubroot severity, average individual plant height, aboveground biomass, clubbed root weight, and yield of the canola hybrids '45H31' and 'CS2000' displayed statistically significant differences between 2019-2020 and 2020-2021, so the greenhouse data were analyzed separately for each year. In both years, all lime treatments significantly reduced disease severity on both '45H31' and 'CS2000' compared with the controls (Figure 3.4). In 2019-2020, the application of 10 t ha^{-1} fall ZG reduced DSI by 61.6% and 30.0% on '45H31' and 'CS2000', respectively. This reduction in clubroot severity was slightly lower compared with the combination of fall ZG and spring HL at 5 t ha^{-1}, as well as the application of spring HL at 10 t ha^{-1}, which resulted in reductions ranging from 68.7% to 73.5% on '45H31' and from 33.3% to 35.8% on 'CS2000'.

In 2019-2020, all lime treatments resulted in numerical yield increases compared with the untreated controls (UTCs). However, only three treatments - fall ZG at 10 t ha^{-1}, spring ZG at 10 t ha^{-1}, or the combination of fall ZG and spring HL at 2.5 t ha^{-1} + 2.5 t ha^{-1}, significantly increased yields on '45H31'. Similarly, only the combination of fall ZG and spring HL at 5 t ha^{-1} + 5 t ha^{-1} significantly increased yields on 'CS2000' (Tables 3.3, 3.4). In 2020-2021, the

application of fall ZG at 10 t ha^{-1}, the combination of fall ZG and spring HL at 5 t ha^{-1} + 5 t ha^{-1}, and the spring HL at 10 t ha^{-1} treatment achieved the greatest reductions in disease severity (68.1% to 71.1% reduction in DSI on '45H31', and 45.5% to 46.9% reduction on 'CS2000'), and resulted in the highest yield increases (126% to 184% on '45H31' and 161% to 211% on 'CS2000') compared with the UTCs. (Figure 3.4, Tables 3.3, 3.4).

Plants in most tubs treated with lime generally showed increased height, greater biomass, and lower clubbed root weight compared with the UTCs, irrespective of the host variety (Table 3.3, 3.4). However, there were significant differences among cultivars and years. On the susceptible '45H31', no treatments resulted in significantly greater plant height relative to the UTCs in 2019-2020, while in 2020-2021, plants in all lime-treated tubs were significantly taller than in the UTCs (Table 3.3). The plants in tubs treated with 10 t ha^{-1} fall ZG, or the combination of fall ZG and spring HL at either 5 t ha^{-1} or 10 t ha^{-1}, produced significantly greater biomass for '45H31' in 2019-2020. In 2020-2021, plants in all lime-treated tubs produced significantly more biomass than the UTCs, except for the spring and fall ZG applications at 5 t ha^{-1}. The UTCs of '45H31' exhibited greater gall weight compared with the lime treatments in both years, but no significant differences were observed amongst the lime treatments.

In the case of the moderately resistant 'CS2000', no significant increase in plant height was observed among treatments in 2019-2020, while in 2020-2021, plants in all lime-treated tubs were significantly taller than plants in the UTCs (Table 3.4). In 2020-2021, a significantly greater biomass was observed for 'CS2000' across all of the lime treatments relative to the UTCs, except for the spring ZG application at 5 t ha^{-1}. In 2019-2020, the 'CS2000' plants in tubs treated with spring ZG or the combination of fall ZG and spring HL at both rates had lower clubbed root weights than the UTCs. In 2020-2021, the UTCs exhibited greater clubbed root

weight than the lime treatments, with no significant differences observed among the lime treatments. The greatest height and biomass on both host varieties were observed with the combination of fall ZG and spring HL at 5 t ha^{-1} + 5 t ha^{-1}, and with fall or spring ZG at 10 t ha^{-1}, regardless of the year. The spring HL application at 10 t ha^{-1} resulted in the greatest reductions (91.4% to 92.5%) in clubbed root weight for both canola hybrids.

Resting spore densities

Across all four field sites in 2019-2020 and 2021-2022, numerical decreases in *P. brassicae* resting spore densities in the soil of up to 91% were observed following lime treatment. However, these decreases were not always significant (Figure 3.5). The lowest initial inoculum density, recorded at site 1 in 2019-2020, was 6.2×10^5 spores g^{-1} soil. At this site-year, significant reductions in spore levels were achieved with fall ZG at 5 t ha^{-1}, spring ZG at 10 t ha^{-1}, and HL at 10 t ha^{-1}. In contrast, at site 2 in 2019-2020, all treatments significantly reduced inoculum density from the initial average of 1.3×10^6 spores g^{-1} soil (Figure 3.5). The initial resting spore densities at sites 1 and 2 in 2021-2022 were very similar (1.3×10^6 and 1.2×10^6 spores g^{-1} soil, respectively), and significant reductions in spore loads were observed with all treatments, except for fall ZG at 10 t ha^{-1} at site 2 (Figure 3.5).

Correlations between variables

Figure 3.6 illustrates the correlations between clubroot DSI, plant height, aboveground biomass, clubbed root weight, and yield in the field experiments. It also shows the relationship between changes in soil pH and *P. brassicae* resting spore density. These latter parameters exhibited a significant negative correlation, suggesting that an increase in soil pH led to a reduction in soil spore load. Moreover, these two variables showed significant correlations with DSI and root gall weight, indicating their potential impact on clubroot development. Disease severity index was

negatively correlated with plant height, biomass, and yield. In contrast, a positive relationship was noted between DSI and clubbed root weight.

3.4 Discussion

Extensive regions with a soil pH of 6.0 or lower are found across Alberta, Saskatchewan, and northeast British Columbia, and over three million hectares in western Canada are classified as medium to strongly acidic (Canola Council of Canada n.d.). Soil acidification can also result from the repeated application of nitrogen and sulfur, common components of fertilizers used in canola production. These conditions create a favorable environment for clubroot development, as *P. brassicae* prefers acidic soils (Gossen et al. 2014). The pathogen can produce abundant resting spores within severely galled root tissues. After the decay of the galls, these spores are released back into the soil (Hwang et al., 2013), where they can persist for many years, serving as a reservoir of inoculum for future infections. Therefore, liming has been recommended as a farm management practice to enhance plant growing conditions and mitigate clubroot (Fox et al. 2022). This strategy could complement host resistance, which has been overcome in canola on hundreds of fields in western Canada, particularly in Alberta (Hollman et al. 2023).

The generation of a more alkaline environment and the introduction of calcium into the soil both contribute to the reductions in clubroot associated with the application of lime. However, these factors can be influenced by various field conditions, such as weather, lime type and quantity, and the time between application and planting (Myers and Campbell 1985; Donald and Porter 2009). In the field experiment in this study, the plots received much more precipitation in 2022 vs. 2020 (Environment and Climate Change Canada n.d.). As *P. brassicae* prefers wetter conditions (Gossen et al. 2014), clubroot severity was generally higher in 2021-2022 than in 2019-2020. Nonetheless, the effects of liming on pH and clubroot development

remained significant across all sites in both years. Theoretically, hydrated lime exhibits a soil-neutralizing potential equivalent to 120% to 135% of limestone, and releases up to 35% more calcium ions than limestone when applied at the same rate. Hydrated lime typically causes an increase in pH within 3-7 days, while limestone requires at least 3 months (Havlin et al. 2005; Donald and Porter 2009).

In this study, the limestone (ZG) and hydrated lime (HL) treatments, as well as their combinations, were applied at the same rates, either 5 t ha^{-1} or 10 t ha^{-1}. In contrast, the fall and spring lime applications resulted in different durations for the treatments to alter the pH of the soil - six months and one week, respectively. Since hydrated lime has a greater neutralizing potential than limestone, and fall limestone applications require a longer period to achieve soil pH changes relative to spring applications, the observed pH increases in the greenhouse trials followed the order: spring HL > fall ZG + spring HL > fall ZG > spring ZG. This trend corresponded with the varying potential of different lime types and application timings to raise pH levels. Although the spring HL treatment resulted in more substantial increases in pH compared to spring ZG treatments across all four site years in the field experiment, it did not significantly elevate pH more than fall ZG or the combination of spring ZG and HL under the more diverse environmental conditions in the field.

The connection between the occurrence and severity of clubroot symptoms and reductions in plant height, biomass, and yield has been established in prior research (Botero-Ramírez et al. 2022; Fox et al. 2022; Hennig et al. 2022). In this study, increases in DSI and root gall weight were also found to be associated with a decline in these growth parameters. As soil pH increased, the *P. brassicae* resting spore density declined, consistent with previous reports (Hennig et al. 2022). Furthermore, clubroot severity also decreased with reductions in resting

spore density. While clubroot severity has been observed to increase linearly with the concentration of inoculum (Webster and Dixon 1991), Niwa et al. (2008) suggested that an increase in soil pH and calcium levels may inhibit resting spore germination and affect the responsiveness of *P. brassicae* to root exudates in the rhizosphere.

In both the field and greenhouse experiments, the application of limestone in the fall showed promise for clubroot management. In the field, all lime applications resulted in reduced clubroot severity compared with the UTCs. The most significant reductions in disease severity and increases in yield were observed with fall ZG or spring HL application alone, or fall ZG in combination with spring HL. A recent report found that spring applications of hydrated lime effectively reduced clubroot severity in the field (Fox et al. 2022). Furthermore, under greenhouse conditions, hydrated lime applications proved more effective than limestone. In the present study, spring applications of hydrated lime were also more successful in reducing disease and increasing yield compared to spring limestone treatments under field conditions. However, treatments incorporating a fall limestone application showed similar or greater reductions in clubroot severity, increases in yield, and improvements in plant growth parameters such as plant height and biomass, compared to hydrated lime applications.

The effectiveness of lime applications for reducing clubroot was also observed on both canola hybrids '45H31' and 'CS2000' under greenhouse conditions. The response of the clubroot-susceptible '45H31' in the greenhouse was similar to the field results and consistent across 2019-2020 and 2020-2021 at an inoculum density of approx. 1×10^6 spores g^{-1} soil. Treatments involving fall limestone application not only achieved a comparable reduction in disease levels to hydrated lime, but also demonstrated greater improvements in yield compared with hydrated lime. Since 'CS2000' exhibits moderate resistance to clubroot, differences

amongst lime treatments were not apparent on this host; all lime treatments successfully reduced DSI to below 20% at 5 t ha^{-1}, with treatments involving fall limestone and/or spring hydrated application at 10 t ha^{-1} resulting in further reductions in DSI to less than 10%. Based on these results, a combination of genetic resistance and liming appears particularly promising for clubroot management, regardless of product type. Indeed, the combination of spring hydrated lime and clubroot-resistant canola been shown to be an effective strategy for disease reduction, as evidenced by studies conducted under both greenhouse (Fox et al. 2022) and field conditions (Hennig et al. 2022). However, genetic resistance was emphasized as the primary factor contributing to lower DSIs.

The timing of seeding or initial exposure to pathogen inoculum after lime application is critical for the efficacy of clubroot management. Webster (1986) found that exposing Chinese cabbage seedling transplants to alkaline pH environments within 3-7 days of inoculation minimized root hair infection by *P. brassicae*. Prolonged exposure to alkaline pH did not result in further reductions in root hair infection, but did suppress clubroot severity. A follow-up study by Webster and Dixon (1991) found that the most significant reductions in root hair infection, clubroot incidence, and severity occurred within the first 14 days following exposure to elevated calcium levels and a pH of 7.2, with the greatest effects seen in the initial 7 days post-inoculation. Attempts to increase the soil pH beyond 9 days post-inoculation were ineffective in suppressing clubroot symptoms (Webster and Dixon 1991). Therefore, preparing a high-calcium, alkaline environment prior to seeding is important for minimizing *P. brassicae* infection and clubroot severity.

In this study, the spring application of ZG and HL was conducted at one week prior to seeding, a timeframe previously suggested as sufficient for hydrated lime (Fox et al. 2022).

However, it was observed that applying limestone in the fall resulted in a greater increase in soil pH compared to using the same product in the spring. Additionally, the fall application showed advantages in both clubroot management and increasing crop yield over the spring application. Therefore, allowing several months for the limestone to react in the soil appears to be more effective than a short 7-day period.

In addition to efficacy, another consideration of any clubroot management strategy is its associated cost. From a western Canadian perspective, Fox et al. (2022) indicated that while hydrated lime seemed to offer superior control against clubroot, it is significantly more expensive than limestone. However, the results of the present study, which show that fall application of limestone or a combination of fall limestone and spring hydrated lime can provide a similar level of control as hydrated lime, suggest that growers might be able to achieve good results with a less expensive limestone product. Typically, liming is followed by tillage to incorporate the lime into the soil. This could represent another limitation to the adoption of this practice in western Canada, where direct seeding is more common. However, some growers conduct tillage post-harvest in the fall, to bury residues and control weeds (Canola Council of Canada n.d.). In such cases, opting for fall limestone application could be more feasible and save time for spring seeding.

Overall, this study highlighted the complex interactions involving lime products, application timing, soil pH, pathogen resting spore density, and host resistance. The application of lime treatments significantly reduced both clubroot severity and the spore density of *P. brassicae* in the soil. These effects not only enhance plant health, but also have the potential for long-term clubroot management by alleviating selection pressure on pathogen populations and controlling soil inoculum levels. Furthermore, considering that limestone is more cost-effective

than hydrated lime, incorporating a fall limestone treatment in canola production may be a more effective and economical approach to manage clubroot. Therefore, limestone applications in the fall should be considered as part of an integrated management strategy for clubroot of canola.

3.5 Tables

Table 3.1. Average individual plant height, aboveground biomass, clubbed root (gall) weight, and yield of the canola hybrid '45H31' in field trials conducted in clubroot-infested soil in Edmonton, Alberta, at two sites in 2019-2020.

Treatment	Plant height (cm)		Biomass (g)		Gall weight (g)		Yield (kg ha^{-1})	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
UTC	109.89b	60.99b	65.98c	26.38b	8.19a	4.44a	2194.31c	178.42d
Fall ZG (5 t ha^{-1})	133.11a	77.02a	127.96ab	46.70ab	0.26b	1.75bc	3284.72ab	854.31abc
Fall ZG (10 t ha^{-1})	130.95a	81.16a	149.91a	53.24a	0.41b	0.62c	3741.78a	1196.69ab
Spring ZG (5 t ha^{-1})	130.51a	77.14a	102.60bc	41.82ab	1.06b	2.45b	2894.94bc	517.44cd
Spring ZG (10 t ha^{-1})	131.43a	75.62a	118.83ab	40.44ab	1.38b	2.06bc	3135.00ab	820.47abc
Spring HL (5 t ha^{-1})	132.81a	75.62a	93.80bc	45.00ab	1.38b	1.77bc	3402.25ab	746.08bc
Spring HL (10 t ha^{-1})	132.99a	74.41a	104.80bc	42.26ab	1.13b	1.28bc	3449.14ab	862.36abc
Fall ZG + Spring HL (2.5+2.5 t ha^{-1})	132.25a	73.77ab	131.18ab	47.67ab	0.28b	0.97bc	3619.61ab	860.47abc
Fall ZG + Spring HL (5+5 t ha^{-1})	135.70a	81.71a	135.61ab	59.74a	0.11b	1.31bc	3962.86a	1221.08a

Note: UTC, untreated control; ZG, Zero Grind limestone; HL, hydrated lime. Treatments followed by the same letter are not significantly different at $P < 0.05$. 'Fall' and 'Spring' refer to application timings in the fall and spring, respectively. The application rate is indicated in parentheses by each treatment.

Table 3.2. Average individual plant height, biomass, root gall weight, and yield of the canola hybrid '45H31' in field trials conducted in clubroot-infested soil in Edmonton, Alberta, at two sites in 2021-2022.

Treatment	Plant height (cm)		Biomass (g)		Gall weight (g)		Yield (kg ha^{-1})	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
UTC	88.35c	85.94a	30.92d	29.16c	4.36a	4.67a	388.44c	278.86d
Fall ZG (5 t ha^{-1})	93.53bc	94.52a	44.21bc	37.83bc	2.18bc	1.82bc	1051.78ab	615.25bc
Fall ZG (10 t ha^{-1})	103.56abc	102.31a	56.22a	52.19a	0.85c	0.69c	1537.06a	921.17a
Spring ZG (5 t ha^{-1})	95.67bc	84.81a	38.98cd	36.22bc	2.49bc	2.65bc	746.39bc	467.03cd
Spring ZG (10 t ha^{-1})	98.58abc	88.31a	44.18bc	40.70b	2.56b	2.42b	1047.83ab	646.00abc
Spring HL (5 t ha^{-1})	105.65ab	94.83a	42.10bcd	42.63ab	2.14bc	1.73bc	882.44bc	665.53abc
Spring HL (10 t ha^{-1})	103.20abc	97.11a	50.60ab	43.96ab	1.65bc	1.32bc	1176.17ab	708.44abc
Fall ZG + Spring HL (2.5+2.5 t ha^{-1})	100.89abc	88.93a	41.45bcd	37.97bc	1.07bc	1.07bc	873.61bc	617.86bc
Fall ZG + Spring HL (5+5 t ha^{-1})	115.78a	102.15a	51.17ab	46.58ab	1.59bc	1.34bc	1455.44a	811.89ab

Note: UTC, untreated control; ZG, Zero Grind limestone; HL, hydrated lime. Treatments followed by the same letter are not significantly different at $P < 0.05$. 'Fall' and 'Spring' refer to application timings in the fall and spring, respectively. The application rate is indicated in parentheses by each treatment.

Table 3.3. Average individual plant height, biomass, root gall weight, and yield of the clubroot-susceptible canola hybrid '45H31' in greenhouse trials conducted in 2019-2020 and 2020-2021.

Treatment	Plant height (cm)		Biomass (g)		Gall weight (g)		Yield (kg ha^{-1})	
	2020	2021	2020	2021	2020	2021	2020	2021
UTC	96.37ab	84.21d	9.89b	8.24d	2.24a	3.61a	84.59b	207.90d
Fall ZG (5 t ha^{-1})	96.09ab	100.20bc	10.21b	11.52bcd	1.07b	1.32b	175.99ab	427.90bc
Fall ZG (10 t ha^{-1})	108.94a	115.18a	15.52a	16.29a	0.82b	0.93b	295.86a	590.80a
Spring ZG (5 t ha^{-1})	99.83ab	98.14c	12.44ab	10.98cd	0.45b	1.61b	193.19ab	370.86c
Spring ZG (10 t ha^{-1})	104.55ab	107.08abc	13.12ab	13.00abc	0.35b	1.25b	275.58a	472.51abc
Spring HL (5 t ha^{-1})	98.19ab	102.39bc	13.76ab	11.15cd	0.81b	1.11b	229.33ab	401.22bc
Spring HL (10 t ha^{-1})	93.63b	107.12abc	11.75ab	13.16abc	0.19b	0.53b	164.21ab	470.13abc
Fall ZG + Spring HL (2.5+2.5 t ha^{-1})	101.37ab	105.61abc	15.01a	11.88bc	0.46b	0.94b	293.69a	410.79bc
Fall ZG + Spring HL (5+5 t ha^{-1})	109.73a	112.08ab	15.48a	14.61ab	0.24b	0.72b	208.68ab	551.89ab

Note: UTC, untreated control; ZG, Zero Grind limestone; HL, hydrated lime. Treatments followed by the same letter are not significantly different at $P < 0.05$. 'Fall' and 'Spring' refer to application timings in the fall and spring, respectively. The application rate is indicated in parentheses by each treatment. The potting mixture was inoculated with *Plasmodiophora brassicae* resting spores at a density of 1×10^6 resting spores per g potting mix.

Table 3.4. Average individual plant height, biomass, root gall weight, and yield of the moderately clubroot-resistant canola hybrid 'CS2000' in greenhouse trials conducted in 2019-2020 and 2020-2021.

Treatment	Plant height (cm)		Biomass (g)		Gall weight (g)		Yield (kg ha^{-1})	
	2020	2021	2020	2021	2020	2021	2020	2021
UTC	103.25a	107.25d	9.53a	10.20c	0.26a	3.09a	96.45b	223.27e
Fall ZG (5 t ha^{-1})	102.26a	120.26bc	10.50a	12.99b	0.10ab	0.56b	259.75ab	437.75cd
Fall ZG (10 t ha^{-1})	106.48a	131.23a	14.23a	16.16a	0.10ab	0.11b	254.55ab	693.35a
Spring ZG (5 t ha^{-1})	108.04a	117.81c	11.07a	12.26bc	0.02b	0.67b	233.31ab	397.60de
Spring ZG (10 t ha^{-1})	113.35a	121.83abc	13.19a	14.38ab	0.01b	0.46b	269.76ab	475.12bcd
Spring HL (5 t ha^{-1})	101.00a	117.33c	9.90a	12.99b	0.16ab	0.54b	183.45ab	443.09cd
Spring HL (10 t ha^{-1})	103.85a	120.12bc	9.59a	14.20ab	0.09ab	0.23b	224.75ab	583.50abc
Fall ZG + Spring HL (2.5+2.5 t ha^{-1})	107.63a	121.11bc	13.16a	12.92b	0.02b	0.48b	259.51ab	429.33cd
Fall ZG + Spring HL (5+5 t ha^{-1})	114.16a	130.42ab	13.86a	16.48a	0.03b	0.09b	323.72a	667.44ab

Note: UTC, untreated control; ZG, Zero Grind limestone; HL, hydrated lime. Treatments followed by the same letter are not significantly different at $P < 0.05$. 'Fall' and 'Spring' refer to application timings in the fall and spring, respectively. The application rate is indicated in parentheses by each treatment. The potting mixture was inoculated with *Plasmodiophora brassicae* resting spores at a density of 1×10^6 resting spores per g potting mix.

3.6 Figures

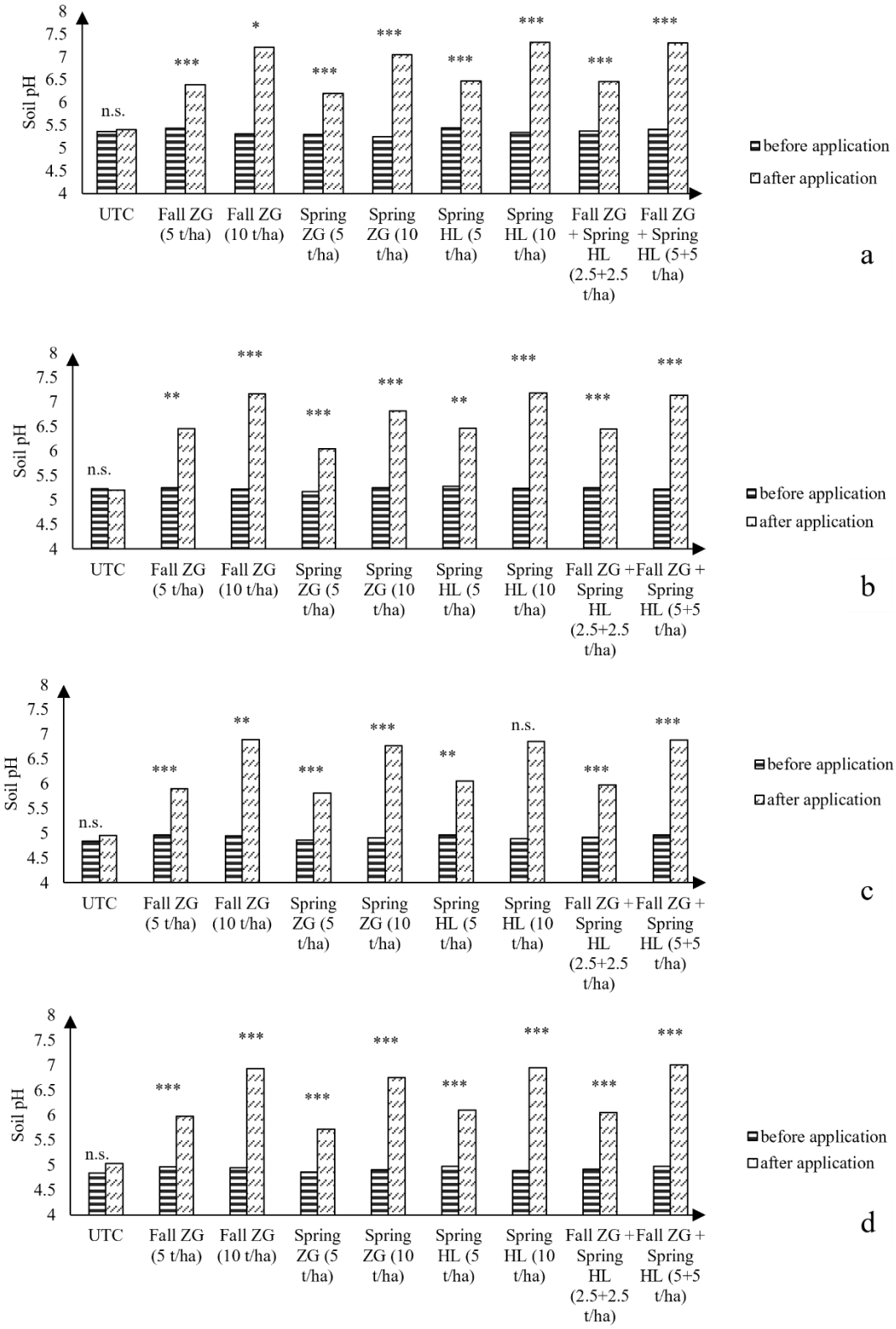

Soil pH
8
7.5
7
6.5
6
5.5
5
4.5
4
n.s.
***
*
***
***
***
***
***
UTC
Fall ZG (5 t/ha)
Fall ZG (10 t/ha)
Spring ZG (5 t/ha)
Spring ZG (10 t/ha)
Spring HL (5 t/ha)
Spring HL (10 t/ha)
Fall ZG + Spring HL (2.5+2.5 t/ha)
Fall ZG + Spring HL (5+5 t/ha)
before application
after application
a
**
b
n.s.
c
d

Figure 3.1. The change in soil pH before fall and after spring lime applications under field conditions in Edmonton, Alberta, in 2019-2020 site 1 (panel (**a**)), 2019-2020 site 2 (panel (**b**)), 2021-2022 site 1 (panel (**c**)) and 2021-2022 site 2 (panel (**d**)). Labels on top of bars indicate statistical differences between the two time-points, where: n.s., not significant ($P > 0.05$); * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

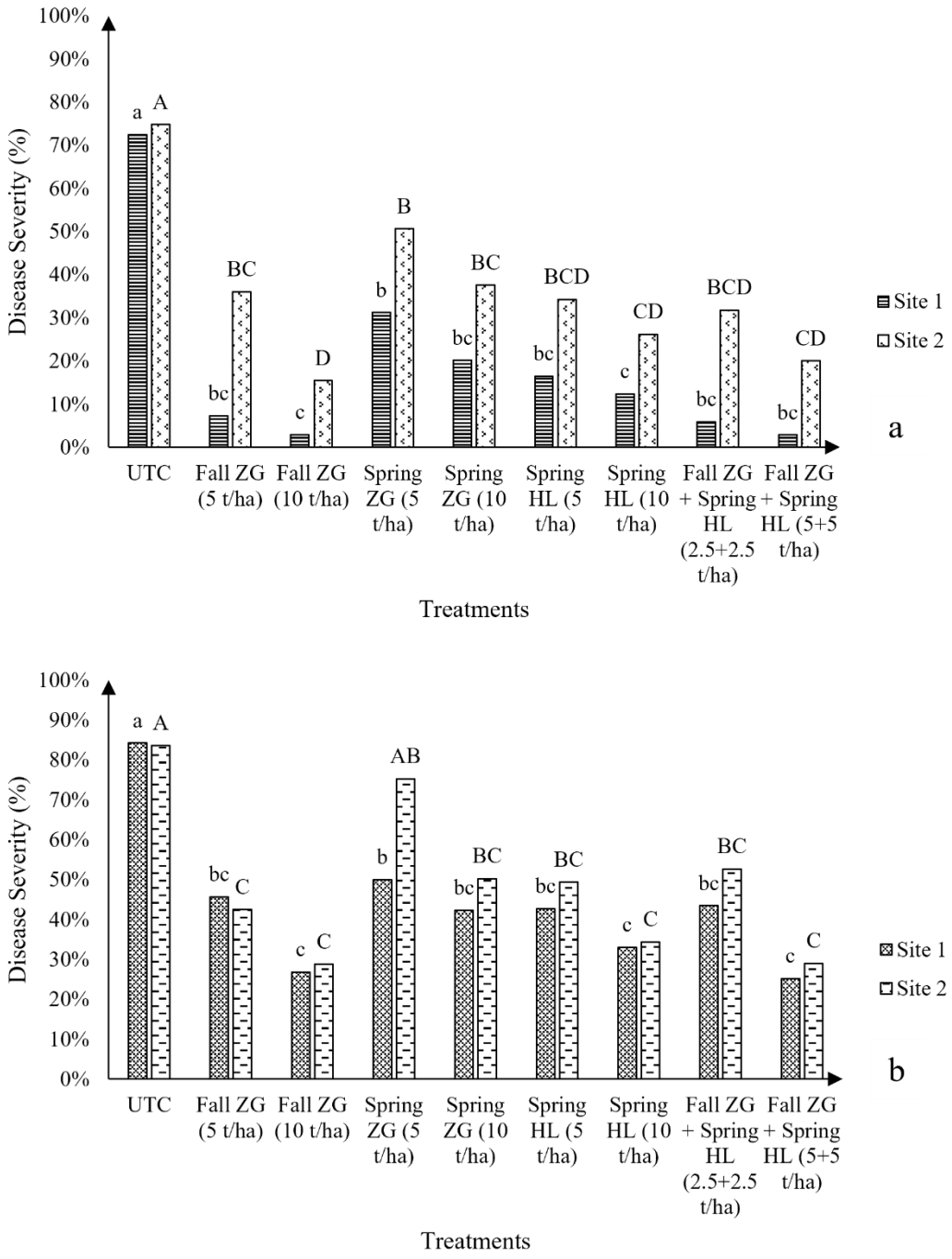


Figure 3.2. Clubroot disease severity index in the canola hybrid '45H31' under field conditions in Edmonton, Alberta, in 2019-2020 (panel (**a**)) and 2021-2022 (panel (**b**)). Bars topped by the same letter are not significantly different at $P < 0.05$; lowercase and uppercase letters indicate

differences among treatments at sites 1 and 2, respectively. UTC, untreated control; ZG: Zero Grind limestone; HL, hydrated lime.

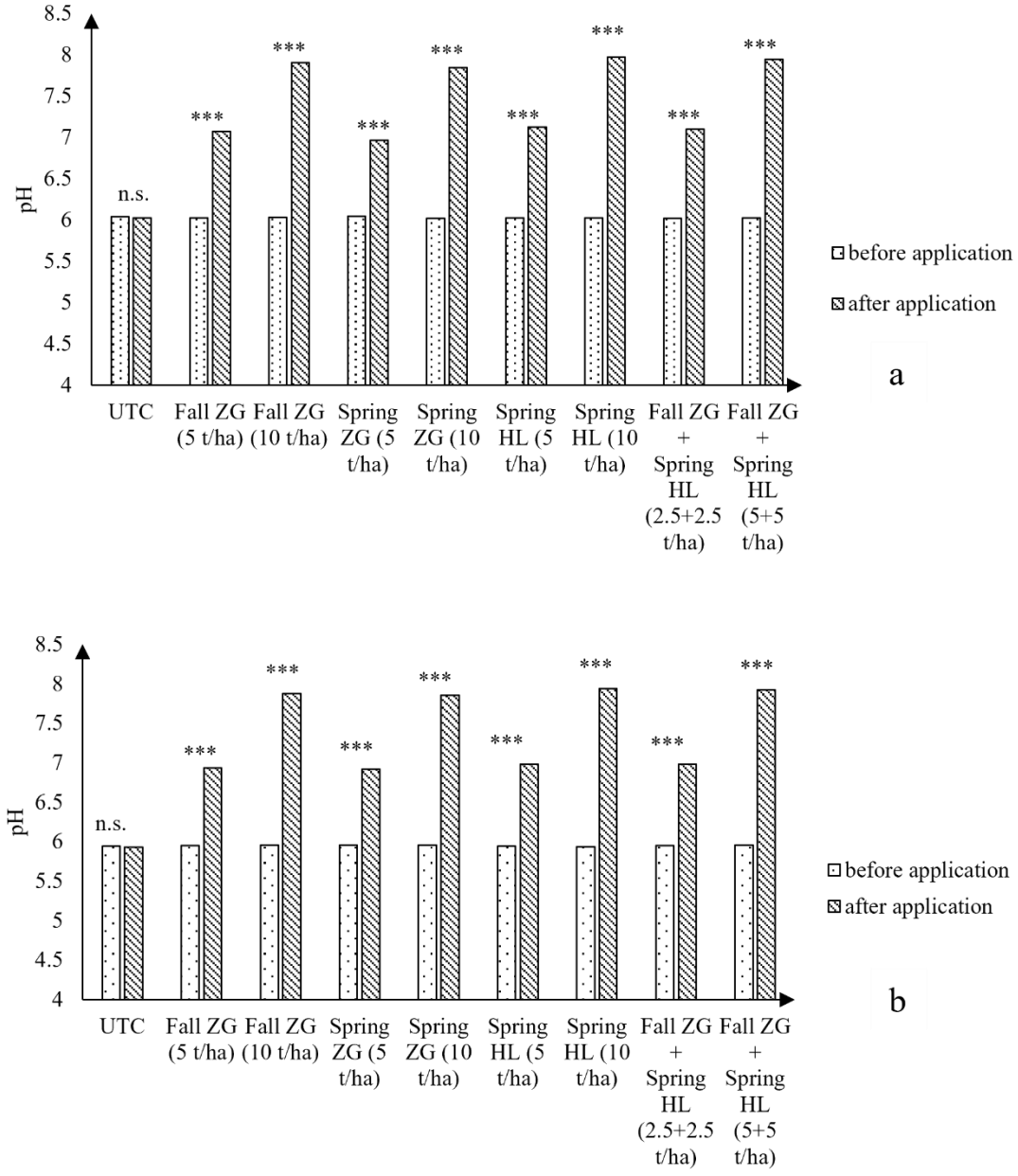


Figure 3.3. The change in soil pH before fall and after spring lime applications under greenhouse conditions in 2019-2020 (panel (**a**)) and 2020-2021 (panel (**b**)). Labels on top of bars indicate statistical differences between the two time-points, where: n.s., not significant ($P > 0.05$); * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

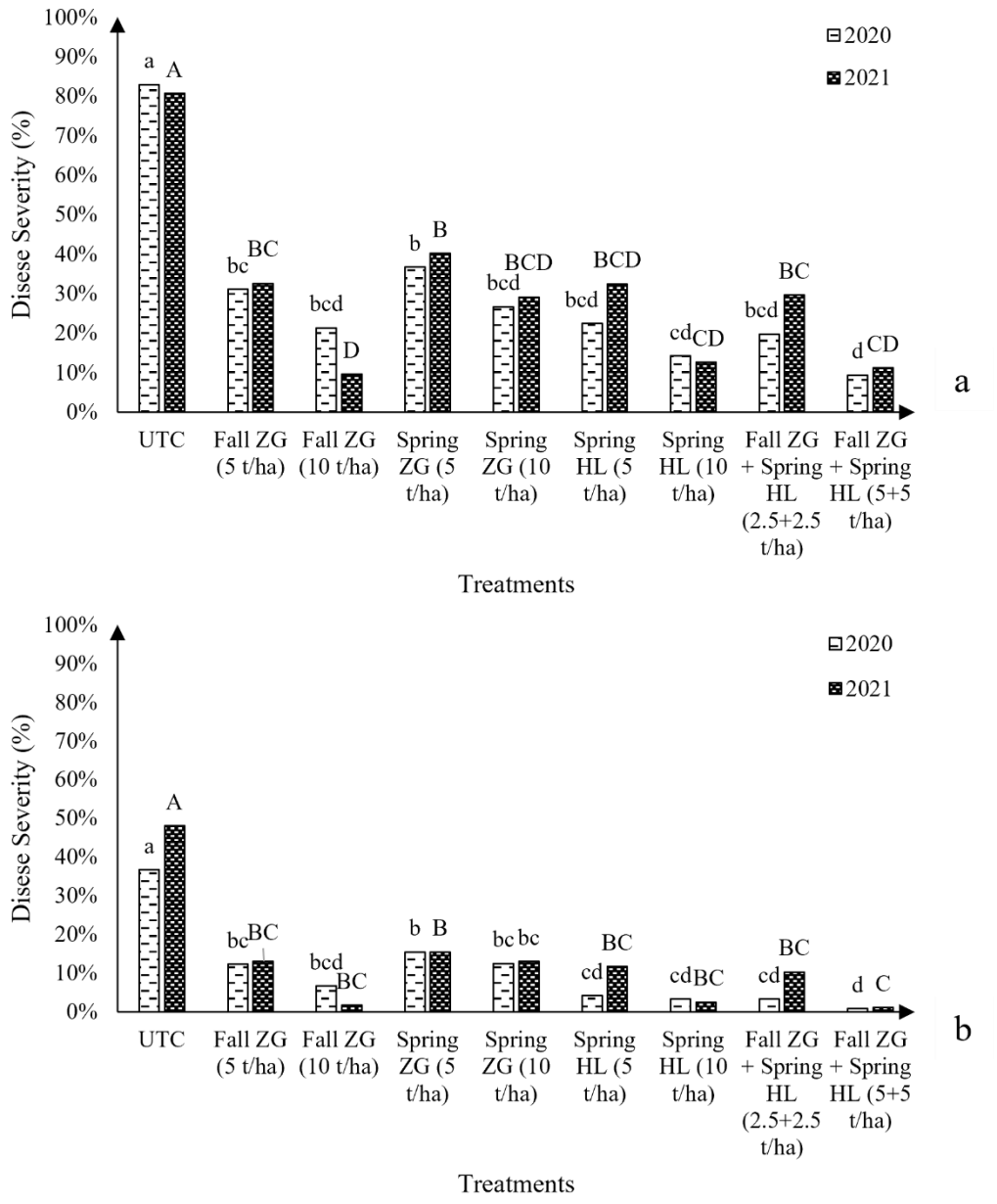


Figure 3.4. Clubroot disease severity index in the canola hybrids '45H31' (panel (**a**)) and 'CS2000' (panel (**b**)) under greenhouse conditions in 2019-2020 and 2020-2021. Bars topped by the same letter are not significantly different at $P < 0.05$; lowercase and uppercase letters indicate differences among treatments in 2019-2020 and 2020-2021, respectively. UTC, untreated control; ZG, Zero Grind limestone; HL, hydrated lime.

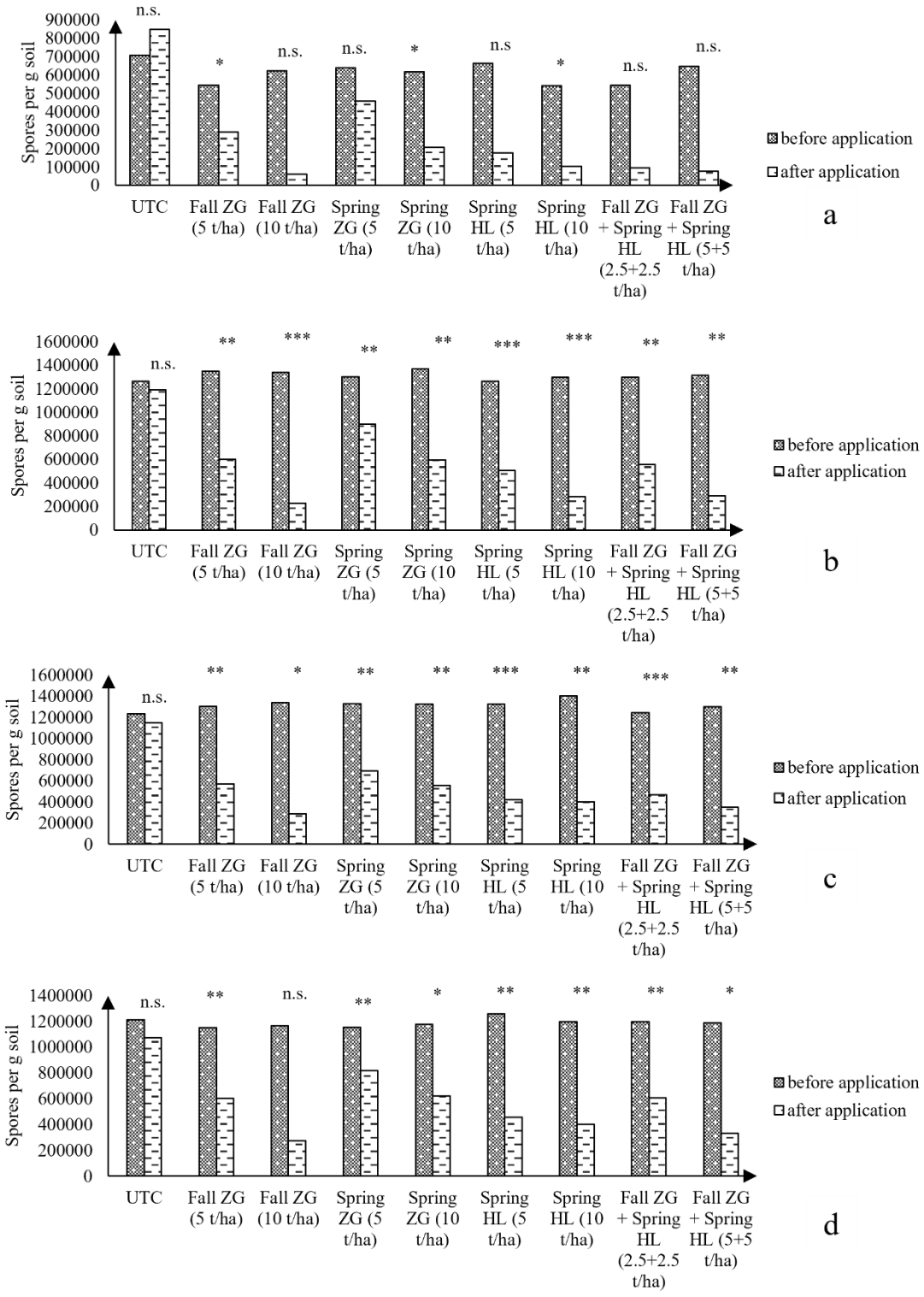


Figure 3.5. Changes in average *Plasmodiophora brassicae* resting spore densities in the soil before fall and after spring lime applications under field conditions in Edmonton, Alberta, at sites

1 (**a**) and 2 (**b**) in 2019-2020, and at sites 1 (**c**) and 2 (**d**) in 2021-2022. Labels on top of the bars indicate statistical differences between the two time-points, where: n.s., not significant ($P > 0.05$); * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

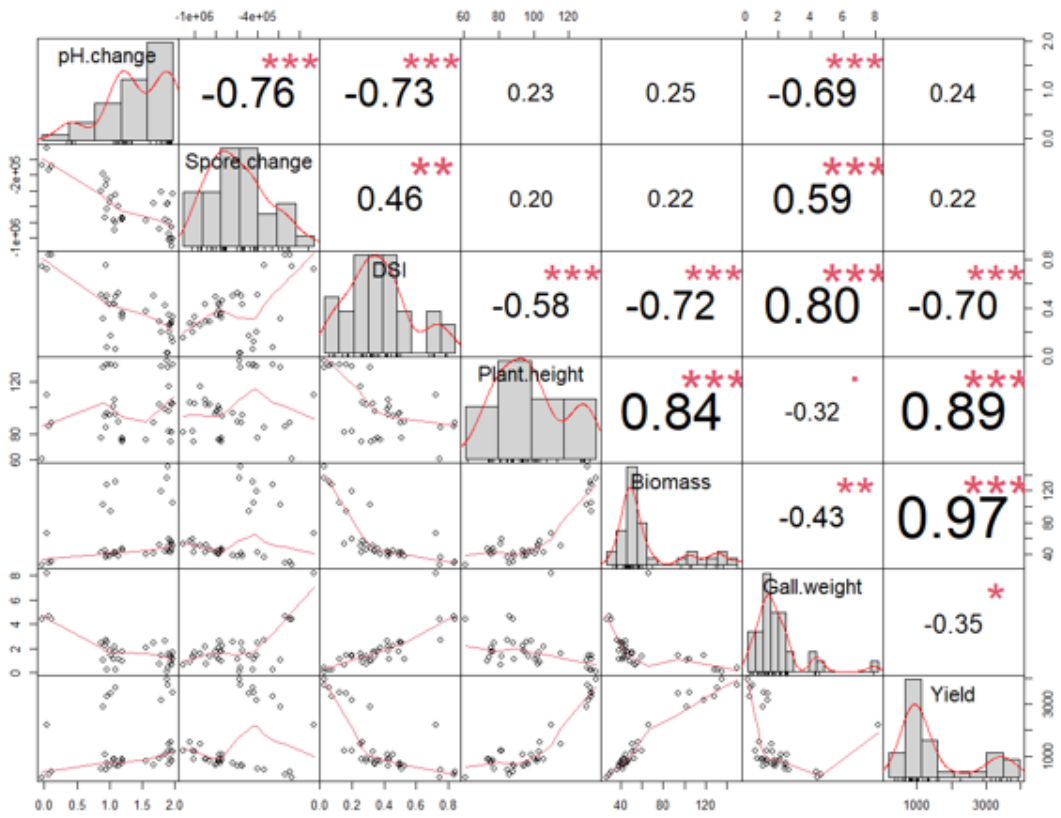


Figure 3.6. Correlations between clubroot disease severity index (DSI), plant height, biomass, clubbed (galled) root weight, and yield of the canola hybrid '45H31', and changes in pH and *Plasmodiophora brassicae* resting spore density in the soil. The experiment was conducted under field conditions in the Edmonton, AB, region in 2019-2020 and 2021-2022. The scatter plots show the relationship between two variables. The bar graphs indicate the frequency distributions across the diagonal. The significance of each correlation coefficient is indicated, where: · significant at $P < 0.1$; * significant at $P < 0.05$; ** significant at $P < 0.01$; *** significant at $P < 0.001$.

Chapter 4 − RNA-Seq bulked segregant analysis of an exotic *B. napus* ssp. *napobrassica* (rutabaga) F_2 population reveals novel QTLs for breeding clubroot-resistant canola

4.1 Introduction

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is an important soilborne disease of cruciferous crops worldwide (Dixon 2009; Javed et al. 2022). Infection by *P. brassicae* results in excessive growth and division of the host root cells, resulting in formation of root galls and an eventual reduction in the plant's capacity for water and nutrient uptake (Strelkov et al. 2006; Kageyama and Asano 2009). The cruciferous genus *Brassica* is known for its economically important agricultural and horticultural crops (Warwick 2011). These include Chinese cabbage, turnip, Polish canola and other crops belonging to the species *Brassica rapa* (A-genome), cabbage, cauliflower, broccoli, kale, Brussels sprouts and others classified as *B. oleracea* (C-genome), and rutabaga and canola/oilseed rape which are *B. napus* (AC-genome) (Dixon 2009; Warwick 2011; Hasan et al. 2021). Globally, average yield losses caused by clubroot are estimated at 10% to 15%, but may be as high as to 30% to 100% under favourable conditions (Dixon 2009; Strelkov and Hwang 2014; Strehlow et al. 2015). The clubroot pathogen survives as resting spores that can persist in the soil for many years, making the management of this disease difficult (Wallenhammar 1996; Dixon 2014).

In Alberta and other Canadian provinces, clubroot has emerged as a constraint to canola (*B. napus* var. *napus* L.) production (Strelkov et al. 2018; Hollman et al. 2023; Cornelsen et al. 2023). The number of *P. brassicae*-infested fields in Alberta has increased from 12 in 2003 (Tewari et al. 2005) to 3894 individual fields by 2022 (Strelkov et al. 2023). Although clubroot resistant canola varieties represent the most effective and environmentally-friendly strategy for

clubroot management (Rahman et al. 2014; Cornelsen et al. 2023), *P. brassicae* populations show high diversity in terms of virulence and can quickly adapt to overcome host resistance (Strelkov et al. 2018; Hollman et al. 2023; Mcdonald et al. 2021). Over the past decade, 'resistance-breaking' pathotypes have been documented in hundreds of fields across Alberta (Hollman et al. 2023, 2021; Strelkov et al. 2018). Forty-three pathotypes of *P. brassicae*, as classified on the Canadian Clubroot Differential (CCD) set (Strelkov et al. 2018), have been reported to date from Canadian collections of the pathogen (Hollman et al. 2023). A majority of these pathotypes are highly virulent on canola cultivars carrying 'first-generation' type resistance (Hollman et al. 2023), which appears to be derived from the European oilseed rape cv. 'Mendel' (Fredua-Agyeman et al. 2018).

Genetic mapping is important for the identification of clubroot resistance (CR) gene loci and for the development of molecular markers for marker assisted selection (MAS). Conventional PCR-based markers, such as amplified fragment length polymorphisms (AFLP), cleaved amplified polymorphic sequences (CAPS), random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), sequence characterized amplified regions (SCAR), sequence tagged sites (STS) and simple sequence repeats (SSR), were used widely for linkage-based identification and mapping clubroot-resistance gene loci before the era of sequencing technologies (Suwabe et al. 2003; Fredua-Agyeman et al. 2020b; Yu et al. 2021; Piao et al. 2004; Kato et al. 2013; Rocherieux et al. 2004; Voorrips et al. 1997). Next-generation sequencing (NGS) for genetic mapping has facilitated the development and application of genomics tools in plant breeding, such as genotyping-by-sequencing, single nucleotide polymorphism (SNP) arrays, and bulked segregant analysis (BSA) (Bolger et al. 2014). Bulked segregant RNA sequencing (BSR-seq) is one of the most cost-effective methods for mapping

genes of interest via BSA. This process evaluates two bulk DNAs or RNAs of plants with different phenotypes for differentially expressed genes (DEGs) and maps QTLs by variant calling (Liu et al. 2012). Multiple CR gene loci against Canadian pathotypes of *P. brassicae*, such as *Rcr1*, *Rcr2*, *Rcr3*, *Rcr6* and *Rcr9*^{wa}, have been identified via BSA or BSR-seq (Yu et al. 2016; Huang et al. 2017; Chang et al. 2019; Karim et al. 2020).

Rutabaga (*B. napus* ssp. *napobrassica*) could be a good source of clubroot resistance genes for emerging virulent *P. brassicae* pathotypes. Old rutabaga varieties like 'Wilhelmsburger' are known to carry resistance effective against most Canadian *P. brassicae* pathotypes (Williams 1966; Strelkov et al. 2018). Hasan and Rahman (2016) found a Canadian rutabaga cv. 'Brookfield', which was resistant to all five 'old' pathotypes (2F, 3H, 5I, 6M and 8N) found in Canada prior to the introduction of clubroot resistant canola. Wang et al. (2022) used a rutabaga cv. 'Polycross' as a resistance donor to breed for canola populations resistant to three Canadian pathotypes. Fredua-Agyeman et al. (Fredua-Agyeman et al. 2020b) observed that 87.9% of 124 rutabaga accessions from Nordic countries showed resistance to at least one of 16 Canadian *P. brassicae* pathotypes.

In this study, an F_2 population derived from a rutabaga accession FGRA106 that was reported to be resistant to 17 isolates representing 16 pathotypes of *P. brassicae* (Fredua-Agyeman et al. 2020b) was evaluated for its reaction to pathotypes 3A, 3D and 3H. The inheritance of the resistance was determined based on segregation ratios. The genomic regions that co-segregated with resistance were determined based on the number of differentially expressed genes (DEGs), and the quantitative trait loci (QTLs) were mapped by BSR-seq.

4.2 Materials and Methods

Plant materials

The parental materials consisted of the clubroot-resistant *B. napus* ssp. *napobrassica* accession FGRA106 (identified as the cultivar 'Wilhemsburger') (Fredua-Agyeman et al. 2020b; Yu et al. 2021), and the susceptible *B. napus* ssp. *napus* accession FG769 (spring canola cv. 'Sedo') (Fredua-Agyeman et al. 2019). FGRA106 was reported to be resistant (disease severity index (DSI) ≤ 30%) to isolates of *P. brassicae* representing pathotypes 2F, 5I, 6M, 5X (LG-2), 5L, 2B, 3A, 8E, 5K, 3O and 8P, and moderately resistant (30% < DSI ≤ 50%) to pathotypes 3H, 5X (LG-1), 8N, 5C, 5G and 8J (Fredua-Agyeman et al. 2020b; Yu et al. 2021). Genetic crosses were carried out by emasculation followed by hand pollination as follows: FGRA106 (♂) × FG769 (♀). An F_1 hybrid plant that was resistant to *P. brassicae* pathotype 3H was vernalized for 10 weeks at 4ºC under a 12 h photoperiod and self-pollinated to obtain F_2 seeds.

Phenotyping assays

The parents and the F_2 population were screened against one single-spore isolate each of *P. brassicae* pathotypes 3A and 3H, and a field isolate of pathotype 3D, as classified based on the CCD set (Strelkov et al. 2018). A total of 1620 F_2 individuals were tested in three rounds of bioassays, in which 180 plants were inoculated with each pathotype in each experiment. The inoculations were conducted as described previously by Strelkov et al. (Strelkov et al. 2016, 2006) with slight modifications. Briefly, clubbed roots were blended in sterile water and filtered through two layers of cheesecloth to generate a resting spore suspension. The spore concentration was then adjusted to 1×10^7 resting spores/mL with sterile water. For inoculation, the roots of 7-day-old seedlings were dipped into the spore suspension for about 10 s and then planted in plastic pots (6 cm × 6 cm × 6 cm) filled with Sunshine Mix#4 potting mixture (Sun Gro Horticulture, Seba Beach, AB, Canada) at a density of one seedling per pot. One millilitre of the resting spore suspension was then pipetted in the potting mix around each seedling to ensure

infection and minimize disease escape. In addition to the resistant and susceptible parents FGRA106 and FG769, respectively, the susceptible *B. napus* cv. 'Westar' was also included as a positive control in all experiments.

The inoculated plants were kept in a greenhouse maintained at 25°C/18°C day/night with a 16-h light period (natural light supplemented with artificial lighting), and assessed for clubroot symptoms at 7 weeks after inoculation (wai) on a 0-3 disease severity scale as described by Kuginuki et al. (Kuginuki et al. 1999) and Strelkov et al. (Strelkov et al. 2006), where: 0 = no galling, 1 = slight galling on side roots, 2 = moderate galling on main and side roots, and 3 = severe galling with almost no observable side roots.

Bulk construction and RNA extraction

RNA extraction from the R (resistant) and S (susceptible) plant pools was based on the phenotypic reactions of individual plants to the three pathotypes. For each pathotype, 15 plants with a disease rating of 0 were pooled into an R bulk, while each S bulk consisted of 15 plants with a rating of 2 or 3. Three biological replicates of both the R and S bulks were assigned for each pathotype.

Leaf samples of each bulk collected at 7 weeks after inoculation were mixed and ground into powder in liquid nitrogen. Total RNA from each bulk replicate was extracted from 0.1 mL (~100 mg) of powdered root tissue of each sample using an RNeasy Plant Mini Kit (Qiagen; Toronto, ON) and purified with an RNase-Free Dnase kit (Qiagen; Toronto, ON). The RNA concentration was measured with a NanoDrop 2000c Spectrophotometer (Thermo Scientific; Waltham, MA) and its quality (RNA integrity numbers (RIN) $\geq$ 6.5 and a 28S/18S ratio $\geq$ 1.0) confirmed using an Agilent 2200 TapeStation system (Agilent, Santa Clara, CA).

RNA sequencing

The cDNA library preparation and RNA sequencing were performed by the Oklahoma Medical Research Foundation NGS Core (Oklahoma City, OK, USA) with an IDT xGen RNA Library kit (Integrated DNA Technologies, Inc., San Diego, CA, USA) and an Illumina NovaSeq 6000 S4 platform (Illumina; San Diego, CA). Pair-end read sequences (2 × 150 bp) in 'fastq' format were generated for further analysis.

Sequence alignment, identification of DEGs, and variant calling

The adaptors were removed from the raw sequences using GATK v4.2.2.0 (McKenna et al. 2010) and quality checked with FastQC v0.12.1 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The trimmed sequences were then aligned to the *B. napus* cv. 'ZS11' reference genome v2.0 (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000686985.2/) using STAR v2.7.9a (Dobin et al. 2013). Three R and three S bulks of each pathotype were analyzed as replicates to identify differentially expressed genes (DEGs) and pooled for variant calling. Gene expression read counts were calculated using RSEM v1.3.3 (Li and Dewey 2011), and normalized with the R package 'DESeq2' (Love et al. 2014). The significance of differentially expressed genes (DEGs) between the R and S bulks was determined based on the log2 fold change ($|\log2\ FC| > 2$) for the bulk pairs of each pathotype. Volcano plots of DEG counts was generated using the R package 'ViDGER' (McDermaid et al. 2018). Enrichment analyses of Gene Ontology (GO; http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG; https://www.genome.jp/kegg/) databases was conducted with eggNOG-mapper v2 (Cantalapiedra et al. 2021) and KOBAS-i (Bu et al. 2021) for annotating disease-related biological processes and pathways.

Variant calling was performed using the GATK v4.2.2.0 function 'HaplotypeCaller', with the SNPs detected subsequently filtered using the GATK 'VariantFiltration' function under proper standards ("QD<2.0 || MQ<40.0 ||FS>60.0 || SOR>3.0"). The final *.vcf* files were converted to *.table* format using the GATK 'VariantsToTable' tool for analysis in *R* 4.2.1 (McKenna et al. 2010; Poplin et al. 2018). SN*P* frequency calling was carried out on resistant and susceptible bulks as described by Liu et al. (Liu et al. 2012), Yu et al. (Yu et al. 2016), and Wu et al. (Wu et al. 2022), with modifications. Comparisons of SN*P* variants between the bulks were performed with the R package 'QTLseqr' (Mansfeld and Grumet 2018) with the following filter settings: refAlleleFreq = 0.20, minTotalDepth = 50, maxTotalDepth = 500, minSampleDepth = 80, minGQ = 99. Detection of QTLs was based on the SNP-index and the Δ(SNP-Index) in a 1-Mb sliding window (Takagi et al. 2013). The SNP-index statistic calculates marker association differences in genotype frequencies of mixed pools, where a value of 0 indicates that the short reads contain genomic fragments from the reference parent, while a value of 1 indicates that all the short reads represent the genome from the other parent (Takagi et al. 2013). A Δ(SNP-index) graph was used to detect the differences between the 'highest' and 'lowest' pools of extreme phenotypes, where Δ(SNP-index) = 1 and -1 indicates bulk DNA from one parent and the other parent, respectively, while Δ(SNP-index) = 0 if both parents have the same SNP-indices at the genomic regions (Takagi et al. 2013). The physical positions of the detected QTLs were visualized with MapChart v2.3.2 (Voorrips 2002) and compared with previously reported QTLs for clubroot resistance.

Statistical analyses

The phenotypic data from different replicates of the same inoculum (pathotype) were subjected to Chi-square tests of homogeneity. A Chi-square goodness of fit test was performed to

determine the segregation of the phenotypic data for all three pathotypes using *R* 4.2.1. Other built-in R packages including ggplot2, reshape2 and ggrepel were also used for data analysis or visualization.

4.3 Results

Clubroot tests

The Chi-square tests of homogeneity indicated that the phenotypic data of F_2 plants inoculated with all three pathotypes were not significantly different in the three replicates (Table S4.1). Therefore, data for the same pathotype were pooled for analysis. The frequency distribution of disease ratings to the three pathotypes is presented in Figure 4.1. The inoculation conducted on the F_2 plants with *P. brassicae* pathotype 3A showed that 12.3%, 7.4%, 32.1% and 48.3% (n = 408) of the plants were rated as 0, 1, 2, or 3, respectively (Table S4.1). The screening results with pathotype 3D indicated that 29.4%, 11.4%, 17.8%, and 41.4% of the F_2 plants (n = 411) exhibited disease ratings of 0, 1, 2, or 3, respectively (Table S4.1). In response to *P. brassicae* pathotype 3H, 23.0%, 9.8%, 13.0% and 54.2% of the F_2 population showed disease ratings of 0, 1, 2, or 3 (n = 439) (Table S4.1). Based on Fisher's LSD test on the disease reaction data, the virulence of the pathotypes on the F_2 population was of the order 3A > 3H = 3D.

Inheritance of clubroot resistance in F_2 populations

The Chi-square goodness of fit test was carried out in two ways following the protocol of Fredua-Agyeman et al. (Fredua-Agyeman et al. 2020a). The first method grouped plants with a disease rating = 0 or 1 as resistant (R), and those with scores 2 or 3 as susceptible (S); while the second method grouped plants with a disease rating = 0 as R, and all others as S. The results obtained with the first method showed that the segregation of clubroot resistance in

the F_2 population was not significantly (P<0.05) different from the expected Mendelian segregation ratios (R : S) of 3 : 13, 7 : 9, and 5 : 11 for pathotypes 3A, 3D, and 3H, respectively, all of which fit two gene models (Table 4.1). The second method indicated that the R : S ratios for pathotypes 3D and 3H were not significantly different from 5 : 11 (two-gene model) or 1 : 3 (one-gene model), respectively, while the ratio for pathotype 3A significantly deviated from all assumptions (Table 4.1).

RNA sequencing, filtering, and sequence alignment

The raw RNA sequences of the resistant and susceptible bulks were filtered and the adapters removed. Subsequently, the number of clean reads retained in the resistant bulks ranged from 24.2 - 28.7 Gb, 23.5 - 26.5 Gb and 22.3 - 25.0 Gb, while in the susceptible bulks this number ranged from 21.5 - 42.9 Gb, 20.7 - 23.2 Gb and 21.8 - 23.8 Gb for pathotypes 3A, 3D and 3H, respectively (Table S4.2). Therefore, the RNA sequencing data yielded 20× to 30× the genome size of *B. napus*. The GC content ranged from 47% to 48%. Approximately 89.7% to 93.1% of these reads were mapped to the *B. napus* cv. 'ZS11' reference genome v2.0, of which 68.6% to 71.9% mapped only to exonic gene regions (Table S4.2). The mismatch rate per base ranged from 0.9% to 1.1% (Table S4.2). Therefore, the sequencing data were of adequate quality for the subsequent analysis.

SNP calling and marker distribution

A total of 338177, 331344 and 325623 SN*P* markers were obtained for the comparisons between the reference (*B. napus* cv. 'ZS11') genome and the resistant and susceptible bulks from the inoculation experiments with pathotypes 3A, 3D and 3H, respectively (Table 4.2, Figure 4.2). The SN*P* marker densities on all 19 *B. napus* chromosomes are presented in Table 2 and Figure 2. The SN*P* densities for pathotypes 3A, 3D and 3H bulks on the different chromosomes ranged

from 190.78 to 732.31, 187.07 to 719.08 and 189.14 to 692.25 SNPs/Mb, respectively (Table 4.2). The highest SN*P* density was found on chromosome A10 for the three pathotypes, while the lowest occurred on chromosome C02 (Table 4.2). Consistently high SN*P* densities were observed at the beginning of chromosomes A01, A02, and A03, as well as at the end of chromosome A10, across all three pathotypes. Conversely, a region of low coverage density was noted on chromosome C09 (Figure 4.2).

Differentially expressed genes

Totals of 73607, 72644, and 72361 differentially expressed genes were identified between the resistant and susceptible bulks for pathotypes 3A, 3D and 3H, respectively (Figure 4.3). About 1.15% (850), 0.17% (120) and 0.21% (151) of these genes were significantly ($P_{adj} < 0.05$) differentially expressed (Figure 4.3). Based on a 95% confidence threshold ($P_{adj} < 0.05$) and |log2 FC| > 2 as the criteria, 428, 67, and 98 DEGs were identified in the bulks of pathotypes 3A, 3D and 3H (Table S4.3, Figure 4.4). Among these, 81, 27 and 36 genes were upregulated in the R bulks of 3A, 3D and 3H, respectively, while 347, 40, 62 were upregulated in the S bulks (Figure 4.5a,b). One DEG was consistently identified in the R bulks, S bulks, and both R and S bulks. Two, nine and 13 DEGs were found between the bulks of pathotypes 3A and 3D, 3D and 3H, and 3A and 3H, respectively. About 72% (412/569), 13% (76/569) and 10% (56/569) of the DEGs were detected for pathotypes 3A, 3H and 3D, respectively (Figure 4.5c).

Identification of QTLs associated with clubroot resistance

Based on Δ(SNP-index) statistics at a 99% confidence interval (CI) from the variant calling between R and S bulks of the three pathotypes, a total of 12 QTLs associated with resistance to the three pathotypes were detected on seven of the 19 chromosomes of *B. napus* (Figure 4.6, Table S4.6). These QTLs were located on chromosomes A01 (1), A05 (1), A08 (4), C01 (3), C07

(2), C08 (1) and C09 (1) (Table S6). The peak |Δ(SNP-index)| values ranged from ~0.23 to 0.53 (Table S4.6). The higher the |Δ(SNP-index)|, the stronger the correlation between marker SNPs and traits. In this study, the QTLs were classified as major if the |Δ(SNP-index)| > 0.32 and the peak was clearly above the 99% confidence interval; conversely, QTLs were classified as minor if the |Δ(SNP-index)| < 0.30 and the peak fell between the 95% and 99% confidence intervals (Table S6).

The QTLs were named following the *Brassica* gene nomenclature system proposed by Østergaard and King (Østergaard and King 2008), as modified by Fredua-Agyeman et al. (Fredua-Agyeman et al. 2020a). For example, the QTL on chromosome A01 was designated *BnaA1P3D.CRX1.1*, where the first letter denotes the genus (*Brassica*), the second and third letters the species (*napus*), the fourth letter the genome (*A*), the fifth letter the chromosome (*1*), and the sixth, seventh and eighth letters (*P3D*) indicate the pathotype of *P. brassicae* used for inoculation; these are followed by the name(s) of the closest published CR gene(s) (3-8 letters) or the letter *X* if no previous markers have been reported (*CRX*), and finally the number of the QTL number (2 digits, *1.1*).

Based on this gene nomenclature, two major effect QTLs, *BnaA5P3A.CRX1.1* on chromosome A05 and *BnaC7P3A.CrrA51.1* on chromosome C07, conferred resistance to the isolate representing pathotype 3A (Figure 4.7, Table 4.3, Table S4.6). Four major and five minor QTLs were identified for resistance to the isolate representing pathotype 3D. The four major QTLs, *BnaA8P3D.CRX1.1*, *BnaA8P3D.RCr91.2*, *BnaA8P3D.Crr11.3*, and *BnaA8P3D.qBrCR381.4*, were all situated on chromosome A8. The five minor QTLs, *BnaA1P3D.CRX1.1, BnaA5P3D.CRX1.1*, *BnaC7P3D.CRX1.1*, *BnaC8P3D.CRX1.1*, and *BnaC9P3D.CRX1.1*, were located on chromosomes A01, A05, C07, C08, and C09, respectively

(Figure 4.7, Table 4.3, Table S4.6). Seven QTLs (3 major and 4 minor) conferred resistance to the isolate representing *P. brassicae* pathotype 3H. Two of the major QTLs *BnaA8P3H.RCr91.2*, *BnaA8P3H.Crr11.3*, along with one minor QTL *BnaA8P3H.qBrCR381.4*, were located on chromosome A08. Additionally, one major QTL *BnaC1P3H.CRX1.2*, and two minor QTL *BnaC1P3H.CRX1.1* and *BnaC1P3H.CRX1.3*, were found on chromosome C01. One minor QTL, *BnaC9P3H.CRX1.1*, was located on chromosome C09 (Figure 4.7, Table 4.3, Table S4.6).

QTLs located within 2 cM (~1000 kb) of each other were regarded as coincident and treated as the same QTL. Four of these coincident genomic regions provided resistance to two pathotypes. The coincident QTLs *BnaA5P3A.CRX1.1* (12,272,166 nt)/*BnaA5P3D.CRX1.1* (13,623,271 nt), on chromosome A05, conferred resistance to isolates representing pathotypes 3A and 3D. Similarly, the coincident QTLs *BnaA8P3D.RCr91.2* (10,275,090 nt)/*BnaA8P3H.RCr91.*2 (11,310,428 nt) and *BnaA8P3H.Crr11.3* (15,908,289nt)/*BnaA8P3D.Crr11.3* (16,016,864 nt) on chromosome A08, along with *BnaC9P3D.CRX1.1*/*BnaC9P3H.CRX1.1* (31,610,782 nt) on chromosome C09, conferred resistance to isolates representing pathotypes 3D and 3H (Figure 4.7, Table 4.3, Table S4.6).

Genes identified in clubroot resistance QTL regions

Eleven of the 12 identified QTL regions contained genes with the exception of *BnaC8P3D.CRX1.1* (Table 4.3, Table S4.6). These included genes involved in the plant disease response such as ethylene-responsive transcription factor ERF109 (gene ID: 106361033), serine/threonine-related genes (106418155), heat shock protein (HSP) genes (gene ID: 106361308, 106362025), polyubiquitin 11 (gene ID: 106349084), GLABROUS1 enhancer-binding protein-like genes (gene ID: 106376056), UDP-glucosyltransferase genes (gene ID: 106361045), and 60S ribosomal protein genes (gene ID: 106406248, 111202359).

DEGs identified in clubroot resistance QTL regions

Seventeen annotated DEGs were found in four of the identified QTLs regions associated with clubroot resistance. Thirteen of these genes were located on chromosome C07, while three genes were on chromosome A08 (Table 4.4). Eight of the DEGs (IDs: 106348481, 106348998, 106390302, 106410495, 106410578, 106410663, 106410664, 111198409), all on chromosome C07, were identified in the hosts inoculated with the isolate representing pathotype 3A. One of these genes (ID# 106348481) encoded DOWNY MILDEW RESISTANCE 6 protein (Table 4.4). Two genes, IDs: 106407096, and 106348764, on chromosome C07, were expressed in the reactions to pathotype 3D. The latter of these, which encoded tesmin/TSO1-like CXC 7, was also differentially expressed in response to pathotype 3H (Table 4.4). Three other genes on chromosome C07 (IDs: 106349452, 106440113, 111204564) were also expressed following inoculation with the isolate representing pathotype 3H. Another three genes on chromosome A08 (IDs: 106360694, 106381656, and 106416269) also were upregulated in response to pathotype 3H (Table 4.4). Other genes in the QTL region associated with the plant disease response included ethylene-responsive transcription factor 1A-like (gene ID: 106381656) on *BnaA8P3D.RCr91.2*, 60S ribosomal protein L7-2 (gene ID: 106349452) on *BnaC7P3D.CRX1.1*, and heat stress transcription factor A-7a-like (gene ID: 111198409) on *BnaC7P3A.CRX1.1*.

Functional enrichment analyses of differentially expressed genes

The Gene Ontology (GO) analysis indicated a significant enrichment (FDR < 0.05) of GO terms for pathotypes 3A, 3D, and 3H, with 19, 22, and 59 enriched terms, respectively (see Table S4). Figure 4.8 illustrates the top GO terms with the highest number of enriched DEGs. The results showed that DEGs in the pathotype 3A bulks were involved in bioprocesses previously reported to induce or enhance plant immunity, such as response to sucrose (GO:0009744), aminoglycan

metabolism (GO:0006022) and catabolism (GO:0006026), chitin metabolism (GO:0006030) and catabolism (GO:0006032), and defense response (GO:0006952) (Chisholm et al. 2006; Payá et al. 2024; Pusztahelyi 2018; Tauzin and Giardina 2014). The DEGs in the pathotype 3D bulks were involved in methionine regulation related bioprocesses (GO:0019509, GO:0071267, GO:0071265, GO:0009086, GO:0006555), actin filament bundle assembly (GO:0051017) and organization (GO:0061572), and sulfur amino acid biosynthetic (GO:0000097) and metabolic process (GO:0000096). The DEGs in the pathotype 3H bulks were involved in tRNA or mitochondrial tRNA-related processes (GO:0034414, GO:0042779, GO:0042780, GO:0072684, GO:0000963, GO:0000959, GO:1905267), inositol phosphate-related processes (GO:0046855, GO:0071545, GO:0046854, GO:0043647, GO:0046856, GO:0006661), phospholipid biosynthetic (GO:0008654), metabolic (GO:0006644) and dephosphorylation processes (GO:0046839), and chitin metabolism (GO:0006030) and catabolism (GO:0006032).

The KEGG pathway analyses indicated that 14, 7 and 10 pathways were significantly (FDR<0.05) associated with the DEGs in response to pathotypes 3A, 3D and 3H, respectively (Table S4.5, Figure 4.9). Metabolic (bna01100) and ribosome (bna03010) pathways were associated with DEGs for all three pathotypes. In contrast, the plant-pathogen interaction (bna04626) and glycine, serine and threonine metabolism (bna00260) pathways were associated only with DEGs for pathotype 3A. Glutathione metabolism (bna00480) was linked to the DEGs in response to both pathotypes 3A and 3H. Phenylpropanoid (bna00940) and flavonoid (bna00941) biosynthesis were associated with DEGs for pathotypes 3D and 3H.

4.4 Discussion

The German rutabaga cv. ‘Wilhelmsburger’ (ECD 10) was originally proposed as a differential host by Williams (1966), and subsequently was included in both the European Clubroot

Differential (ECD; (Buczacki et al. 1975)) and CCD (Strelkov et al. 2018) sets. However, Yu et al. (2021) and Fredua-Agyeman et al. (2020b) observed different resistance phenotypes in seven 'Wilhemsburger' accessions from Denmark, FGRA106, FGRA107, FGRA108, FGRA109, FGRA110, FGRA111 and FGRA112, when these were challenged with the same set of isolates representing 16 different *P. brassicae* pathotypes from Canada. Only the 'Wilhemsburger' accession FGRA106 from Denmark showed broad-spectrum clubroot resistance comparable to that of 'Wilhelmsburger' (ECD 10) from Germany; both were resistant to the 16 pathotypes tested by Fredua-Agyeman et al. (2020b). Given the proximity of Denmark to Germany as neighboring countries and the potential for germplasm movement, it is plausible that the 'Wilhelmsburger' accession FGRA106, based on its reactions, could be equivalent to 'ECD 10' and thus might harbor the same CR gene(s).

'Wilhelmsburger' has been used as a clubroot resistance donor in breeding programs worldwide for many decades (Spaner 2002). Lammerink (1967) evaluated F_2 progenies derived from 'Wilhelmsburger' with a *P. brassicae* isolate designated "Race B", and suggested that the resistance was controlled by one dominant gene based on a 3R:1S segregation ratio. Similarly, Ayers and Lelacheur (1972) reported, based on segregation ratios of an F_2 population derived from 'Wilhelmsburger', that the resistance to *P. brassicae* race 2 (*sensu* Williams 1966) was controlled by two dominant genes, whereas resistance to race 3 was controlled by one dominant gene. In a separate study involving an F_2 population, Gustafsson and Falt (1986) reported that the resistance of 'Wilhelmsburger' to a less virulent isolate 'Pb3' may have been conferred by two genes, whereas resistance to a highly virulent isolate 'Pb7' appeared to involve only one gene. In contrast, Crute et al. (1983) suggested that 'Wilhelmsburger' possesses three clubroot resistance genes. These observations indicate that 'Wilhelmsburger' may carry multiple CR genes that

could be differentially effective depending on the virulence of specific *P. brassicae* isolates. In the current study, the segregation ratios of F_2 plants inoculated with Canadian *P. brassicae* isolates representing various pathotypes were analyzed. The results suggested that the resistance inherited from FGRA106 to pathotypes 3A and 3D was likely determined by two genes, whereas resistance to pathotype 3H was conferred by either one or two genes.

Crute et al. (1983) suggested that the CR genes in *B. rapa* (A-genome) and *B. napus* (AC-genome) are qualitative or race-specific, while resistance in *B. oleracea* (C-genome) is quantitative or race-nonspecific (Crute et al. 1983; Piao et al. 2009). In this study, two major QTLs, *BnaA5P3A.CRX1.1* on chromosome A05 and *BnaC7P3A.CRX1.1* chromosome C07, conferred resistance to an isolate representing pathotype 3A. The QTL *BnaC7P3A.CRX1.1* mapped to a position proximal to the *CrrA5* gene on chromosome A05 (Nguyen et al. 2018), while the QTL *BnaC7P3A.CRX1.1* mapped distal to the *Rcr7*, *qCRc7-1*, *qCRc7-2*, *qCRc7-3*, and *qCRc7-4* genes or QTLs on chromosome C07 (Dakouri et al. 2018; Ce et al. 2021). This indicates the possibility of novel CR genes in these regions. In the case of the isolates representing pathotypes 3D and 3H, the major QTLs *BnaA8P3D.Rcr91.2* and *BnaA8P3H.Rcr91.2* mapped to the same genomic location as *Rcr1* (Yu et al. 2022, 2017), while *BnaA8P3H.Crr11.3* and *BnaA8P3D.Crr11.3* mapped to the same genomic location as *Crr1* (Chen et al. 2013; Suwabe et al. 2003). Another major QTL, *BnaA8P3D.qBrCR381.4*, detected in response to pathotype 3D, mapped to the same genomic location on chromosome A08 as *qBrCR38-2* (Karim et al. 2020; Laila et al. 2019), while the major QTL *BnaA8P3D.CRX1.1* mapped to a position proximal to the aforementioned genes on this chromosome. This suggests that two additional genomic regions on chromosome A08 were needed to confer resistance to pathotype 3D.

Chromosome A08 has been reported to harbour major CR loci in rutabaga (Fredua-Agyeman et al. 2020b). Four major QTLs in this study were positioned on the A08 chromosome. Despite the differences in mapping methods and reference genomes, the identified QTLs were located in genomic regions where *Crr1* (Chen et al. 2013; Suwabe et al. 2003), *CRs* (Laila et al. 2019), *PbBa8.1* (Chen et al. 2013), *Rcr9* (Yu et al. 2017), *Rcr9*^{wa} (Karim et al. 2020), and *Rcr9*^{ECD01} (Yu et al. 2022) were previously mapped. The *Crr1* gene was identified from progenies of *B. rapa* 'Siloga' and conferred resistance to a Japanese isolate of *P. brassicae* classified as pathotype/race 4 (Suwabe et al. 2003). The gene *CRs* (Laila et al. 2019) was mapped in inbred lines derived from an unknown CR turnip donor, which provided resistance to pathotype 4 (Williams 1966); while *Rcr9* (Yu et al. 2017), conferring resistance to pathotype 5X (Strelkov et al. 2018), was derived from a German turnip cultivar 'Pluto'. Additionally, *Rcr9*^{wa} or *PbBa8.1*, which confers resistance to pathotype 5X (Strelkov et al. 2018) or pathotypes 4 and 7 (Williams 1966), was inherited from *B. rapa* ECD 04 (Chen et al. 2013; Karim et al. 2020). The gene *Rcr9*^{ECD01}, derived from *B. rapa* ECD 01, provided resistance to pathotypes 3A, 3D, 3H and 5X, which includes the three pathotypes examined in this study (Yu et al. 2022).

Two major effect QTLs on the C-genome were mapped to chromosomes C01 and C07. The first major QTL, *BnaC1P3H.CRX1.2*, which conferred resistance to pathotype 3H, was distant from the QTL *Rcr_C01-1* reported on chromosome C01 of *B. oleracea* (Karim and Yu 2023). Two minor effect QTLs, *BnaC1P3H.CRX1.1* and *BnaC1P3H.CRX1.3* on chromosome C01, which also conferred resistance to pathotype 3H, were identified as proximal and distal, respectively, to a major effect QTL *BnaC1P3H.CRX1.2*. The second major effect QTL, *BnaC7P3A.CRX1.1*, which conferred resistance to pathotype 3A, was located on the bottom half of chromosome C07. A minor QTL, *BnaC7P3D.CRX1.1*, was also located on the bottom half of

chromosome C07. These results confirm the bottom half of chromosome C07 as a genomic hotspot for several clubroot resistance genes, including *Rcr7*, *qCRc7-1*, *qCRc7-2*, *qCRc7-3*, and *qCRc7-4* (Dakouri et al. 2018; Ce et al. 2021). Therefore, the clubroot resistance derived from the donor FGRA106 in this study appears to be conferred not only by major QTLs on the A-genome, but also by major and minor QTLs located on the C-genome.

The plant immune system is generally considered to comprise two layers: pathogen-associated molecular patterns (PAMPs)-triggered immunity (PTI), which offers basal protection against many pathogens, and effector-triggered immunity (ETI), which results in robust and localized responses against specific pathogens (Dodds and Rathjen 2010). The GO and KEGG enrichment analyses conducted in this study suggested that the DEGs between the R and S bulks were associated with both of these layers of defense. The GO analysis revealed the differential expression of genes involved in bioprocesses related to sucrose, actin filaments and sulfur amino acids, while enriched KEGG pathways were associated with metabolic pathways, ribosomes and tRNA. These processes and pathways have been implicated as common initial defense signaling processes in eukaryotes (Nagaraj et al. 2016; Ramu et al. 2020; Soprano et al. 2018; Zheng et al. 2024; Cittadino et al. 2023). Additionally, pathways involving inositol phosphate, glycine, serine, and threonine were also identified and have been implicated in the recognition of PAMPs by pattern recognition receptors (PRRs) in the host and in subsequent defense responses (Hasanuzzaman et al. 2018; Henty-Ridilla et al. 2013; Nagaraj et al. 2016; Rojas et al. 2014; Zhai et al. 2022). Several GO processes and KEGG pathways were also identified that have been implicated in ETI, included GO bioprocesses related to methionine, phospholipid and chitin, and KEGG pathways associated with glutathione, phenylpropanoid and flavonoids (Soprano et al.

2018; Williams et al. 2015; Seth et al. 2024; Pusztahelyi 2018; Gullner et al. 2017; Dixon et al. 2002).

Some of the genes identified in the QTL regions through the SNPs and differential gene expression analyses have also been associated with PTI and ETI. For instance, heat shock proteins (HSPs) function as chaperones, playing roles in protein folding, assembly, translocation, and degradation during both abiotic and biotic stress. These processes are vital for the formation of PRRs and intracellular responsive proteins essential for resistance (Park and Seo 2015). Polyubiquitin modulates cellular protein turnover and homeostasis in basal host defense to abiotic and biotic stresses, and is involved in responsive modification of proteins in both PTI and ETI (Zhang and Zeng 2020). The ethylene-responsive transcription factor ERF109 plays a key role in ethylene-mediated defense pathways during ETI (Yang et al. 2022). GLABROUS1 enhancer-binding protein-like, UDP-glucosyltransferase, and 60S ribosome proteins are implicated in ETI, and silencing of these genes can activate plant defense pathways (Fakih et al. 2023; García-Cano et al. 2018; Lukan et al. 2020; Xing et al. 2018). Additionally, DOWNY MILDEW RESISTANCE 6 serves as a resistance gene in ETI (Zeilmaker et al. 2015). In a recent study of differential gene expression in 'Wilhelmsburger' in response to inoculation with pathotype 3A, Zhou et al. (2020) found that salicylic acid and ethylene-mediated defense were involved in the host reaction.

4.5 Conclusions

The CR donor FGRA106 and the resistant F_2 progeny evaluated in this study were confirmed to carry resistance to *P. brassicae* pathotypes 3A, 3D and 3H, which are predominant on canola in western Canada (Hollman et al. 2023). The resistance donor FGRA106 exhibited reactions similar to ECD10, and was previously reported to be resistant or moderately resistant to 17

isolates representing 16 pathotypes of *P. brassicae* (Fredua-Agyeman et al. 2020b). Based on the DEGs, QTLs and associated GO terms and KEGG pathways, gene loci conferring resistance to pathotype 3A were mapped to chromosomes A05 and C07, while major QTLs for resistance to pathotypes 3D and 3H co-segregated to at least three genomic regions on chromosome A08. Another major QTL on chromosome C01 was required for resistance to pathotype 3H. The CR donor and the SNP markers identified in this study may serve as valuable resources for clubroot resistance breeding in Canadian canola.

4.6 Tables

Table 4.1. Segregation ratios of resistant (R) and susceptible (S) plants in an F_2 population derived from FGRA106 (♀) × FG769 (♂) evaluated for resistance to *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Pathotype	Total	R (0+1)	S (2+3)	R:S ratio[1]	Chi-square	p-value	R (0)	S (1+2+3)	R:S ratio[2]	Chi-square	p-value
3A	408	80	328	3R:1S	667.6601	<0.00001	50	358	3R:1S	856.6797	<0.00001
				1R:3S	6.326797	0.01189			1R:3S	35.34641	<0.00001
				9R:7S	222.5982	<0.00001			9R:7S	320.8988	<0.00001
				7R:9S	96.62994	<0.00001			7R:9S	164.4544	<0.00001
				5R:11S	25.73975	<0.00001			5R:11S	68.5205	<0.00001
				11R:5S	458.6125	<0.00001			11R:5S	606.1205	<0.00001
				13R:3S	1017.633	<0.00001			13R:3S	1274.888	<0.00001
				3R:13S	0.197084	0.65708			3R:13S	11.29814	0.00078
				15R:1S	3827.712	<0.00001			15R:1S	4624.575	<0.00001
				1R:15S	124.2458	<0.00001			1R:15S	25.1085	<0.00001
3D	411	168	243	3R:1S	255.2482	<0.00001	121	290	3R:1S	454.9886	<0.00001
				1R:3S	55.24818	<0.00001			1R:3S	4.321979	0.03762
				9R:7S	39.4748	<0.00001			9R:7S	120.039	<0.00001
				7R:9S	1.379562	0.24018			7R:9S	34.1977	<0.00001
				5R:11S	17.72568	0.00003			5R:11S	0.626454	0.42866
				11R:5S	148.6348	<0.00001			11R:5S	295.6083	<0.00001
				13R:3S	439.767	<0.00001			13R:3S	724.1655	<0.00001
				3R:13S	132.0747	<0.00001			3R:13S	30.83218	<0.00001
				15R:1S	1960.994	<0.00001			15R:1S	2900.964	<0.00001
				1R:15S	840.9942	<0.00001			1R:15S	377.2303	<0.00001
3H	439	144	295	3R:1S	416.918	<0.00001	101	338	3R:1S	632.9301	<0.00001
				1R:3S	14.25133	0.00016			1R:3S	0.930144	0.33483
				9R:7S	98.08038	<0.00001			9R:7S	197.1373	<0.00001
				7R:9S	21.38196	<0.00001			7R:9S	76.7563	<0.00001
				5R:11S	0.492069	0.48301			5R:11S	13.88449	0.00019
				11R:5S	264.0557	<0.00001			11R:5S	427.5572	<0.00001
				13R:3S	676.3863	<0.00001			13R:3S	977.5294	<0.00001
				3R:13S	56.89907	<0.00001			3R:13S	5.221716	0.02231
				15R:1S	2783.138	<0.00001			15R:1S	3749.576	<0.00001
				1R:15S	528.2043	<0.00001			1R:15S	210.3765	<0.00001

[1] Plants with clubroot disease severity ratings of 0 and 1 were regarded as resistant (R), and those with ratings of 2 and 3 as susceptible (S).

[2] Plants with a clubroot disease severing rating of 0 were regarded as R, and those with ratings of 1, 2 and 3 as S.

Table 4.2. Distribution and density of single nucleotide polymorphisms (SNPs) identified in resistant (R) and susceptible (S) bulks in an F_2 population derived from FGRA106 (♀) × FG769 (♂) tested with pathotypes 3A, 3D, or 3H of *Plasmodiophora brassicae*.

Pathotype	**3A**		**3D**		**3H**	
Chromosome	**# SNP[1]**	**SNP/Mb[2]**	**# SN*P***	**SNP/Mb**	**# SN*P***	**SNP/Mb**
A01	20939	584.52	20597	574.97	19994	558.14
A02	22146	626.78	21668	613.25	21502	608.56
A03	31343	637.84	30494	620.56	29862	607.70
A04	12572	533.69	12345	524.05	12040	511.11
A05	16995	539.98	16512	524.63	16373	520.22
A06	20115	557.50	19809	549.02	19356	536.46
A07	15927	580.71	15526	566.09	15439	562.91
A08	11782	424.70	11737	423.08	11348	409.06
A09	23060	501.92	22594	491.78	22382	487.16
A10	16270	732.31	15976	719.08	15380	692.25
C01	11826	232.90	11388	224.28	11236	221.28
C02	13049	190.78	12795	187.07	12937	189.15
C03	26556	330.38	26055	324.15	25843	321.51
C04	16072	227.80	16025	227.14	15571	220.70
C05	12100	274.10	11924	270.11	11722	265.54
C06	11473	252.12	11417	250.89	11086	243.62
C07	13456	215.49	12956	207.49	12986	207.97
C08	13499	291.26	13316	287.31	12989	280.26
C09	10939	211.59	10503	203.16	10074	194.86
Scaffolds	18058	NA	17707	NA	17503	NA

[1] # SNP, SN*P* count located on A- and C- chromosomes or scaffolds.

[2] SNP/Mb, SN*P* density per million base pairs.

Table 4.3. QTLs conferring resistance to pathotypes 3A, 3D, or 3H of *Plasmodiophora brassicae* and genes identified by significant single nucleotide polymorphisms (SNPs) in QTLs.

QTL	Chromosome	Position		Gene ID	Gene name	Overlapping QTL
		Start	End			
BnaA5P3A.CRX1.1 (Major)	A05	12272166	12272166	106362025	hsp70-Hsp90 organizing protein 3	
BnaC7P3A.CRX1.1 (Major)	C07	45670744	46185588	106406248	60S ribosomal protein L26-1	
				111208271	alpha-humulene/(-)-(E)-beta-caryophyllene synthase-like	
BnaA1P3D.CRX1.1 (Minor)	A01	14059868	14060851	106361131	cilia- and flagella-associated protein 251	
BnaA5P3D.CRX1.1 (Minor)	A05	13623271	13623271	106411554	proteasome subunit alpha type-4-A-like	
BnaA8P3D.CRX1.1 (Major)	A08	7443712	7443835	106396583	enoyl-CoA delta isomerase 2, peroxisomal	
BnaA8P3D.RCr91.2 (Major)	A08	10275090	11076201	106418916	transcription initiation factor TFIID subunit 1	
BnaA8P3D.Crr11.3 (Major)	A08	16016864	16050956	106361045	UDP-glucosyl transferase 73B2-like	*BnaA8P3H.Crr11.3*
				106361049	polyubiquitin 11	*BnaA8P3H.Crr11.3*
				106361048	polyadenylate-binding protein 2	*BnaA8P3H.Crr11.3*
BnaA8P3D.qBrCR381.4 (Major)	A08	17871036	20312633	106361356	probable beta-1,3-galactosyltransferase 4	
				106361316	31 kDa ribonucleoprotein, chloroplastic	
				106361308	heat shock 70 kDa protein 6, chloroplastic-like	*BnaA8P3H.qBrCR381.4*
				106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like	*BnaA8P3H.qBrCR381.4*
				106361295	aconitate hydratase 1	
				106361282	phosphoserine aminotransferase 1, chloroplastic	*BnaA8P3H.qBrCR381.4*
				106361274	50S ribosomal protein L25-like	
				106405582	protein PLASTID MOVEMENT IMPAIRED 1	*BnaA8P3H.qBrCR381.4*
				106361258	bZI*P* transcription factor 60	*BnaA8P3H.qBrCR381.4*
				106451550	GLABROUS1 enhancer-binding protein-like 1	
				106384864	uncharacterized LOC106384864	
				106384865	uncharacterized LOC106384865	

				106361389	NAD(P)H-quinone oxidoreductase subunit M, chloroplastic	
				106361360	apoptotic chromatin condensation inducer in the nucleus	
BnaC7P3D.CRX1.1 (Minor)	C07	34501975	34509210	106418155	serine/threonine-protein kinase prp4	
				106418157	uncharacterized LOC106418157	
BnaC9P3D.CRX1.1 (Minor)	C09	31610782	31611253	106418720	vicilin-like seed storage protein At2g18540	*BnaC9P3H.CRX1.1*
BnaA8P3H.RCr91.2 (Major)	A08	11310428	12506566	106390924	selenium-binding protein 1	
				106397231	UDP-glycosyltransferase 75C1-like	
				106422372	5-amino-6-(5-phospho-D-ribitylamino)uracil phosphatase, chloroplastic-like	
BnaA8P3H.Crr11.3 (Major)	A08	15908289	16050956	106361033	ethylene-responsive transcription factor ERF109	
				106361045	UDP-glucosyl transferase 73B2-like	*BnaA8P3D.Crr11.3*
				106361049	polyubiquitin 11	*BnaA8P3D.Crr11.3*
				106361048	polyadenylate-binding protein 2	*BnaA8P3D.Crr11.3*
BnaA8P3H.qBrCR381.4 (Major)	A08	18281327	18872996	106361308	heat shock 70 kDa protein 6, chloroplastic-like	*BnaA8P3D.qBrCR381.4*
				106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like	*BnaA8P3D.qBrCR381.4*
				106361282	phosphoserine aminotransferase 1, chloroplastic	*BnaA8P3D.qBrCR381.4*
				106405582	protein PLASTID MOVEMENT IMPAIRED 1	*BnaA8P3D.qBrCR381.4*
				106361258	bZI*P* transcription factor 60	*BnaA8P3D.qBrCR381.4*
BnaC1P3H.CRX1.1 (Minor)	C01	17677705	17678086	106376056	GLABROUS1 enhancer-binding protein-like	
BnaC1P3H.CRX1.2 (Major)	C01	26237303	26648486	106349084	polyubiquitin 11	
				106349049	uncharacterized LOC106349049	
BnaC1P3H.CRX1.3 (Minor)	C01	42700048	42700048	111202359	60S ribosomal protein L27-3	
BnaC9P3H.CRX1.1 (Minor)	C09	31610782	32261061	106418720	vicilin-like seed storage protein At2g18540	*BnaC9P3D.CRX1.1*
				106392952	uncharacterized LOC106392952	

Note: The QTLs are denoted as major or minor in parentheses below each QTL name based on peak |Δ(SNP-index)| values. Gene IDs and names were obtained from the NCBI database

(https://www.ncbi.nlm.nih.gov/), where gene names denote descriptions of gene functions.

Overlapping QTLs denote the same QTL identified in bulks tested with different pathotypes.

Table 4.4. Differentially expressed genes (DEGs) in identified QTL regions of resistant (R) and susceptible (S) bulks in an F_2 population derived from FGRA106 (♀) × FG769 (♂) tested for resistance to *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Gene ID	Symbol	Chromosome	QTL	DEG Pathotype	Description of gene functions
106360694	LOC106360694	A08	*BnaA8P3D.RCr91.2*	3H	RGG repeats nuclear RNA binding protein A
106381656	LOC106381656	A08	*BnaA8P3D.RCr91.2*	3H	ethylene-responsive transcription factor 1A-like
106416269	LOC106416269	A08	*BnaA8P3D.qBrCR381.4*	3H	cysteine protease XCP2
106407096	LOC106407096	C07	*BnaC7P3A.CRX1.1*	3D	protein SMAX1-LIKE 3
106348481	LOC106348481	C07	*BnaC7P3A.CRX1.1*	3A	protein DOWNY MILDEW RESISTANCE 6
106348764	LOC106348764	C07	*BnaC7P3A.CRX1.1*	3D, 3H	protein tesmin/TSO1-like CXC 7
106348998	LOC106348998	C07	*BnaC7P3A.CRX1.1*	3A	peptidyl-prolyl cis-trans isomerase FKBP65
106349452	LOC106349452	C07	*BnaC7P3D.CRX1.1*	3H	60S ribosomal protein L7-2
106390302	LOC106390302	C07	*BnaC7P3D.CRX1.1*	3A	LOB domain-containing protein 37-like
106410495	LOC106410495	C07	*BnaC7P3A.CRX1.1*	3A	glycine-rich protein 5-like
106410578	LOC106410578	C07	*BnaC7P3A.CRX1.1*	3A	pectinesterase inhibitor 7-like
106410663	LOC106410663	C07	*BnaC7P3A.CRX1.1*	3A	bark storage protein A
106410664	LOC106410664	C07	*BnaC7P3D.CRX1.1*	3A	tonoplast dicarboxylate transporter
106440113	LOC106440113	C07	*BnaC7P3A.CRX1.1*	3H	protein CHAPERONE-LIKE PROTEIN OF POR1, chloroplastic
111198409	LOC111198409	C07	*BnaC7P3A.CRX1.1*	3A	heat stress transcription factor A-7a-like
111204564	LOC111204564	C07	*BnaC7P3A.CRX1.1*	3H	ATP-dependent Clp protease ATP-binding subunit CLPT1, chloroplastic-like

Note: Gene IDs, symbols and names were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/), where gene names denote descriptions of gene functions.

4.7 Figures

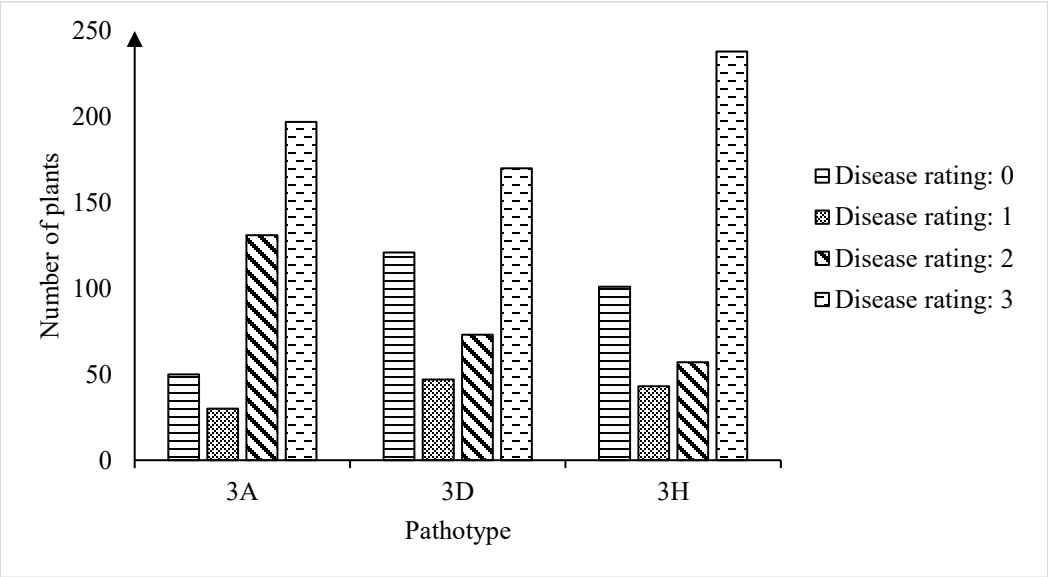
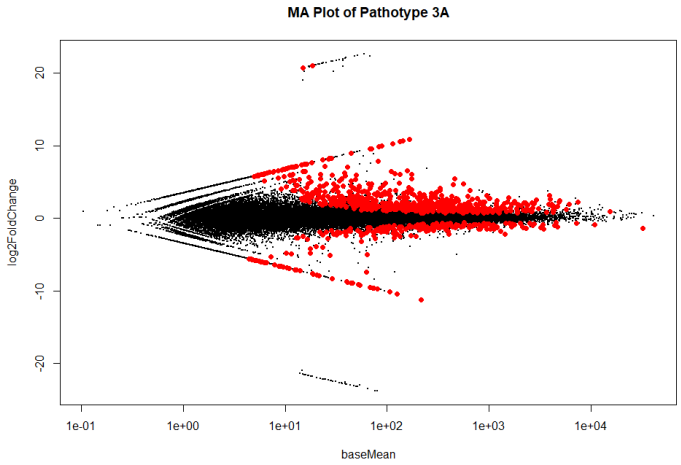
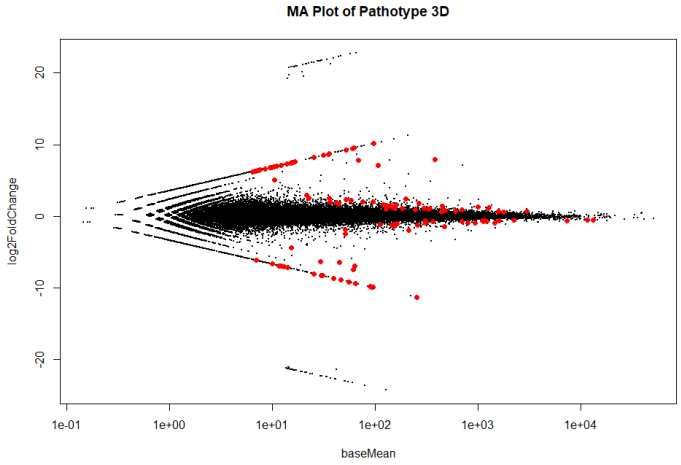


Figure 4.1. Frequency distribution of clubroot disease severity ratings in an F_2 population derived from FGRA106 (♀) × FG769 (♂) to isolates representing pathotypes 3A, 3D, and 3H of *Plasmodiophora brassicae*. Plants were grown under greenhouse conditions and evaluated for clubroot severity on a 0-3 disease severity scale as described by Kuginuki et al. (1999) and Strelkov et al. (2006) at 7 weeks following inoculation with each pathotype.

Figure 4.2. Distribution of polymorphic single nucleotide polymorphisms (SNPs) on 19 *Brassica napus* chromosomes identified between resistant (R) and susceptible (S) bulks in an

F_2 population derived from FGRA106 (♀) × FG769 (♂) tested with *Plasmodiophora brassicae* isolates representing pathotypes 3A (a), 3D (b) and 3H (c). The colors indicate SN*P* density (SNPs/Mb) as per the scale on the right-hand side.





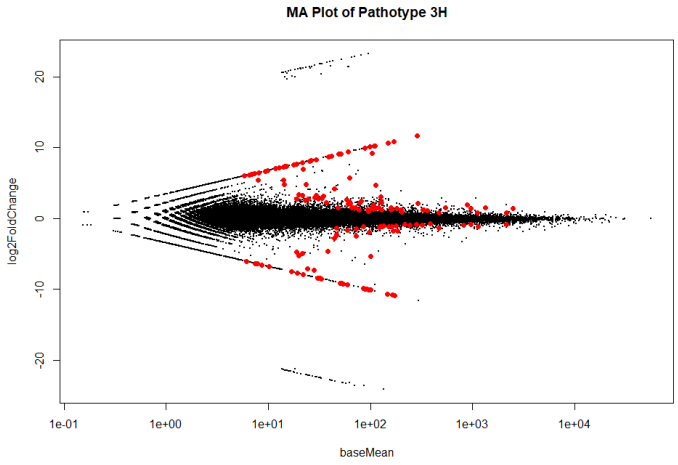
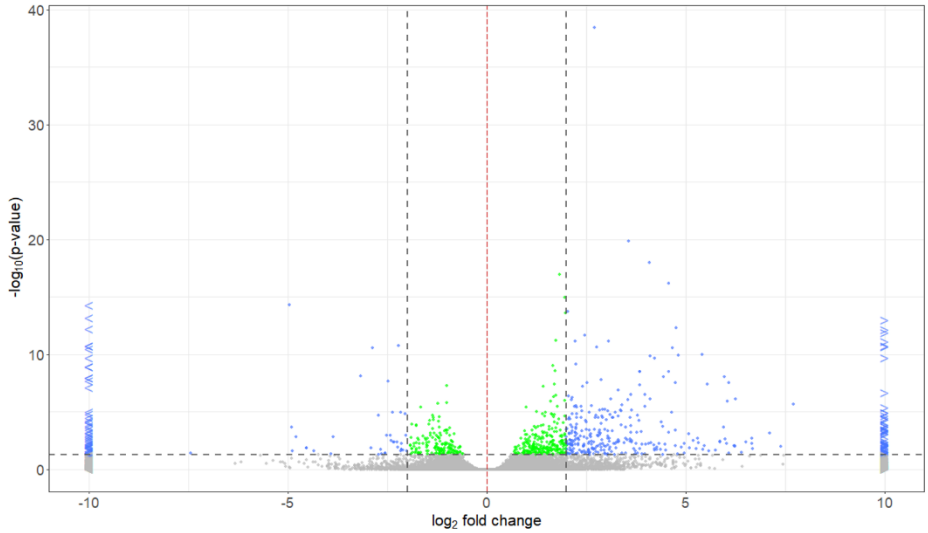
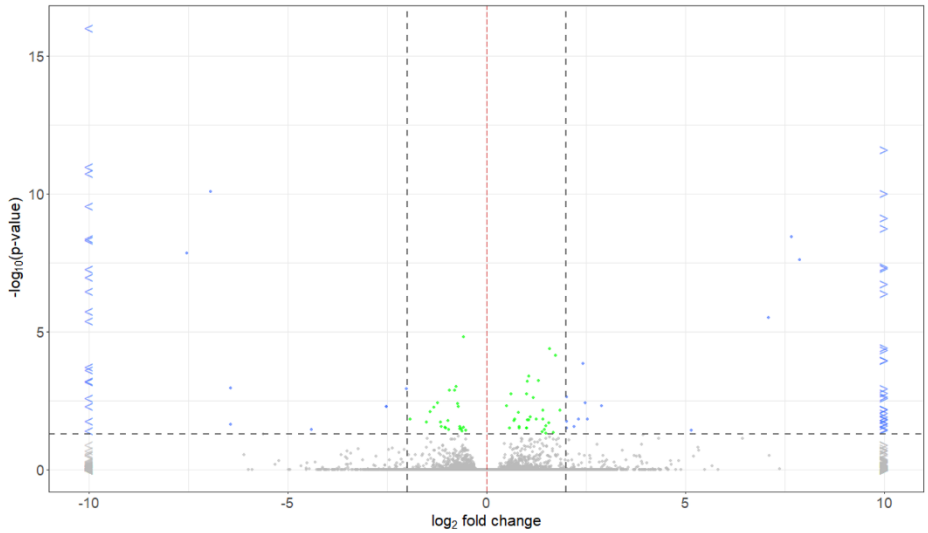


Figure 4.3. MA plots from base means (*x*-axis; 'M') and the average of log fold changes (*y*-axis; 'A'), indicating differentially expressed genes in resistant (R) and susceptible (S) bulks

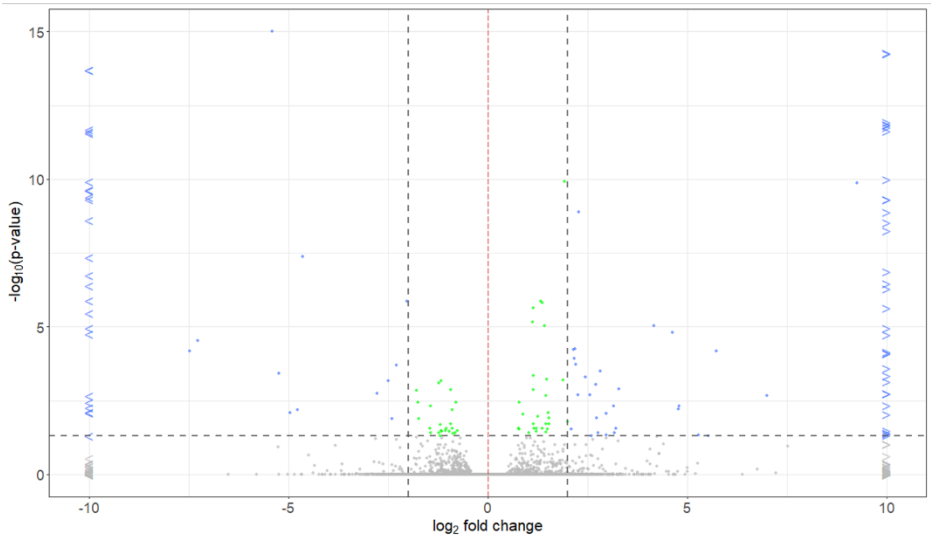
of F_2 plants derived from FGRA106 (♀) × FG769 (♂) tested with isolates representing *P. brassicae* pathotypes 3A (top), 3D (middle), and 3H (bottom). Red spots indicate genes with $P_{adj} < 0.05$.


40
30
20
10
0
-log10(p-value)
-10
-5
0
5
10
log2 fold change

(a)


15
10
5
0
-log10(p-value)
-10
-5
0
5
10
log2 fold change

(b)


15
10
5
0
-log10(p-value)
-10
-5
0
5
10
log2 fold change

(c)

Figure 4.4. Volcano plots from the log2 fold change (*x*-axis) and -log10 P_{adj} (*y*-axis) values for selection of 428, 67, and 98 differentially expressed genes (DEGs) in resistant (R) or susceptible (S) bulks of F_2 plants derived from FGRA106 (♀) × FG769 (♂) tested with *Plasmodiophora brassicae* pathotypes 3A (a), 3D (b), and 3H (c), respectively. The log2 fold change indicates the mean expression level for each gene; each dot represents one gene. Grey dots: genes of $P_{adj} > 0.05$; green dots: genes of $P_{adj} < 0.05$ and |log2 fold change| < 2; blue dots and arrows: genes of $P_{adj} < 0.05$ and |log2 fold change| > 2. The criteria for selection of DEGs in this study was $P_{adj} < 0.05$ and |log2 fold change| > 2.

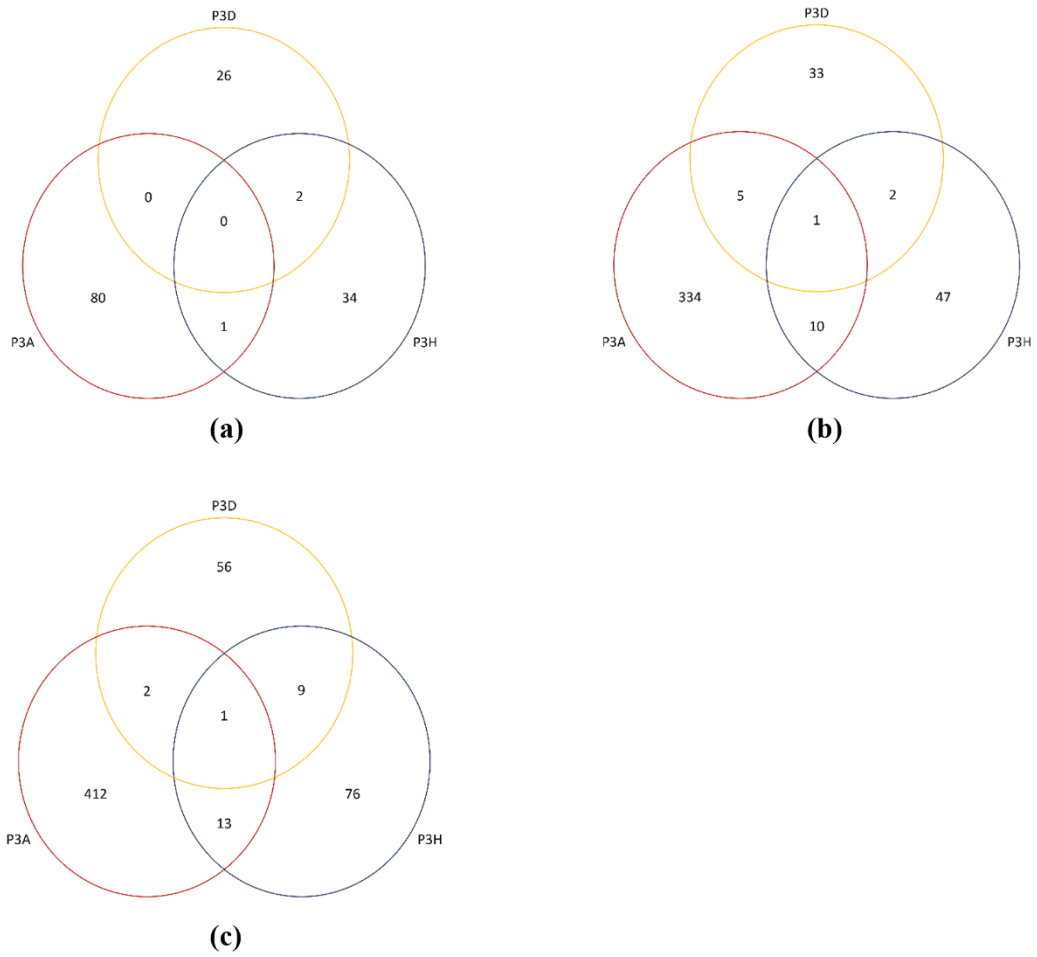


Figure 4.5. Venn diagrams indicating the overlaps of differentially expressed genes among resistant (a), susceptible (b) and all (c) bulks of F_2 plants derived from FGRA106 (♀) × FG769 (♂) tested with *Plasmodiophora brassicae* pathotypes. P3A, P3D and P3H denote pathotypes 3A, 3D and 3H, respectively.

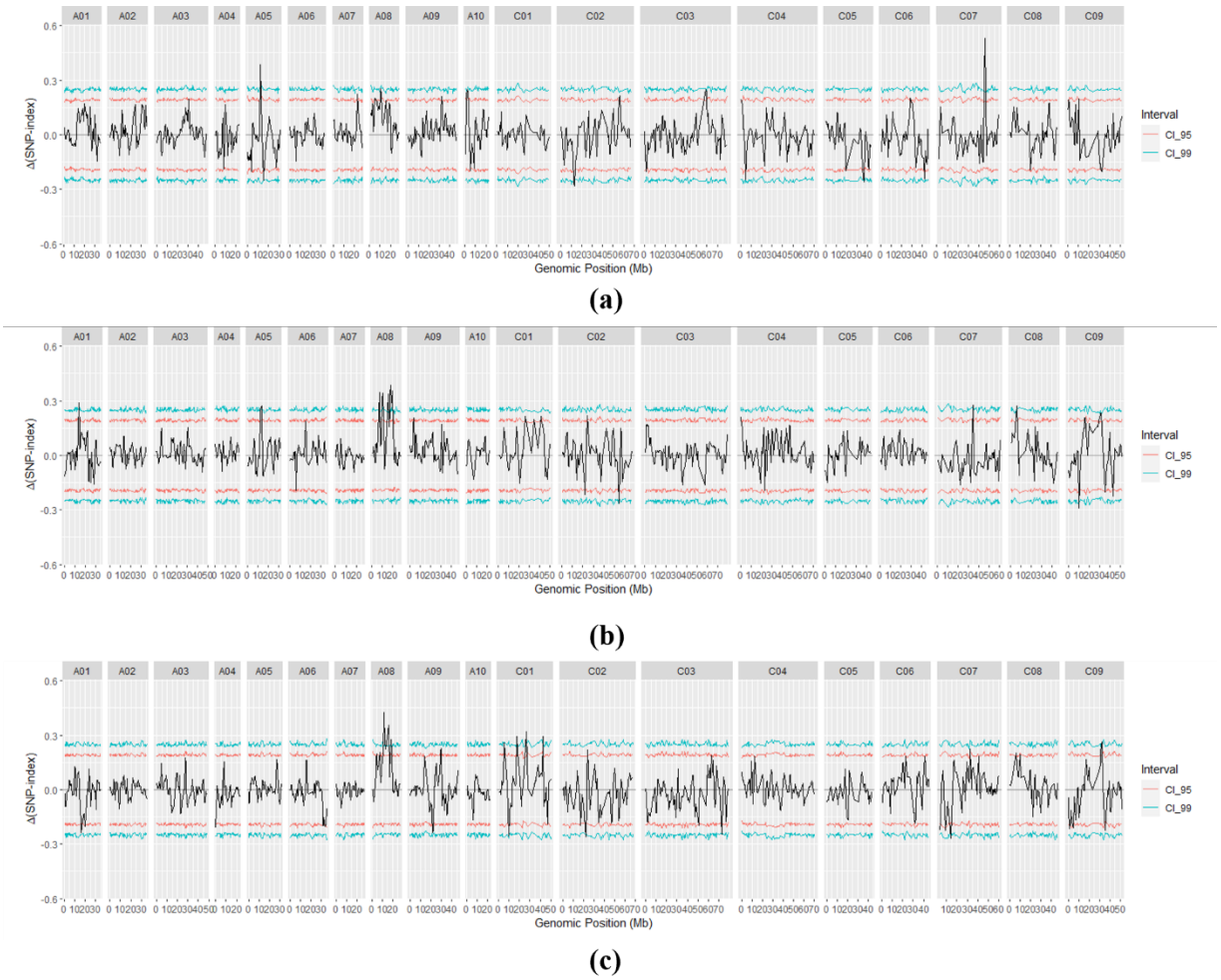
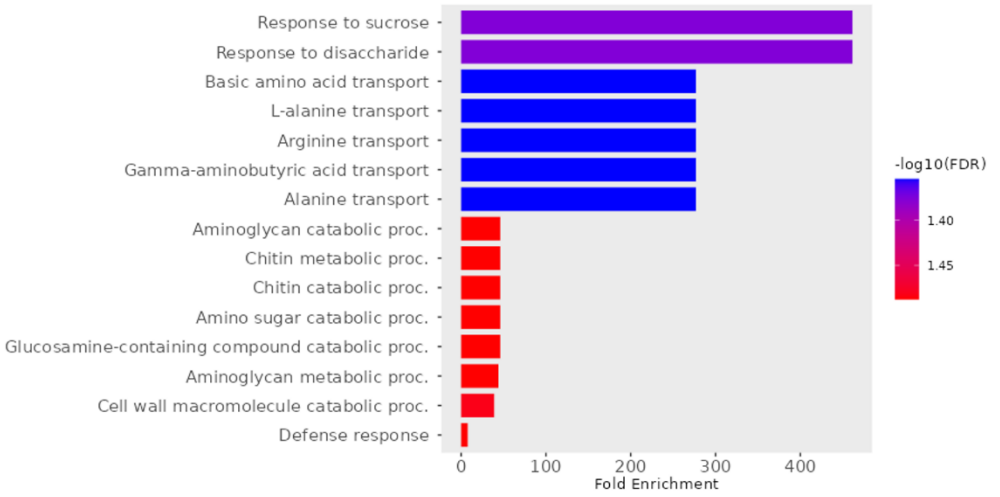


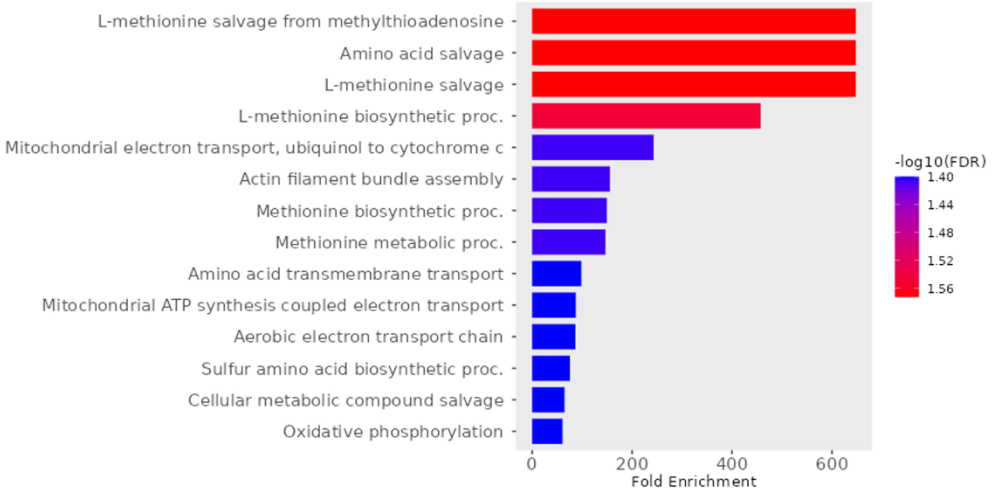
Figure 4.6. Variant calling of polymorphic single nucleotide polymorphisms (SNPs) differing between clubroot-resistant (R) and susceptible (S) bulks of F_2 plants derived from FGRA106 (♀) × FG769 (♂) tested with *Plasmodiophora brassicae* pathotypes 3A (a), 3D (b) and 3H (c) on 19 *B. napus* chromosomes based on Δ(SNP-index) statistics (Takagi et al., 2013) at a 99% confidence interval. The *x*-axis indicates the position on the chromosomes, and the *y*-axis denotes the Δ(SNP-index), where CI_95 = 95% confidence interval and CI_99 = 99% confidence interval.

Figure 4.7. The single nucleotide polymorphisms (SNPs) and QTLs identified from clubroot-resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H on chromosomes A01, A05, A08, C01, C07, C08 and C09. The positions and names of SNPs are denoted on each chromosome (left and right, respectively),

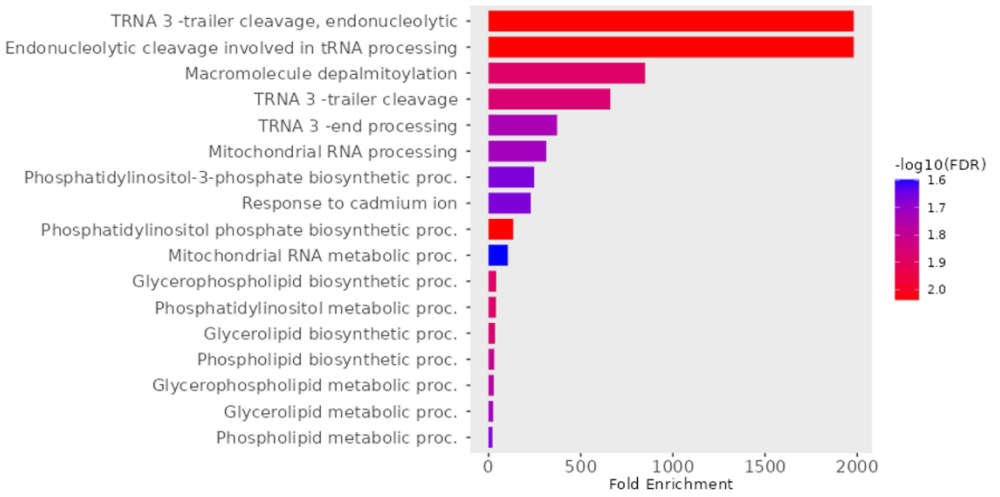
while the flanking regions of identified QTLs are represented as red bars, both on and to the right of each chromosome.


Response to sucrose
Response to disaccharide
Basic amino acid transport
L-alanine transport
Arginine transport
Gamma-aminobutyric acid transport
Alanine transport
Aminoglycan catabolic proc.
Chitin metabolic proc.
Chitin catabolic proc.
Amino sugar catabolic proc.
Glucosamine-containing compound catabolic proc.
Aminoglycan metabolic proc.
Cell wall macromolecule catabolic proc.
Defense response
0
100
200
300
400
Fold Enrichment
-log10(FDR)
1.40
1.45

(a)

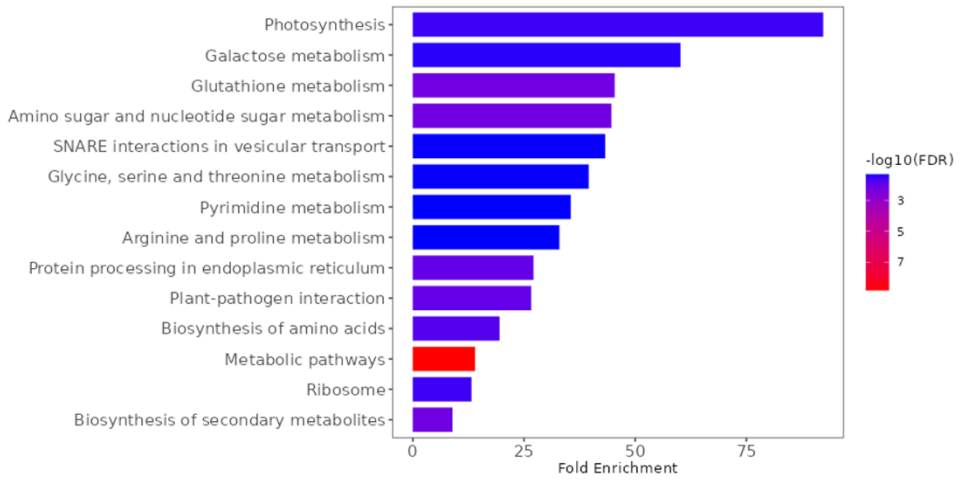

L-methionine salvage from methylthioadenosine
Amino acid salvage
L-methionine salvage
L-methionine biosynthetic proc.
Mitochondrial electron transport, ubiquinol to cytochrome c
Actin filament bundle assembly
Methionine biosynthetic proc.
Methionine metabolic proc.
Amino acid transmembrane transport
Mitochondrial ATP synthesis coupled electron transport
Aerobic electron transport chain
Sulfur amino acid biosynthetic proc.
Cellular metabolic compound salvage
Oxidative phosphorylation
0
200
400
600
Fold Enrichment
-log10(FDR)
1.40
1.44
1.48
1.52
1.56

(b)

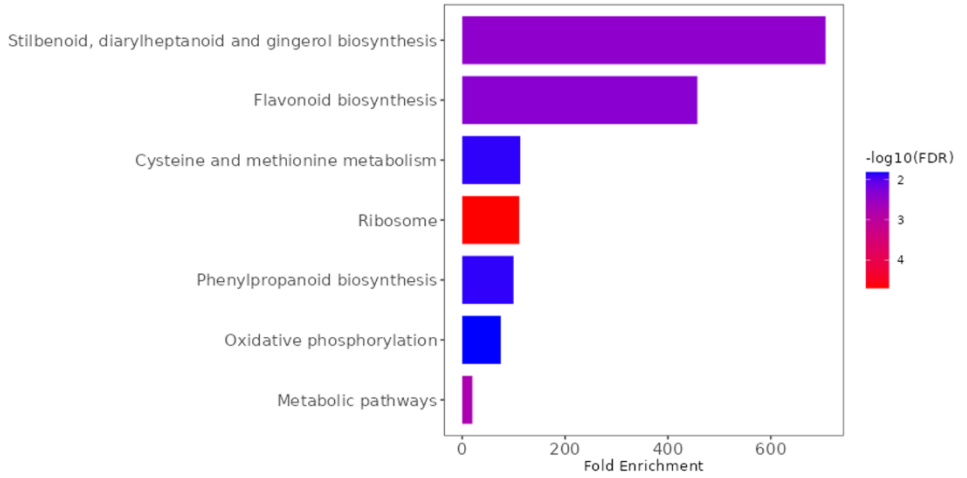

TRNA 3 -trailer cleavage, endonucleolytic
Endonucleolytic cleavage involved in tRNA processing
Macromolecule depalmitoylation
TRNA 3 -trailer cleavage
TRNA 3 -end processing
Mitochondrial RNA processing
Phosphatidylinositol-3-phosphate biosynthetic proc.
Response to cadmium ion
Phosphatidylinositol phosphate biosynthetic proc.
Mitochondrial RNA metabolic proc.
Glycerophospholipid biosynthetic proc.
Phosphatidylinositol metabolic proc.
Glycerolipid biosynthetic proc.
Phospholipid biosynthetic proc.
Glycerophospholipid metabolic proc.
Glycerolipid metabolic proc.
Phospholipid metabolic proc.
0
500
1000
1500
2000
Fold Enrichment
-log10(FDR)
1.6
1.7
1.8
1.9
2.0

(c)

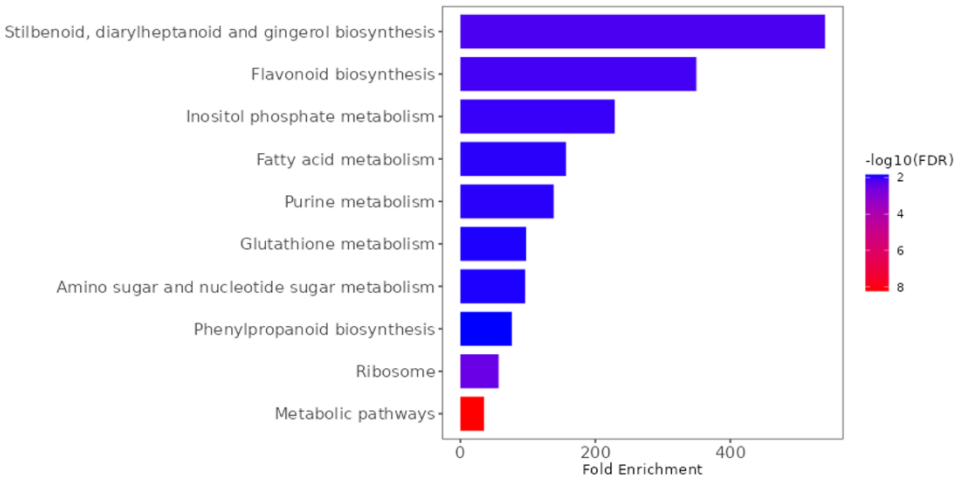
Figure 4.8. GO enrichment with differentially expressed genes between clubroot-resistant and susceptible bulks of F_2 plants derived from FGRA106 (♀) × FG769 (♂) tested with *Plasmodiophora brassicae* pathotypes 3A (a), 3D (b) and 3H (c). Visualization was based on the GO biological processes sorted by fold enrichment. The colors indicate -log10 false discovery rate (FDR) as per the scale on the right-hand side.


Photosynthesis
Galactose metabolism
Glutathione metabolism
Amino sugar and nucleotide sugar metabolism
SNARE interactions in vesicular transport
Glycine, serine and threonine metabolism
Pyrimidine metabolism
Arginine and proline metabolism
Protein processing in endoplasmic reticulum
Plant-pathogen interaction
Biosynthesis of amino acids
Metabolic pathways
Ribosome
Biosynthesis of secondary metabolites
0
25
50
75
Fold Enrichment
-log10(FDR)
3
5
7

(a)


Stilbenoid, diarylheptanoid and gingerol biosynthesis
Flavonoid biosynthesis
Cysteine and methionine metabolism
Ribosome
Phenylpropanoid biosynthesis
Oxidative phosphorylation
Metabolic pathways
0
200
400
600
Fold Enrichment
-log10(FDR)
2
3
4

(b)


Stilbenoid, diarylheptanoid and gingerol biosynthesis
Flavonoid biosynthesis
Inositol phosphate metabolism
Fatty acid metabolism
Purine metabolism
Glutathione metabolism
Amino sugar and nucleotide sugar metabolism
Phenylpropanoid biosynthesis
Ribosome
Metabolic pathways
0
200
400
Fold Enrichment
-log10(FDR)
2
4
6
8

(c)

Figure 4.9. KEGG pathways associated with differentially expressed genes between clubroot-resistant and susceptible bulks of F_2 plants derived from FGRA106 (♀) × FG769 (♂) tested with *Plasmodiophora brassicae* pathotypes 3A (a), 3D (b) and 3H (c). Visualization of pathways was sorted by fold enrichment. The colors indicate -log10 false discovery rate (FDR) as per the scale on the right-hand side.

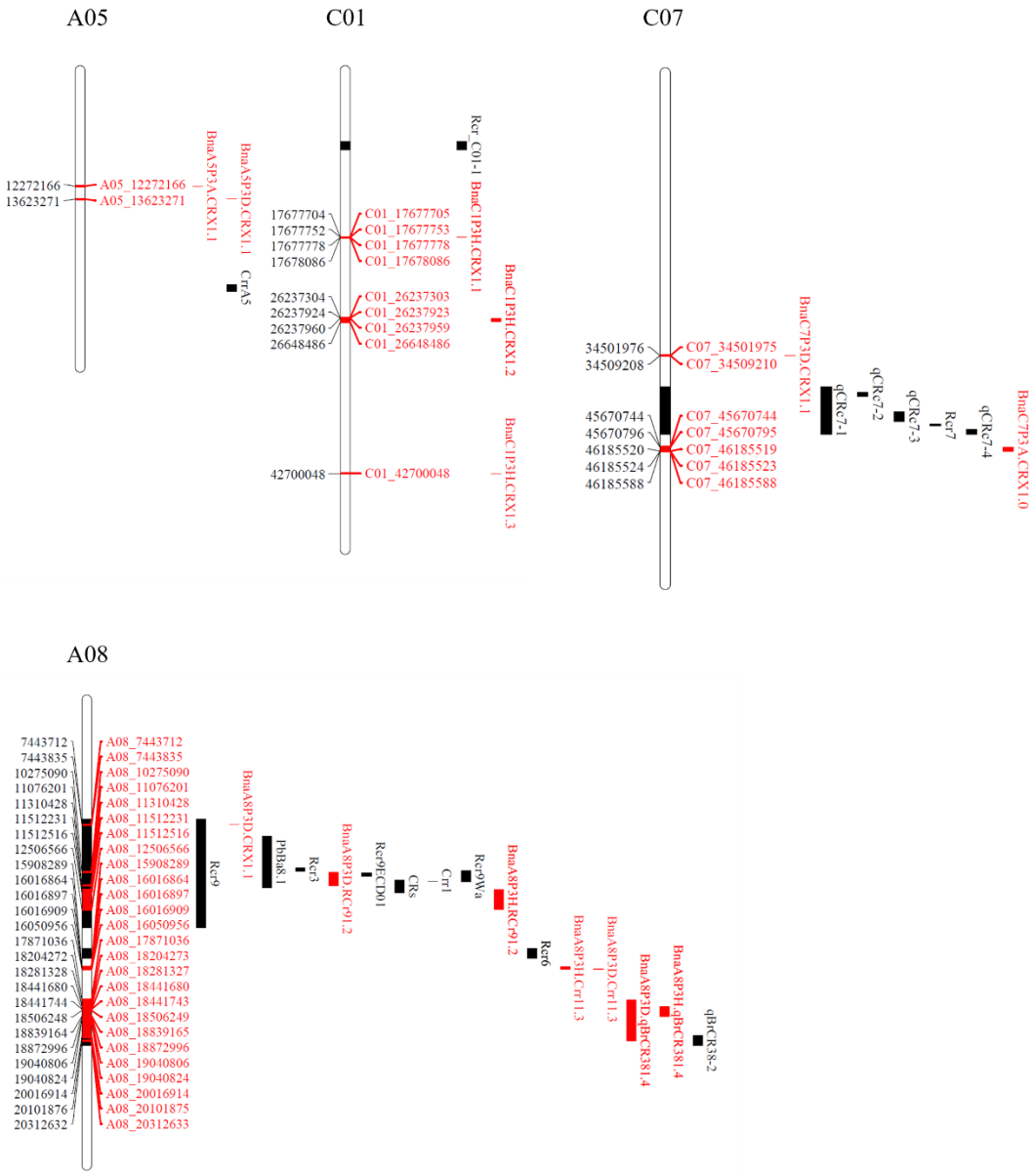


Figure 4.10. Comparison between previously reported clubroot-resistant (CR) gene loci and QTLs identified in this study on chromosomes A05, A08, C01 and C07. The position and name of SNPs identified in this study are indicated on each chromosome (left and right, respectively), and the flanking regions of previously reported CR gene loci and QTLs identified in this study are denoted by black and red bars, respectively, both on and to the right of each chromosome.

4.8 Supplementary Tables

Table S4.1. Chi-square tests of homogeneity on the phenotypic data of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H in three replicates.

Table S4.2. Alignment statistics by STAR v2.7.9a for resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H aligned to the *B. napus* cv. "ZS11" reference genome v2.0.

Table S4.3. Statistics of differentially expressed genes identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Table S4.4. GO terms significantly (FDR < 0.05) enriched with differentially expressed genes identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Table S4.5. KEGG pathways significantly (FDR < 0.05) enriched with differentially expressed genes identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Table S4.6. QTLs and single nucleotide polymorphisms (SNPs) identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H. QTLs are distinguished using different colours, whereas multiple nomenclatures marked with the same colour represent the same QTL identified from bulks of plants inoculated with different pathotypes.

Chapter 5 – Conclusions and future directions

5.1 General conclusions

Clubroot, caused by *Plasmodiophora brassicae* Wor., poses a significant global challenge to the production *Brassica* crops, particularly in Canada, where it represents a substantial threat to the highly profitable canola (*Brassica napus* L.) industry. Currently, clubroot management in western Canadian cropping systems relies heavily on the deployment of genetically resistant canola cultivars. This thesis investigated several different strategies for clubroot control, including fungicide applications, soil amendments with lime, and the identification of novel genetic resistance for breeding purposes. By exploring and evaluating these strategies, this research aimed to contribute to the development of effective and comprehensive clubroot management approaches tailored to the needs of the Canadian canola industry.

Fungicides and soil amendments have been shown to provide variable levels of clubroot control (Hwang et al. 2014a). In Chapter 2 of the thesis, the QiI fungicide amisulbrom was assessed for its effectiveness in reducing clubroot severity. The results indicated that amisulbrom inhibited resting spore germination by up to 79% and reduced viable spores by 31%, relative to the control treatments. Both granular and liquid formulations of amisulbrom reduced disease severity and increased yields in the susceptible canola cv. ‘45H31’ and the moderately resistant cv. ‘CS2000’, under both field and greenhouse conditions. These findings were consistent with previous studies on *B. rapa* (Kawasaki et al. 2014; Murakami et al. 2005), suggesting that amisulbrom holds promise as part of an integrated clubroot management approach in western Canada. Additionally, the availability of amisulbrom in granular fertilizer form, compatible with seeding equipment commonly used in this region, offers farmers a convenient option for its application.

In Chapter 3, the efficacy of fall and spring limestone and spring hydrated lime treatments was evaluated and compared. Hydrated lime achieved greater disease reductions and yield increases compared to limestone when both were applied in spring. However, fall limestone application showed similar or even greater efficacy in reducing clubroot and increasing yields. These results confirmed the hypothesis of Hwang et al. (2014) that allowing for an extended period for limestone to increase soil pH in the field can lead to improved clubroot control. Importantly, this study was the first to investigate the effect of different lime application timings for managing clubroot of canola in Canada. The findings suggest that fall limestone application can be a more cost-effective option for clubroot management than the more costly hydrated lime.

The planting of clubroot-resistant cultivars is the preferred method for clubroot management in canola, as it is environmentally friendly and does not require additional practices or investments by growers (Hasan et al. 2021; Hwang et al. 2014a). However, single-gene resistance to *P. brassicae* in Canada has been rapidly eroded in the past (Hollman et al. 2023). In Chapter 4, an F_2 population derived from the rutabaga (*B. napus* var. *napobrassica*) accession FGRA106 'Wilhelmsburger' was found to harbor resistance to three prevalent *P. brassicae* pathotypes in western Canada: 3A, 3D, and 3H. Analysis of gene expression profiles indicated 431, 67, and 98 differentially expressed genes (DEGs) between resistant (R) and susceptible (S) bulks for pathotypes 3A, 3D and 3H, respectively. In addition, variant calling identified a total of 12 QTLs (7 major and 5 minor) across seven chromosomes. The results suggested that clubroot resistance in FGRA106 may be controlled by major and minor genes on both the A- and C-genomes. While the DEGs offer insights into clubroot resistance mechanisms, the single nucleotide polymorphisms (SNPs) identified in this study can serve as valuable resources for

marker-assisted breeding. This will facilitate the development of additional clubroot-resistant canola cultivars for use in western Canada.

5.2 Future directions

As canola growers increasingly face challenges from clubroot and the emergence of resistant-breaking pathotypes of *P. brassicae*, there has been a growing interest in utilizing fungicides and liming as disease management tools. The fungicide amisulbrom and ZG limestone investigated in this thesis could offer novel options for clubroot control. However, their effectiveness may vary depending on factors such as soil type, precipitation level, and application rates (Hwang et al. 2014b; Peng et al. 2014). Future studies will be necessary to evaluate the efficacy of these products under varying soil conditions and environments across the Canadian prairies, as well as to determine the optimal application rates. Furthermore, the rutabaga accession FGRA106 demonstrated promising clubroot resistance, with an F_2 population derived from this genotype confirmed to possess resistance against pathotypes 3A, 3D, and 3H of *P. brassicae*. Additional breeding efforts to develop clubroot-resistant canola varieties incorporating this resistance are anticipated. It will also be important to assess the resistance of other host pedigrees to emerging pathotypes in Canada for effective clubroot management. Continued exploration and development of innovative solutions will allow growers to manage clubroot effectively, ensuring the long-term sustainability of the canola industry.

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Appendix A: Supplementary materials for Chapter 4

Table S4.1. Chi-square tests of homogeneity on the phenotypic data of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H in three replicates.

Phenotype rounds	No. of F2 plants with disease rating					Observed values[1]		Expected values		*(O-E)2/E				Observed values[2]		Expected values		*(O-E)2/E			
	0	1	2	3	Total	R (0+1)	S (2+3)	R-Expected	S-Expected	R	S	Chi-squre	p-value	R (0)	S (1+2+3)	R-Expected	S-Expected	R	S	Chi-squre	p-value
3A-1	15	7	38	87	147	22	125	28.824	118.176	1.615	0.394			15	132	18.015	128.985	0.505	0.070		
3A-2	16	13	39	69	137	29	108	26.863	110.137	0.170	0.041			16	121	16.789	120.211	0.037	0.005		
3A-3	19	10	54	41	124	29	95	24.314	99.686	0.903	0.220			19	105	15.196	108.804	0.952	0.133		
3A total	50	30	131	197	408	80	328					3.344	0.188	50	358					1.702	0.427
3D-1	46	12	26	71	155	58	97	63.358	91.642	0.453	0.313			46	109	45.633	109.367	0.003	0.001		
3D-2	40	18	23	39	120	58	62	49.051	70.949	1.633	1.129			40	80	35.328	84.672	0.618	0.258		
3D-3	35	17	24	60	136	52	84	55.591	80.409	0.232	0.160			35	101	40.039	95.961	0.634	0.265		
3D total	121	47	73	170	411	168	243					3.920	0.141	121	290					1.778	0.411
3H-1	36	12	22	95	165	48	117	54.123	110.877	0.693	0.338			36	129	37.961	127.039	0.101	0.030		
3H-2	25	19	16	62	122	44	78	40.018	81.982	0.396	0.193			25	97	28.068	93.932	0.335	0.100		
3H-3	40	12	19	81	152	52	100	49.859	102.141	0.092	0.045			40	112	34.970	117.030	0.723	0.216		
3H total	101	43	57	238	439	144	295					1.757	0.415	101	338					1.507	0.471

Note: [1] Plants with clubroot disease severity ratings of 0 and 1 were regarded as resistant (R), and those with ratings of 2 and 3 as susceptible (S).

[2] Plants with a clubroot disease severing rating of 0 were regarded as R, and those with ratings of 1, 2 and 3 as S.

* O = observed values; E = expected values

Table S4.2. Alignment statistics by STAR v2.7.9a for resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H aligned to the *B. napus* cv. "ZS11" reference genome v2.0.

Sample#	3AR1	3AR2	3AR3	3AS1	3AS2	3AS3	3DR1	3DR2	3DR3	3DS1	3DS2	3DS3	3HR1	3HR2	3HR3	3HS1	3HS2	3HS3
Number of input reads	27345137	28657071	24179570	21495575	42898722	27036146	26536093	24848921	23484229	20681757	23182934	22271799	22341970	25040277	22748073	23834592	21842769	23681421
Average input read length	302	302	302	302	302	302	302	302	302	302	302	302	302	302	302	302	302	302
UNIQUE READS:																		
Uniquely mapped reads number	19487750	20327537	17230462	15449790	30588098	18933874	18821155	17626307	16099261	14680133	16522818	15810352	16002816	17837604	16276308	16880671	15630125	16987236
Uniquely mapped reads %	71.27%	70.93%	71.26%	71.87%	71.30%	70.03%	70.93%	70.93%	68.55%	70.98%	71.27%	70.99%	71.63%	71.24%	71.55%	70.82%	71.56%	71.73%
Average mapped length	288.29	288.16	288.2	288.93	288.3	287.43	288.27	287.99	288.13	287.92	288.41	288.2	288.4	288.33	288.42	288.29	288.2	288.52
Number of splices: Total	18984335	18627887	15073443	13558869	28122645	17274310	17426405	16464800	15435118	13422011	15368857	14518918	15003666	16549022	14170000	15540593	14329907	15716149
Number of splices: Annotated (sjdb)	18203364	17868490	14478809	12998545	26938045	16587222	16687966	15777374	14786419	12852902	14716781	13885239	14404254	15891669	13576114	14935298	13751314	15086299
Number of splices: GT/AG	18328502	17953014	14498140	13053679	27101567	16649195	16799864	15884983	14903192	12946195	14822173	14006695	14470239	15961511	13642619	14982703	13821394	15155219
Number of splices: GC/AG	208535	202954	166992	157538	313285	196760	195470	183423	168192	148107	166836	158550	172294	185283	162409	175388	160531	175387
Number of splices: AT/AC	29089	31072	28473	22798	42637	26810	27273	24017	21976	20452	23942	23549	19934	24334	22915	23655	21921	25669
Number of splices: Non-canonical	418209	440847	379838	324854	665156	401545	403798	372377	341758	307257	355906	330124	341199	377894	342057	358847	326061	359874
Mismatch rate per base, %	0.87%	0.89%	0.91%	0.91%	0.91%	1.06%	0.91%	0.91%	0.90%	0.90%	0.87%	0.87%	0.90%	0.88%	0.89%	0.89%	0.90%	0.89%
Deletion rate per base	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%	0.04%
Deletion average length	2.73	2.73	2.76	2.72	2.71	2.75	2.73	2.7	2.75	2.77	2.78	2.64	2.74	2.76	2.73	2.73	2.74	2.73
Insertion rate per base	0.03%	0.03%	0.03%	0.02%	0.03%	0.02%	0.02%	0.02%	0.02%	0.03%	0.03%	0.02%	0.02%	0.03%	0.03%	0.02%	0.03%	0.03%
Insertion average length	3.21	3.16	3.18	3.21	3.16	3.2	3.2	3.19	3.19	3.18	3.19	3.18	3.15	3.12	3.24	3.14	3.19	3.17
MULTI-MAPPING READS:																		
Number of reads mapped to multiple loci	5855192	6055462	5099388	4529742	9147369	5726669	5660520	5342334	4960457	4400003	5000546	4829831	4799308	5383802	4823568	5063540	4640112	5053257
% of reads mapped to multiple loci	21.41%	21.13%	21.09%	21.07%	21.32%	21.18%	21.33%	21.50%	21.12%	21.27%	21.57%	21.69%	21.48%	21.50%	21.20%	21.24%	21.24%	21.34%

Number of reads mapped to too many loci	123167	160732	159174	151668	227154	168578	146124	130501	256971	101153	146365	155281	141159	192261	134468	163006	121132	110073
% of reads mapped to too many loci	0.45%	0.56%	0.66%	0.71%	0.53%	0.62%	0.55%	0.53%	1.09%	0.49%	0.63%	0.70%	0.63%	0.77%	0.59%	0.68%	0.55%	0.46%
UNMAPPED READS:																		
Number of reads unmapped: too many mismatches	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of reads unmapped: too many mismatches	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Number of reads unmapped: too short	1876499	2110075	1687869	1362389	2931672	2204732	1905737	1747518	2163966	1498770	1510898	1474055	1396608	1623868	1511594	1725048	1449380	1528954
% of reads unmapped: too short	6.86%	7.36%	6.98%	6.34%	6.83%	8.15%	7.18%	7.03%	9.21%	7.25%	6.52%	6.62%	6.25%	6.49%	6.64%	7.24%	6.64%	6.46%
Number of reads unmapped: other	2529	3265	2677	1986	4429	2293	2557	2261	3574	1698	2307	2280	2079	2742	2135	2327	2020	1901
% of reads unmapped: other	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.02%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%
CHIMERIC READS:																		
Number of chimeric reads	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
% of chimeric reads	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Note: R = resistant; S = susceptible; R1-3 and S1-3 represent replicates 1-3 of R and S bulks.

Table S4.3. Statistics of differentially expressed genes identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Pathotype	NCBI Gene ID	Gene	baseMean	log2FoldChange	lfcSE	pvalue	padj	R1_normalized	R2_normalized	R3_normalized	S1_normalized	S2_normalized	S3_normalized
3A	106401597	LOC106401597	18.44229	21.02466	3.909278	7.53E-08	3.01E-05	0	0	0	110.6537	0	0
3A	106430940	LOC106430940	14.84963	20.72402	3.909888	1.16E-07	4.37E-05	0	0	0	89.0978	0	0
3A	111207414	LOC111207414	166.0771	10.84387	1.315851	1.71E-16	6.33E-13	0	0	0	497.2232	120.5241	378.7154
3A	106347739	LOC106347739	141.9867	10.6236	1.36993	8.84E-15	1.89E-11	0	0	0	79.03837	530.8054	242.0766
3A	106347217	LOC106347217	133.0586	10.53068	1.239629	1.98E-17	9.17E-14	0	0	0	194.0033	347.2092	257.1392
3A	106371070	LOC106371070	113.833	10.30273	1.263187	3.46E-16	1.20E-12	0	0	0	260.1081	291.6307	131.2593
3A	106347958	LOC106347958	89.06542	9.948242	2.439617	4.55E-05	0.006032	0	0	0	4.311184	335.3441	194.7372
3A	111201423	LOC111201423	82.99283	9.841971	1.244405	2.60E-15	7.21E-12	0	0	0	216.9962	148.6255	132.3352
3A	111212170	LOC111212170	69.97907	9.60485	1.294034	1.15E-13	1.88E-10	0	0	0	117.839	219.1914	82.844
3A	111213645	LOC111213645	68.42283	9.567815	1.228331	6.74E-15	1.64E-11	0	0	0	132.2096	137.3849	140.9424
3A	106352980	LOC106352980	44.34034	8.943295	2.350397	0.000142	0.014234	0	0	0	7.185306	229.8075	29.04919
3A	106377126	LOC106377126	28.44362	8.301855	1.519684	4.68E-08	2.10E-05	0	0	0	12.93355	31.84833	125.8798
3A	106368242	LOC106368242	27.22622	8.229401	1.296505	2.19E-10	1.90E-07	0	0	0	71.85306	43.08891	48.41532
3A	111204222	LOC111204222	26.35208	8.198372	1.414743	6.83E-09	4.13E-06	0	0	0	15.80767	68.06799	74.23683
3A	106381593	LOC106381593	23.45172	8.035211	1.435083	2.15E-08	1.09E-05	0	0	0	18.6818	88.67573	33.35278
3A	106402676	LOC106402676	81.66551	7.893839	1.329835	2.92E-09	2.00E-06	0	0	2.320014	342.0206	26.22803	119.4245
3A	106439752	LOC106439752	18.07597	7.66107	1.438623	1.01E-07	3.92E-05	0	0	0	20.11886	66.81904	21.51792
3A	106352412	LOC106352412	16.29155	7.4877	1.495132	5.50E-07	0.000174	0	0	0	37.36359	8.742678	51.64301
3A	106358201	LOC106358201	15.87902	7.473285	1.697343	1.07E-05	0.001966	0	0	0	7.185306	80.55753	7.531273
3A	106387857	LOC106387857	15.86267	7.470955	1.464329	3.36E-07	0.000113	0	0	0	10.05943	47.46025	37.65636
3A	106451348	LOC106451348	15.80753	7.454263	1.331479	2.16E-08	1.09E-05	0	0	0	28.74122	30.59937	35.50457
3A	106412006	LOC106412006	15.36869	7.420675	1.548011	1.64E-06	0.000421	0	0	0	30.17829	55.57845	6.455376
3A	106381239	LOC106381239	14.39433	7.303712	1.463882	6.06E-07	0.000188	0	0	0	44.5489	10.61611	31.20099
3A	106421983	LOC106421983	12.83321	7.140722	1.805165	7.63E-05	0.008855	0	0	0	66.10482	8.742678	2.151792
3A	106404679	LOC106404679	24.64261	7.131743	1.81968	8.88E-05	0.009895	0.877903	0	0	1.437061	138.0094	7.531273
3A	106388950	LOC106388950	12.60373	7.115615	1.759499	5.25E-05	0.006696	0	0	0	60.35657	13.11402	2.151792
3A	106346827	LOC106346827	12.31028	7.076464	1.662978	2.09E-05	0.003344	0	0	0	57.48245	5.620293	10.75896

3A	106431930	LOC106431930	11.9491	7.053958	1.489184	2.17E-06	0.000539	0	0	0	28.74122	34.34623	8.607169
3A	106416488	LOC106416488	12.05319	7.051633	1.448496	1.13E-06	0.000315	0	0	0	38.80065	20.60774	12.91075
3A	106418471	LOC106418471	12.05319	7.051633	1.448496	1.13E-06	0.000315	0	0	0	38.80065	20.60774	12.91075
3A	106386755	LOC106386755	11.80488	7.045586	1.509497	3.05E-06	0.000711	0	0	0	18.6818	42.46443	9.683065
3A	106416234	LOC106416234	40.75062	7.017174	1.500004	2.90E-06	0.000688	1.755807	0	0	4.311184	171.7312	66.70556
3A	106394966	BNAC05G09720D	11.52894	7.005195	1.732833	5.29E-05	0.006723	0	0	0	1.437061	26.85251	40.88405
3A	106346424	LOC106346424	11.61676	7.004547	1.38582	4.32E-07	0.000141	0	0	0	22.99298	18.73431	27.9733
3A	106375305	LOC106375305	10.66501	6.899949	1.956686	0.000421	0.033079	0	0	0	1.437061	59.32531	3.227688
3A	106367791	LOC106367791	10.40412	6.853343	1.701931	5.65E-05	0.007047	0	0	0	2.874122	14.36297	45.18764
3A	111213367	LOC111213367	10.16089	6.824326	1.470254	3.46E-06	0.000778	0	0	0	10.05943	21.85669	29.04919
3A	106382943	LOC106382943	10.24494	6.818093	1.412292	1.38E-06	0.000367	0	0	0	25.8671	16.2364	19.36613
3A	106423379	LOC106423379	9.925176	6.782919	1.411085	1.53E-06	0.000402	0	0	0	21.55592	21.85669	16.13844
3A	106370295	LOC106370295	9.516333	6.716808	1.604774	2.85E-05	0.004184	0	0	0	11.49649	6.869247	38.73226
3A	106356363	LOC106356363	9.299489	6.670814	1.672057	6.62E-05	0.007927	0	0	0	41.67478	8.742678	5.37948
3A	106367971	LOC106367971	8.481849	6.574176	1.849226	0.000378	0.030351	0	0	0	2.874122	43.71339	4.303584
3A	106376116	LOC106376116	158.5758	6.560878	1.557805	2.54E-05	0.003926	0	0.926019	9.280056	662.4852	86.17782	192.5854
3A	106425272	BNAA01G26300D	8.584733	6.55647	1.835277	0.000354	0.028613	0	0	0	43.11184	6.24477	2.151792
3A	106419867	LOC106419867	15.7951	6.46524	1.573117	3.96E-05	0.005502	0	0.926019	0	45.98596	3.746862	44.11174
3A	106385345	LOC106385345	108.7377	6.423379	1.812942	0.000395	0.031451	0	1.852038	5.800035	4.311184	386.5513	253.9115
3A	106407722	LOC106407722	15.00083	6.419625	1.46153	1.12E-05	0.002004	0.877903	0	0	12.93355	56.82741	19.36613
3A	106429831	LOC106429831	7.773366	6.410955	1.600241	6.17E-05	0.007504	0	0	0	24.43004	4.995816	17.21434
3A	106425571	LOC106425571	15.15017	6.402803	1.691075	0.000153	0.015101	0.877903	0	0	77.60131	8.118201	4.303584
3A	106387512	LOC106387512	7.621626	6.385253	1.559944	4.25E-05	0.005739	0	0	0	20.11886	6.24477	19.36613
3A	111199834	LOC111199834	7.023477	6.299126	1.536935	4.16E-05	0.005707	0	0	0	8.622367	20.60774	12.91075
3A	106353789	LOC106353789	38.02031	6.25408	1.025175	1.06E-09	8.05E-07	0.877903	0.926019	1.160007	109.2167	33.09728	82.844
3A	106430144	LOC106430144	6.672394	6.205376	1.631681	0.000143	0.014314	0	0	0	20.11886	15.61192	4.303584
3A	106446123	LOC106446123	6.340072	6.144843	1.53676	6.37E-05	0.007709	0	0	0	11.49649	16.86088	9.683065
3A	106347176	LOC106347176	6.239743	6.11342	1.533537	6.71E-05	0.007982	0	0	0	10.05943	11.24059	16.13844
3A	106405532	LOC106405532	119.5855	6.111719	1.775844	0.000578	0.041266	0	0	10.44006	248.6116	206.7019	251.7597
3A	111215203	LOC111215203	6.192107	6.106755	1.544224	7.67E-05	0.008879	0	0	0	12.93355	15.61192	8.607169
3A	106353266	LOC106353266	6.084278	6.083222	1.755081	0.000528	0.038762	0	0	0	14.37061	19.98326	2.151792

3A	106419168	LOC106419168	172.4664	6.069913	0.912277	2.86E-11	2.94E-08	0.877903	12.96426	1.160007	80.47543	380.931	558.3901
3A	106452107	LOC106452107	134.4723	6.067255	1.005628	1.61E-09	1.16E-06	1.755807	5.556113	4.640028	14.37061	547.0418	233.4694
3A	106429751	LOC106429751	11.76363	6.046612	1.520198	6.96E-05	0.008271	0	0.926019	0	33.05241	31.22385	5.37948
3A	106357926	LOC106357926	22.071	6.035069	1.382662	1.27E-05	0.002238	0.877903	0	1.160007	84.78661	6.869247	38.73226
3A	106435387	LOC106435387	5.834742	6.015185	1.754164	0.000606	0.042504	0	0	0	17.24473	15.61192	2.151792
3A	106446503	LOC106446503	11.39507	5.982383	1.413678	2.32E-05	0.003642	0	0	1.160007	34.48947	18.73431	13.98665
3A	106431919	LOC106431919	11.19383	5.96976	1.525543	9.11E-05	0.010084	0.877903	0	0	35.92653	24.97908	5.37948
3A	106428166	LOC106428166	11.01826	5.959198	1.50365	7.40E-05	0.008674	0.877903	0	0	24.43004	34.34623	6.455376
3A	106385355	LOC106385355	5.521237	5.938589	1.597346	0.000201	0.018686	0	0	0	12.93355	13.73849	6.455376
3A	106366282	LOC106366282	50.0334	5.935208	0.867604	7.87E-12	9.31E-09	3.511613	0	1.160007	152.3285	86.17782	57.02249
3A	106421318	LOC106421318	5.512453	5.919242	1.590335	0.000198	0.018464	0	0	0	14.37061	6.869247	11.83486
3A	106452820	LOC106452820	5.383298	5.912023	1.646438	0.00033	0.027188	0	0	0	10.05943	16.86088	5.37948
3A	106452819	LOC106452819	5.383298	5.912023	1.646438	0.00033	0.027188	0	0	0	10.05943	16.86088	5.37948
3A	106437575	LOC106437575	28.71686	5.896689	1.189858	7.20E-07	0.000214	0.877903	1.852038	0	10.05943	87.42678	72.08504
3A	106379154	LOC106379154	5.425949	5.888781	1.716931	0.000604	0.042496	0	0	0	21.55592	5.620293	5.37948
3A	106382401	LOC106382401	38.51176	5.869978	1.395387	2.59E-05	0.003968	0.877903	1.852038	1.160007	0	109.9079	117.2727
3A	106356151	LOC106356151	5.227791	5.848468	1.622567	0.000313	0.026069	0	0	0	10.05943	6.24477	15.06255
3A	106414273	LOC106414273	9.663934	5.771914	1.41111	4.31E-05	0.005797	0.877903	0	0	12.93355	23.73013	20.44203
3A	106391875	LOC106391875	4.943153	5.766101	1.607852	0.000336	0.027507	0	0	0	12.93355	8.118201	8.607169
3A	111215171	LOC111215171	4.943153	5.766101	1.607852	0.000336	0.027507	0	0	0	12.93355	8.118201	8.607169
3A	106454693	LOC106454693	17.07317	5.635768	1.559683	0.000302	0.025445	0	0.926019	1.160007	68.97894	1.248954	30.12509
3A	106353158	LOC106353158	8.463167	5.543272	1.511659	0.000245	0.021482	0	0.926019	0	30.17829	9.991632	9.683065
3A	111202235	LOC111202235	461.8937	5.46779	1.382191	7.63E-05	0.008855	35.11613	25.92853	0	1421.254	544.5439	744.5201
3A	106394749	LOC106394749	35.80484	5.449482	0.825957	4.17E-11	4.07E-08	2.63371	1.852038	0	81.91249	45.58682	82.844
3A	106444565	BNAC09G50070D	173.2144	5.427227	0.72126	5.29E-14	9.48E-11	2.63371	12.96426	8.120049	91.97192	387.8002	535.7962
3A	106359782	LOC106359782	22.19956	5.423034	1.306322	3.30E-05	0.004771	0	0.926019	2.320014	8.622367	103.0387	18.29023
3A	106443275	LOC106443275	13.70356	5.374977	1.444342	0.000198	0.018478	0	1.852038	0	34.48947	4.995816	40.88405
3A	106374540	LOC106374540	48.71016	5.340395	1.21396	1.09E-05	0.001981	0	3.704075	3.480021	4.311184	205.4529	75.31273
3A	106369123	LOC106369123	11.85727	5.190315	1.384641	0.000178	0.016962	0.877903	0.926019	0	25.8671	38.0931	5.37948
3A	106426361	LOC106426361	12.24154	5.18559	1.362872	0.000142	0.014234	0	0.926019	1.160007	5.748245	38.71757	26.8974
3A	106422817	LOC106422817	33.79355	5.149313	1.203215	1.87E-05	0.003076	2.63371	2.778057	0	7.185306	161.1151	29.04919

3A	106402187	LOC106402187	44.17843	5.134678	1.109829	3.72E-06	0.000827	0.877903	0.926019	5.800035	211.248	26.85251	19.36613
3A	106370642	LOC106370642	6.223676	5.100829	1.477881	0.000558	0.040148	0	0	1.160007	12.93355	12.48954	10.75896
3A	106423637	LOC106423637	11.03978	5.036995	1.223006	3.81E-05	0.005354	0.877903	0	1.160007	21.55592	24.3546	18.29023
3A	111198409	LOC111198409	17.92494	5.031898	1.302535	0.000112	0.011874	0	0	3.480021	58.91951	7.493724	37.65636
3A	106426402	BNAC04G41400D	132.2074	4.831807	0.644094	6.30E-14	1.09E-10	1.755807	13.89028	11.60007	126.4614	356.5764	282.9607
3A	106381160	LOC106381160	96.60213	4.793396	0.57811	1.12E-16	4.78E-13	3.511613	5.556113	11.60007	265.8563	108.0345	185.0541
3A	106437574	LOC106437574	66.06154	4.726864	0.710003	2.78E-11	2.92E-08	0.877903	11.11223	2.320014	89.0978	169.2333	123.728
3A	106424671	LOC106424671	16.88057	4.721655	1.378222	0.000613	0.042793	3.511613	0	0	35.92653	3.746862	58.09839
3A	106436185	LOC106436185	69.76917	4.704757	0.839255	2.07E-08	1.08E-05	0.877903	4.630094	10.44006	53.17127	266.6517	82.844
3A	106350277	LOC106350277	29.64542	4.70053	1.190552	7.87E-05	0.009039	5.26742	0	1.160007	77.60131	5.620293	88.22348
3A	106436186	LOC106436186	25.43561	4.69889	0.972329	1.35E-06	0.000362	0.877903	4.630094	0	30.17829	86.8023	30.12509
3A	106416308	LOC106416308	555.2809	4.685372	0.609382	1.49E-14	2.85E-11	28.97081	12.96426	83.52051	1345.089	604.4937	1256.647
3A	106415613	LOC106415613	442.6211	4.586112	0.655432	2.61E-12	3.30E-09	27.215	30.55862	48.72029	794.6949	1570.56	183.9782
3A	106370941	LOC106370941	12.68266	4.583756	1.239771	0.000218	0.019698	0.877903	0	2.320014	22.99298	6.869247	43.03584
3A	106351535	LOC106351535	91.81543	4.565322	0.486318	6.14E-21	6.83E-17	5.26742	11.11223	5.800035	153.7656	143.6297	231.3177
3A	106402227	LOC106402227	13.062	4.551341	1.346986	0.000728	0.048043	0	0	3.480021	31.61535	5.620293	37.65636
3A	106429220	LOC106429220	21.70258	4.476877	0.947205	2.29E-06	0.000562	5.26742	0	0	44.5489	40.591	39.80815
3A	106360791	LOC106360791	37.17653	4.449902	0.650444	7.85E-12	9.31E-09	4.389517	1.852038	3.480021	68.97894	51.83159	92.52706
3A	106440574	LOC106440574	44.30897	4.434891	1.092717	4.94E-05	0.006367	11.41274	0	0	140.832	51.20711	62.40197
3A	106367679	LOC106367679	69.00955	4.433887	0.888547	6.04E-07	0.000188	0.877903	8.33417	9.280056	218.4333	24.3546	152.7772
3A	106374996	LOC106374996	23.78533	4.4175	1.073303	3.86E-05	0.005389	0	0.926019	5.800035	86.22367	17.48536	32.27688
3A	106387856	LOC106387856	53.15655	4.395103	0.847358	2.14E-07	7.48E-05	1.755807	9.260189	3.480021	28.74122	196.0858	79.61631
3A	111203374	LOC111203374	31.50557	4.359036	1.17328	0.000203	0.018775	5.26742	0	3.480021	104.9055	4.371339	71.00914
3A	106426779	LOC106426779	32.28696	4.325779	1.166129	0.000208	0.019079	0.877903	2.778057	5.800035	7.185306	159.8661	17.21434
3A	106438680	LOC106438680	10.25035	4.311164	1.111695	0.000105	0.011345	0	1.852038	1.160007	15.80767	16.86088	25.82151
3A	111206280	LOC111206280	163.9088	4.251461	0.574234	1.32E-13	2.04E-10	5.26742	16.66834	27.84017	331.9611	425.2688	176.447
3A	106345698	LOC106345698	52.70418	4.245531	1.124964	0.000161	0.015654	4.389517	4.630094	6.960042	7.185306	274.7699	18.29023
3A	106386997	LOC106386997	16.18794	4.225273	1.143717	0.00022	0.019862	2.63371	0	2.320014	24.43004	7.493724	60.25018
3A	106427885	LOC106427885	46.04568	4.221306	1.05203	6.01E-05	0.007355	1.755807	5.556113	6.960042	30.17829	221.0649	10.75896
3A	106423096	BNAA10G01350D	47.3931	4.144488	0.676575	9.03E-10	7.07E-07	3.511613	2.778057	9.280056	142.2691	51.20711	75.31273
3A	106375825	LOC106375825	68.02745	4.135192	1.001033	3.61E-05	0.00515	0.877903	17.59436	3.480021	35.92653	287.8839	62.40197

3A	106453102	LOC106453102	86.03526	4.130972	0.553588	8.51E-14	1.43E-10	7.023227	7.408151	13.92008	171.0103	232.9299	83.91989
3A	106345353	LOC106345353	328.4224	4.092177	0.414803	5.88E-23	1.09E-18	23.70339	40.74483	45.24027	678.2929	812.4445	370.1082
3A	111214866	LOC111214866	68.28081	4.088656	0.996579	4.08E-05	0.005638	8.779033	3.704075	10.44006	15.80767	332.2218	38.73226
3A	106387169	LOC106387169	9.133206	4.071319	1.193824	0.000649	0.044365	0	0.926019	2.320014	22.99298	8.118201	20.44203
3A	106454694	LOC106454694	47.78827	4.026455	0.993972	5.10E-05	0.006542	3.511613	1.852038	11.60007	10.05943	185.4697	74.23683
3A	106380061	LOC106380061	30.07144	3.985619	0.636557	3.82E-10	3.22E-07	2.63371	4.630094	3.480021	63.23069	65.57008	40.88405
3A	106385357	LOC106385357	174.4662	3.974342	0.68128	5.42E-09	3.43E-06	28.09291	15.74232	18.56011	514.4679	64.32113	405.6128
3A	106425779	LOC106425779	20.21729	3.967335	0.98075	5.23E-05	0.00668	0.877903	0.926019	5.800035	43.11184	12.48954	58.09839
3A	106359822	LOC106359822	14.23178	3.94436	1.05175	0.000177	0.016928	0.877903	0	4.640028	30.17829	13.11402	36.58047
3A	106454850	LOC106454850	106.3126	3.860535	0.587491	4.99E-11	4.78E-08	7.023227	14.8163	19.72012	205.4998	304.7448	86.07169
3A	106432730	LOC106432730	29.20427	3.860488	0.816311	2.25E-06	0.000557	3.511613	6.482132	1.160007	30.17829	100.5408	33.35278
3A	106399167	LOC106399167	121.7076	3.85816	0.550458	2.40E-12	3.10E-09	9.656937	20.37241	17.40011	132.2096	390.2981	160.3085
3A	106399166	LOC106399166	121.7076	3.85816	0.550458	2.40E-12	3.10E-09	9.656937	20.37241	17.40011	132.2096	390.2981	160.3085
3A	106351149	LOC106351149	21.11073	3.856364	0.818651	2.47E-06	0.000592	0	2.778057	5.800035	41.67478	31.22385	45.18764
3A	106423530	LOC106423530	644.3415	3.831632	0.728819	1.46E-07	5.40E-05	31.60452	22.22445	200.6812	1566.397	725.0178	1320.124
3A	106366946	LOC106366946	12.48982	3.824434	1.109025	0.000564	0.040385	0.877903	1.852038	2.320014	28.74122	36.84414	4.303584
3A	106404176	LOC106404176	11.86988	3.823989	1.120469	0.000643	0.044212	3.511613	0.926019	0	40.23771	16.86088	9.683065
3A	106406355	LOC106406355	329.472	3.796527	0.693946	4.48E-08	2.02E-05	79.8892	26.85455	25.52015	814.8137	136.7605	892.9937
3A	106353610	LOC106353610	52.17492	3.772983	0.914754	3.71E-05	0.005266	1.755807	8.33417	11.60007	18.6818	209.1998	63.47787
3A	106438995	LOC106438995	21.93396	3.761856	1.031161	0.000264	0.022975	0.877903	3.704075	4.640028	5.748245	38.0931	78.54041
3A	106401947	LOC106401947	59.58671	3.73376	1.093159	0.000636	0.04406	12.29065	0	12.76008	143.7061	11.24059	177.5229
3A	106451635	LOC106451635	15.04598	3.671552	0.974884	0.000166	0.016027	4.389517	0.926019	1.160007	21.55592	49.33368	12.91075
3A	106386585	LOC106386585	63.38305	3.665813	0.642283	1.15E-08	6.64E-06	2.63371	13.89028	11.60007	60.35657	164.8619	126.9557
3A	106435837	LOC106435837	41.86201	3.664989	0.874267	2.76E-05	0.004137	3.511613	4.630094	10.44006	56.04539	16.2364	160.3085
3A	106406764	LOC106406764	43.25941	3.659656	0.582656	3.36E-10	2.88E-07	5.26742	3.704075	10.44006	77.60131	54.95397	107.5896
3A	106348379	LOC106348379	57.06832	3.63819	0.851169	1.92E-05	0.003134	2.63371	20.37241	2.320014	64.66776	191.09	61.32608
3A	106431045	LOC106431045	42.72905	3.634313	0.771862	2.50E-06	0.000595	5.26742	4.630094	9.280056	158.0767	27.47699	51.64301
3A	106375268	LOC106375268	32.9798	3.625184	0.830917	1.28E-05	0.002245	0.877903	8.33417	5.800035	60.35657	99.91632	22.59382
3A	106372086	LOC106372086	155.3908	3.624058	0.719163	4.67E-07	0.000151	21.06968	17.59436	31.32019	605.0028	63.69665	193.6613
3A	111210393	LOC111210393	309.2411	3.616921	0.671934	7.33E-08	3.01E-05	33.36033	50.93104	55.68034	912.5339	705.0345	97.90654
3A	106353089	LOC106353089	94.2917	3.611025	0.853327	2.32E-05	0.003642	11.41274	8.33417	23.20014	405.2513	87.42678	30.12509

3A	106386098	LOC106386098	24.4173	3.581256	0.831726	1.66E-05	0.002802	3.511613	0	8.120049	61.79363	34.34623	38.73226
3A	106451021	LOC106451021	297.6043	3.559558	0.344263	4.66E-25	1.30E-20	64.08694	39.81881	34.80021	682.6041	456.4927	507.8229
3A	106390896	LOC106390896	160.7702	3.549651	0.584338	1.24E-09	9.23E-07	14.92436	33.33668	27.84017	346.3318	442.1297	100.0583
3A	106357119	LOC106357119	18.53765	3.515446	1.011062	0.000507	0.037633	4.389517	0	4.640028	53.17127	39.34205	9.683065
3A	106392222	LOC106392222	171.9843	3.504169	0.799267	1.16E-05	0.002061	24.58129	3.704075	55.68034	386.5695	103.0387	458.3317
3A	106391585	LOC106391585	80.55569	3.502715	0.704744	6.69E-07	0.000203	15.80226	8.33417	15.08009	231.3669	180.4738	32.27688
3A	106358304	LOC106358304	16.58557	3.419698	0.82781	3.61E-05	0.00515	5.26742	1.852038	1.160007	35.92653	38.0931	17.21434
3A	106346535	LOC106346535	208.8735	3.412193	0.577224	3.39E-09	2.30E-06	35.11613	28.70658	44.08027	412.4366	618.8567	114.045
3A	106384098	LOC106384098	20.49995	3.403768	0.983033	0.000535	0.039086	0	8.33417	2.320014	20.11886	59.94979	32.27688
3A	106452536	LOC106452536	144.5502	3.396835	0.671894	4.29E-07	0.000141	6.145323	22.22445	47.56029	238.5522	414.0282	138.7906
3A	111204439	LOC111204439	270.5551	3.396402	0.598232	1.37E-08	7.76E-06	19.31387	49.079	73.08044	182.5068	692.545	606.8054
3A	106360549	LOC106360549	103.676	3.349848	0.589146	1.30E-08	7.46E-06	26.3371	24.07649	4.640028	142.2691	281.6391	143.0942
3A	106394826	LOC106394826	66.098	3.326458	0.767939	1.48E-05	0.002547	4.389517	6.482132	25.52015	215.5592	49.95816	94.67885
3A	106420597	LOC106420597	74.93075	3.321405	0.780163	2.07E-05	0.003324	17.55807	18.52038	4.640028	30.17829	256.0356	122.6522
3A	106410495	LOC106410495	95.65551	3.309504	0.5163	1.45E-10	1.28E-07	19.31387	13.89028	19.72012	91.97192	281.6391	147.3978
3A	106410578	LOC106410578	101.78	3.2908	0.603125	4.86E-08	2.16E-05	29.84871	15.74232	10.44006	268.7305	71.81485	214.1033
3A	106402853	LOC106402853	88.39783	3.287505	0.780852	2.55E-05	0.00394	14.04645	15.74232	19.72012	117.839	337.2176	25.82151
3A	106389800	LOC106389800	18.66383	3.277888	0.779755	2.63E-05	0.004009	2.63371	6.482132	1.160007	38.80065	42.46443	20.44203
3A	106429649	LOC106429649	21.25523	3.262549	0.966736	0.000739	0.048592	2.63371	2.778057	6.960042	14.37061	86.8023	13.98665
3A	111212114	LOC111212114	17.62201	3.257266	0.854455	0.000138	0.014004	2.63371	2.778057	4.640028	38.80065	10.61611	46.26353
3A	106368591	LOC106368591	16.62285	3.232319	0.78576	3.89E-05	0.005426	1.755807	5.556113	2.320014	20.11886	43.08891	26.8974
3A	106371020	LOC106371020	229.5235	3.23085	0.574804	1.90E-08	1.02E-05	57.06372	41.67085	33.6402	507.2826	616.9833	120.5004
3A	106396912	LOC106396912	84.04249	3.202129	0.594167	7.07E-08	2.98E-05	11.41274	7.408151	31.32019	102.0313	126.1443	225.9382
3A	106419211	LOC106419211	182.9018	3.201992	0.781884	4.22E-05	0.005732	28.97081	22.22445	56.84034	146.5802	767.4822	75.31273
3A	106404633	LOC106404633	43.27463	3.183161	0.666305	1.78E-06	0.000449	8.779033	4.630094	12.76008	30.17829	116.1527	87.14758
3A	106436972	LOC106436972	88.79343	3.163436	0.586065	6.75E-08	2.86E-05	20.19178	13.89028	19.72012	76.16425	294.1287	108.6655
3A	106366485	LOC106366485	51.27731	3.157637	0.888496	0.00038	0.030443	3.511613	13.89028	13.92008	15.80767	199.2082	61.32608
3A	106397509	LOC106397509	945.7358	3.126075	0.511985	1.02E-09	7.90E-07	154.511	165.7574	263.3216	2309.357	2199.408	582.0598
3A	106413711	LOC106413711	50.80756	3.106171	0.735239	2.39E-05	0.003736	6.145323	7.408151	18.56011	129.3355	118.6506	24.74561
3A	111206459	LOC111206459	78.66311	3.104753	0.798981	0.000102	0.011047	10.53484	16.66834	22.04013	271.6046	131.7646	19.36613
3A	106407579	LOC106407579	3876.207	3.096902	0.561625	3.50E-08	1.66E-05	510.9397	407.4483	1516.129	11017.95	3945.446	5859.33

3A	106366318	LOC106366318	339.8009	3.089179	0.825736	0.000183	0.01731	62.33114	7.408151	145.0009	761.6425	201.7061	860.7169
3A	106445206	LOC106445206	296.6037	3.082878	0.701126	1.10E-05	0.001981	14.04645	76.85957	97.44059	569.0763	839.2971	182.9023
3A	106352386	LOC106352386	33.32186	3.070236	0.761907	5.59E-05	0.006977	0.877903	10.18621	10.44006	54.60833	35.59519	88.22348
3A	106388532	LOC106388532	34.39008	3.054419	0.677042	6.44E-06	0.001288	5.26742	11.11223	5.800035	64.66776	93.67155	25.82151
3A	106438906	LOC106438906	49.85849	3.052881	0.904866	0.000741	0.048646	10.53484	0	22.04013	50.29714	120.5241	95.75475
3A	106366164	BNAA09G06210D	35.99255	3.05254	0.638406	1.74E-06	0.000442	4.389517	7.408151	11.60007	104.9055	42.46443	45.18764
3A	106393967	LOC106393967	23.82715	3.049827	0.895899	0.000664	0.045091	9.656937	0	5.800035	22.99298	59.32531	45.18764
3A	106399437	LOC106399437	147.2987	3.049279	0.386078	2.83E-15	7.50E-12	32.48242	43.52289	18.56011	294.5976	289.1328	205.4961
3A	106433554	BNAA10G27330D	26.24355	3.048754	0.693911	1.11E-05	0.002003	3.511613	6.482132	6.960042	73.29012	23.10565	44.11174
3A	106401519	LOC106401519	218.661	3.048408	0.538602	1.51E-08	8.42E-06	27.215	26.85455	88.16053	521.6532	243.546	404.5369
3A	106398178	LOC106398178	430.2253	3.017315	0.572455	1.36E-07	5.07E-05	61.45323	63.8953	158.921	1310.6	666.9414	319.5411
3A	106366724	LOC106366724	46.18456	3.006506	0.768868	9.22E-05	0.010167	3.511613	5.556113	22.04013	143.7061	54.95397	47.33943
3A	106345209	LOC106345209	16.07095	2.998801	0.757394	7.51E-05	0.008757	2.63371	3.704075	4.640028	15.80767	40.591	29.04919
3A	106372393	LOC106372393	16.863	2.986368	0.8695	0.000593	0.042185	1.755807	2.778057	6.960042	50.29714	12.48954	26.8974
3A	106453030	LOC106453030	272.4146	2.983055	0.521151	1.04E-08	6.15E-06	45.65097	45.37492	92.80056	737.2124	206.7019	506.7471
3A	106361725	LOC106361725	107.1229	2.980686	0.859425	0.000524	0.03852	5.26742	8.33417	59.16036	313.2794	70.5659	186.13
3A	106443195	LOC106443195	615.2157	2.976768	0.613063	1.20E-06	0.00033	98.32517	67.59938	250.5615	1836.564	421.522	1016.722
3A	106431548	LOC106431548	43.13191	2.96667	0.805448	0.00023	0.020513	7.023227	7.408151	15.08009	61.79363	16.86088	150.6255
3A	106376324	LOC106376324	15.49991	2.941438	0.720648	4.47E-05	0.005961	3.511613	2.778057	4.640028	21.55592	36.84414	23.66971
3A	106346968	LOC106346968	141.5	2.931685	0.544998	7.48E-08	3.01E-05	14.04645	35.18872	49.8803	218.4333	384.0533	147.3978
3A	106400975	LOC106400975	18.28557	2.926201	0.677051	1.55E-05	0.002622	5.26742	1.852038	5.800035	33.05241	36.84414	26.8974
3A	106397566	LOC106397566	235.4899	2.922166	0.510119	1.01E-08	6.06E-06	66.72065	37.96677	60.32037	317.5905	723.7688	206.572
3A	106353671	LOC106353671	21.89121	2.900799	0.784706	0.000218	0.019712	3.511613	1.852038	10.44006	61.79363	26.85251	26.8974
3A	106375026	LOC106375026	50.33927	2.888879	0.562918	2.87E-07	9.90E-05	15.80226	6.482132	13.92008	48.86008	125.5199	91.45117
3A	106356981	LOC106356981	36.57338	2.883878	0.74665	0.000112	0.011887	7.023227	6.482132	12.76008	122.1502	50.58264	20.44203
3A	106445096	LOC106445096	50.25066	2.882005	0.528503	4.95E-08	2.18E-05	14.04645	14.8163	6.960042	76.16425	129.2667	60.25018
3A	106410962	LOC106410962	216.6722	2.872475	0.617487	3.29E-06	0.000746	21.94758	77.78558	56.84034	132.2096	608.8651	402.3851
3A	106354304	LOC106354304	65.33726	2.8623	0.7032	4.69E-05	0.006197	5.26742	20.37241	22.04013	156.6397	151.1234	36.58047
3A	106431327	LOC106431327	57.07039	2.855989	0.422882	1.44E-11	1.60E-08	16.68016	13.89028	10.44006	122.1502	82.43096	96.83065
3A	106426297	LOC106426297	30.32117	2.850835	0.804095	0.000392	0.031309	4.389517	0.926019	17.40011	45.98596	56.20293	57.02249
3A	106436445	LOC106436445	298.7005	2.849847	0.499274	1.14E-08	6.64E-06	41.26146	49.079	128.7608	603.5657	327.2259	642.31

3A	106424752	LOC106424752	130.497	2.848844	0.621211	4.52E-06	0.000981	37.74984	14.8163	42.92026	264.4193	75.56171	347.5144
3A	106382183	LOC106382183	157.1806	2.835264	0.589034	1.48E-06	0.000391	61.45323	23.15047	31.32019	106.3425	453.9948	266.8222
3A	106388327	LOC106388327	199.2327	2.830819	0.599215	2.31E-06	0.000566	17.55807	72.22947	58.00035	185.3809	593.2531	268.974
3A	106345222	LOC106345222	29.37529	2.829235	0.526649	7.78E-08	3.07E-05	6.145323	5.556113	10.44006	45.98596	54.3295	53.7948
3A	106421978	LOC106421978	51.99789	2.828985	0.71816	8.17E-05	0.009236	14.04645	2.778057	22.04013	109.2167	123.022	40.88405
3A	106443219	LOC106443219	1553.327	2.814131	0.621258	5.91E-06	0.001198	112.3716	336.1448	712.2443	4453.453	1699.202	2006.546
3A	106411542	LOC106411542	52.71924	2.80367	0.614134	4.99E-06	0.001067	8.779033	9.260189	22.04013	63.23069	58.07636	154.929
3A	106371314	LOC106371314	23.16517	2.764931	0.70584	8.96E-05	0.009958	3.511613	7.408151	6.960042	60.35657	42.46443	18.29023
3A	106400358	LOC106400358	988.1043	2.763442	0.357311	1.04E-14	2.15E-11	263.371	254.6552	242.4415	2639.882	1214.608	1313.669
3A	106390302	LOC106390302	42.52281	2.74576	0.501004	4.24E-08	1.93E-05	15.80226	5.556113	11.60007	86.22367	64.94561	71.00914
3A	106389025	LOC106389025	35.1305	2.740366	0.762698	0.000327	0.027041	14.92436	0.926019	11.60007	56.04539	41.21548	86.07169
3A	106431953	LOC106431953	47.38389	2.733753	0.561712	1.13E-06	0.000315	12.29065	5.556113	19.72012	103.4684	90.54916	52.71891
3A	106382013	LOC106382013	69.80085	2.729901	0.50956	8.44E-08	3.31E-05	17.55807	21.29843	16.2401	66.10482	191.09	106.5137
3A	106438387	LOC106438387	271.8755	2.726347	0.722708	0.000162	0.015712	58.81952	17.59436	138.0408	836.3696	182.3473	398.0815
3A	106354597	LOC106354597	1015.019	2.711881	0.195076	6.19E-44	3.44E-39	252.8362	292.622	261.0016	1794.889	1880.3	1608.465
3A	111206793	LOC111206793	14.40919	2.702491	0.713872	0.000153	0.015107	5.26742	2.778057	3.480021	18.6818	29.35042	26.8974
3A	106348998	LOC106348998	759.2802	2.702482	0.791913	0.000643	0.044212	79.8892	108.3442	418.7625	2958.909	492.7123	497.064
3A	106432729	LOC106432729	62.94779	2.694512	0.640218	2.57E-05	0.003951	14.04645	25.00251	11.60007	57.48245	206.0774	63.47787
3A	106438385	LOC106438385	476.7719	2.687215	0.722976	0.000202	0.018686	40.38355	59.26521	285.3617	1425.565	415.2772	634.7787
3A	106434612	LOC106434612	49.09676	2.664676	0.626994	2.14E-05	0.003406	7.023227	9.260189	24.36015	119.2761	90.54916	44.11174
3A	111211586	LOC111211586	381.3447	2.658746	0.692218	0.000123	0.012811	24.58129	153.7191	134.5608	998.7576	217.9425	758.5067
3A	106445239	LOC106445239	45.24559	2.651578	0.451291	4.21E-09	2.82E-06	9.656937	12.96426	15.08009	58.91951	85.55335	89.29937
3A	106410664	LOC106410664	105.9873	2.638947	0.648545	4.72E-05	0.006204	23.70339	44.44891	19.72012	219.8704	279.7657	48.41532
3A	106440409	LOC106440409	14.86236	2.629913	0.70059	0.000174	0.016759	4.389517	5.556113	2.320014	25.8671	30.59937	20.44203
3A	106348378	LOC106348378	344.4145	2.620933	0.566648	3.74E-06	0.000828	84.27872	139.8288	64.96039	306.094	1137.797	333.5278
3A	106402987	LOC106402987	39.03019	2.61583	0.622589	2.65E-05	0.004037	4.389517	8.33417	20.88013	50.29714	86.8023	63.47787
3A	106416537	LOC106416537	20.55379	2.614477	0.737611	0.000393	0.031368	2.63371	5.556113	9.280056	57.48245	26.85251	21.51792
3A	106427907	LOC106427907	44.02144	2.611322	0.585685	8.25E-06	0.001565	10.53484	9.260189	17.40011	106.3425	35.59519	84.99579
3A	106354381	LOC106354381	3957.916	2.598613	0.571149	5.37E-06	0.001124	606.6312	783.412	1975.492	12183.41	4248.941	3949.614
3A	106439379	LOC106439379	27.62375	2.586193	0.767343	0.000751	0.049215	7.90113	6.482132	9.280056	31.61535	16.86088	93.60296
3A	106446818	LOC106446818	493.8076	2.585507	0.534738	1.33E-06	0.000361	148.3657	121.3085	153.1209	1649.746	411.5303	478.7738

3A	106348915	LOC106348915	686.967	2.577203	0.738311	0.000482	0.03624	66.72065	111.1223	414.1225	2365.403	635.0931	529.3409
3A	106394738	LOC106394738	46.0562	2.576509	0.460217	2.16E-08	1.09E-05	13.16855	14.8163	11.60007	90.53486	92.42259	53.7948
3A	106370644	LOC106370644	59.70547	2.573244	0.726879	0.0004	0.031578	15.80226	23.15047	12.76008	28.74122	211.0732	66.70556
3A	106387580	LOC106387580	2891.404	2.560985	0.685093	0.000185	0.017465	345.016	329.6627	1839.771	8330.644	2776.425	3726.904
3A	106430272	LOC106430272	72.72836	2.558935	0.708816	0.000306	0.025618	27.215	3.704075	32.4802	173.8844	62.4477	136.6388
3A	106435663	LOC106435663	16.99218	2.544433	0.69513	0.000252	0.021977	4.389517	4.630094	5.800035	41.67478	17.48536	27.9733
3A	106372034	LOC106372034	30.98498	2.538609	0.619334	4.15E-05	0.005707	15.80226	6.482132	4.640028	40.23771	45.58682	73.16093
3A	106359838	LOC106359838	14.61832	2.531166	0.718506	0.000427	0.033361	4.389517	1.852038	6.960042	21.55592	24.97908	27.9733
3A	106354028	LOC106354028	151.5827	2.525372	0.571146	9.80E-06	0.001834	27.215	64.82132	42.92026	166.6991	473.3536	134.487
3A	106412321	LOC106412321	426.1958	2.525293	0.379946	3.00E-11	3.03E-08	65.84275	160.2013	153.1209	688.3523	843.0439	646.6135
3A	106421726	LOC106421726	350.3751	2.506323	0.514215	1.09E-06	0.000308	89.54614	91.67587	133.4008	751.583	205.4529	830.5918
3A	106394517	BNAC04G48880D	42.92622	2.497244	0.481153	2.10E-07	7.39E-05	17.55807	9.260189	11.60007	94.84604	66.19456	58.09839
3A	106401417	LOC106401417	205.1048	2.491272	0.473937	1.47E-07	5.40E-05	54.43001	41.67085	90.48055	178.1956	370.9393	494.9122
3A	106427826	LOC106427826	148.176	2.478386	0.714159	0.00052	0.038297	9.656937	37.96677	88.16053	375.073	264.1538	114.045
3A	106453068	LOC106453068	53.91597	2.465893	0.624769	7.92E-05	0.009039	5.26742	25.00251	19.72012	67.54188	139.2584	66.70556
3A	106447056	LOC106447056	494.9454	2.459053	0.30464	6.92E-16	2.26E-12	175.5807	133.3467	147.3209	1109.411	632.5952	771.4175
3A	106399168	LOC106399168	77.77267	2.454731	0.603871	4.80E-05	0.006239	13.16855	31.48464	27.84017	74.72718	239.7992	79.61631
3A	106353835	LOC106353835	24.02146	2.454054	0.620956	7.75E-05	0.008935	7.023227	7.408151	8.120049	27.30416	63.07217	31.20099
3A	106368085	BNAA09G35810D	1186.025	2.452545	0.547654	7.52E-06	0.001462	130.8076	258.3593	711.0843	1701.481	2096.994	2217.422
3A	106345266	LOC106345266	15.62424	2.445031	0.676509	0.000301	0.025413	6.145323	3.704075	4.640028	31.61535	29.35042	18.29023
3A	106390154	LOC106390154	466.7406	2.441329	0.623942	9.13E-05	0.010084	36.87194	225.0226	174.0011	563.328	1290.169	511.0506
3A	106436352	LOC106436352	74.26523	2.414803	0.370231	6.92E-11	6.30E-08	21.06968	25.92853	23.20014	125.0243	98.66736	151.7013
3A	106406688	LOC106406688	4941.045	2.402579	0.678437	0.000398	0.031567	569.7593	720.4427	3425.501	13938.06	4814.718	6177.795
3A	106381130	LOC106381130	50.13159	2.400594	0.671638	0.000351	0.028544	8.779033	9.260189	30.16018	139.3949	44.33787	68.85735
3A	106396831	BNAC04G02880D	206.985	2.393136	0.422805	1.51E-08	8.42E-06	103.5926	33.33668	61.48037	320.4647	369.0659	353.9698
3A	106426783	LOC106426783	244.9512	2.384553	0.497436	1.64E-06	0.000421	74.62178	121.3085	39.44024	615.0622	249.1663	370.1082
3A	106348481	LOC106348481	239.148	2.378867	0.40717	5.14E-09	3.32E-06	61.45323	92.60189	77.72047	385.1324	584.5105	233.4694
3A	106376237	LOC106376237	63.93139	2.366597	0.439709	7.36E-08	3.01E-05	17.55807	20.37241	24.36015	150.8914	96.16945	74.23683
3A	106380668	LOC106380668	23.94784	2.338429	0.541073	1.55E-05	0.002622	7.023227	6.482132	10.44006	44.5489	41.83996	33.35278
3A	106420271	BNAA10G12240D	32.49917	2.333369	0.517509	6.52E-06	0.001299	15.80226	10.18621	5.800035	53.17127	48.7092	61.32608
3A	106397539	LOC106397539	1782.355	2.331249	0.634365	0.000238	0.020958	280.0512	255.5812	1237.727	4513.809	1592.416	2814.544

3A	106362119	LOC106362119	31.14582	2.321728	0.527857	1.09E-05	0.001981	10.53484	9.260189	11.60007	51.7342	69.31694	34.42867
3A	106362118	LOC106362118	31.14582	2.321728	0.527857	1.09E-05	0.001981	10.53484	9.260189	11.60007	51.7342	69.31694	34.42867
3A	106439741	LOC106439741	327.8692	2.302568	0.573806	6.00E-05	0.007355	48.28468	197.242	85.84052	793.2578	575.7678	266.8222
3A	106379149	LOC106379149	472.8738	2.294441	0.557109	3.81E-05	0.005354	115.0053	100.01	265.6416	1368.082	370.9393	617.5643
3A	106368948	LOC106368948	77.62494	2.285022	0.447492	3.29E-07	0.000111	16.68016	19.4464	44.08027	120.7131	129.2667	135.5629
3A	106436039	LOC106436039	49.86069	2.273319	0.49106	3.67E-06	0.000822	14.92436	14.8163	22.04013	81.91249	114.9038	50.56712
3A	106433954	LOC106433954	337.0145	2.273162	0.409063	2.74E-08	1.37E-05	93.05775	82.41568	171.681	780.3243	500.8305	393.778
3A	106406937	LOC106406937	17.40564	2.268691	0.65919	0.000578	0.041266	9.656937	4.630094	3.480021	22.99298	32.4728	31.20099
3A	106401533	BNAC04G24950D	897.995	2.256852	0.419557	7.48E-08	3.01E-05	150.1215	329.6627	453.5627	1172.642	1797.245	1484.737
3A	106348515	LOC106348515	631.1037	2.254999	0.660606	0.000641	0.044212	86.91243	87.04577	482.5629	1520.411	703.7856	905.9045
3A	106451586	LOC106451586	73.86113	2.253521	0.664197	0.000692	0.046491	7.90113	42.59687	26.68016	83.34955	207.3264	75.31273
3A	106416703	LOC106416703	400.5711	2.25168	0.453267	6.78E-07	0.000205	57.06372	193.5379	167.041	620.8105	801.204	563.7695
3A	106370961	LOC106370961	140.5737	2.250676	0.387888	6.54E-09	4.03E-06	60.57533	33.33668	52.20032	275.9158	156.7437	264.6704
3A	106382609	LOC106382609	817.6659	2.249788	0.311182	4.84E-13	7.27E-10	286.1965	263.9154	302.7618	1340.778	1805.363	906.9804
3A	106396164	LOC106396164	1513.477	2.245209	0.458011	9.48E-07	0.000272	600.4859	559.3154	421.0825	4278.131	1219.604	2002.243
3A	106439643	LOC106439643	152.9634	2.243659	0.382539	4.49E-09	2.97E-06	46.52888	35.18872	78.88048	297.4717	207.9508	251.7597
3A	106386060	LOC106386060	349.3426	2.240595	0.512378	1.23E-05	0.002163	69.35436	112.0483	185.6011	254.3598	769.9801	704.7119
3A	111214069	LOC111214069	31.43781	2.232154	0.523702	2.02E-05	0.00327	11.41274	9.260189	12.76008	47.42302	71.19038	36.58047
3A	106450708	LOC106450708	879.5828	2.224269	0.472203	2.47E-06	0.000592	356.4288	358.3693	214.6013	2372.588	665.068	1310.441
3A	106370981	LOC106370981	316.4958	2.222572	0.49341	6.65E-06	0.001321	117.639	74.08151	143.8409	231.3669	501.455	830.5918
3A	106357833	LOC106357833	7410.775	2.219979	0.470655	2.40E-06	0.000584	2305.374	2032.611	3519.461	19212.07	5198.771	12196.36
3A	106450229	LOC106450229	142.733	2.219561	0.547108	4.97E-05	0.006399	36.87194	78.7116	35.96022	168.1362	406.5345	130.1834
3A	106362484	LOC106362484	438.1705	2.214342	0.279334	2.24E-15	6.56E-12	176.4586	175.0176	113.6807	776.0131	779.9717	607.8813
3A	106434319	LOC106434319	4867.307	2.213422	0.379023	5.23E-09	3.34E-06	1595.15	1444.589	2140.213	10983.46	4360.098	8680.33
3A	106388928	LOC106388928	43.05989	2.194185	0.581079	0.000159	0.015593	21.06968	5.556113	19.72012	71.85306	48.7092	91.45117
3A	106449634	LOC106449634	50.16608	2.190708	0.613599	0.000357	0.02873	7.90113	21.29843	25.52015	40.23771	124.2709	81.7681
3A	106375914	LOC106375914	37.64016	2.172062	0.5065	1.80E-05	0.002969	11.41274	10.18621	19.72012	79.03837	62.4477	43.03584
3A	111200555	LOC111200555	123.1793	2.168596	0.627236	0.000545	0.0396	21.94758	42.59687	70.76043	83.34955	366.568	153.8531
3A	106371884	LOC106371884	35.93182	2.168509	0.543601	6.63E-05	0.007927	9.656937	9.260189	20.88013	41.67478	55.57845	78.54041
3A	106440678	LOC106440678	2612.408	2.163007	0.515854	2.75E-05	0.004135	423.1494	608.3944	1830.491	4641.708	3651.941	4518.764
3A	106390092	LOC106390092	2204.777	2.159447	0.450194	1.61E-06	0.000419	708.468	573.2057	1137.967	5970.989	2540.997	2297.038

3A	111211030	LOC111211030	54.59012	2.158907	0.435446	7.13E-07	0.000214	11.41274	22.22445	26.68016	93.40898	78.05962	95.75475
3A	106354632	LOC106354632	70.93991	2.152908	0.546101	8.07E-05	0.009154	23.70339	24.07649	30.16018	202.6256	88.05125	57.02249
3A	106387209	LOC106387209	1793.168	2.150879	0.535862	5.97E-05	0.007355	495.1375	621.3587	860.7252	5672.081	1411.942	1697.764
3A	106445204	LOC106445204	262.2475	2.148646	0.38792	3.04E-08	1.46E-05	71.11017	90.74985	128.7608	375.073	607.6161	300.175
3A	106348250	LOC106348250	309.4547	2.147863	0.348997	7.54E-10	5.98E-07	143.0982	114.8263	83.52051	510.1567	650.0805	355.0457
3A	106440922	LOC106440922	89.46766	2.132758	0.586717	0.000278	0.023729	18.43597	49.079	32.4802	129.3355	242.9215	64.55376
3A	111214991	LOC111214991	223.628	2.131949	0.350982	1.25E-09	9.23E-07	60.57533	76.85957	112.5207	441.1778	378.433	272.2017
3A	106393197	LOC106393197	76.50865	2.126288	0.575953	0.000223	0.019998	41.26146	25.00251	18.56011	202.6256	54.3295	117.2727
3A	106445332	LOC106445332	621.4137	2.126223	0.455876	3.10E-06	0.000712	114.1274	217.6144	364.2422	888.1038	1386.963	757.4308
3A	106396050	LOC106396050	29.61025	2.123755	0.576355	0.000229	0.020445	8.779033	6.482132	18.56011	35.92653	60.57427	47.33943
3A	106361411	LOC106361411	108.2753	2.123076	0.519181	4.33E-05	0.00581	54.43001	25.92853	40.60025	283.1011	157.3682	88.22348
3A	106353373	LOC106353373	28.4489	2.120016	0.528091	5.96E-05	0.007355	10.53484	6.482132	15.08009	56.04539	40.591	41.95995
3A	106349415	LOC106349415	74.03306	2.117652	0.399153	1.12E-07	4.28E-05	17.55807	26.85455	39.44024	116.402	110.5324	133.4111
3A	106346066	LOC106346066	268.5457	2.105769	0.490012	1.73E-05	0.002876	113.2495	68.5254	121.8007	530.2756	179.2249	598.1982
3A	106369277	LOC106369277	274.4007	2.105614	0.621385	0.000703	0.046967	40.38355	82.41568	187.9211	763.0795	222.9383	349.6662
3A	106357615	LOC106357615	454.2549	2.10516	0.513907	4.20E-05	0.005732	146.6099	77.78558	290.0018	1060.551	465.2353	685.3458
3A	106405923	LOC106405923	51.3011	2.10326	0.415394	4.12E-07	0.000137	22.82549	17.59436	17.40011	102.0313	85.55335	62.40197
3A	106352647	LOC106352647	2280.408	2.100907	0.48839	1.69E-05	0.002838	762.898	610.2464	1213.367	6337.44	1792.249	2966.245
3A	106434407	LOC106434407	360.7758	2.094302	0.514142	4.63E-05	0.006132	86.03453	177.7956	147.3209	587.758	927.9728	237.773
3A	106345834	LOC106345834	412.2002	2.091413	0.544812	0.000124	0.012895	114.1274	202.7981	153.1209	949.8975	849.9132	203.3444
3A	106428974	LOC106428974	181.7957	2.089856	0.482422	1.48E-05	0.002547	53.5521	92.60189	61.48037	317.5905	432.1381	133.4111
3A	106427165	LOC106427165	369.8748	2.07297	0.445979	3.35E-06	0.000757	108.86	97.23198	220.4013	870.8591	387.1757	534.7203
3A	106423189	LOC106423189	256.1864	2.067642	0.383759	7.13E-08	2.98E-05	147.4878	74.08151	74.24045	313.2794	505.2019	422.8272
3A	106415678	LOC106415678	72.7587	2.066564	0.442908	3.07E-06	0.000711	32.48242	22.22445	29.00018	175.3215	89.30021	88.22348
3A	106439039	LOC106439039	47.06534	2.063413	0.604098	0.000636	0.04406	34.23823	8.33417	11.60007	61.79363	67.44351	98.98244
3A	106402178	LOC106402178	329.9553	2.057044	0.331476	5.44E-10	4.45E-07	129.0518	114.8263	139.2008	705.5971	350.9561	540.0998
3A	106364601	LOC106364601	28.62367	2.054764	0.589035	0.000486	0.036507	9.656937	11.11223	12.76008	53.17127	62.4477	22.59382
3A	106358437	LOC106358437	163.2688	2.052954	0.386453	1.08E-07	4.18E-05	59.69743	41.67085	89.32054	267.2934	196.7102	324.9206
3A	106366310	LOC106366310	1399.492	2.040112	0.234164	2.98E-18	1.84E-14	617.1661	509.3104	516.2031	1922.788	2703.361	2128.122
3A	106358375	LOC106358375	154.1542	2.028014	0.477411	2.16E-05	0.003426	38.62775	62.96928	81.20049	261.5451	354.7029	125.8798
3A	106421089	LOC106421089	28.62984	2.027718	0.542795	0.000187	0.017577	7.90113	11.11223	15.08009	37.36359	36.84414	63.47787

3A	106435412	LOC106435412	21.93726	2.027603	0.583303	0.000509	0.037711	6.145323	8.33417	11.60007	33.05241	26.22803	46.26353
3A	106406536	LOC106406536	25.00592	2.024469	0.507821	6.70E-05	0.007982	8.779033	9.260189	11.60007	47.42302	37.46862	35.50457
3A	106371106	LOC106371106	143.955	2.020574	0.489884	3.71E-05	0.005266	54.43001	55.56113	61.48037	114.9649	396.5429	180.7505
3A	106410663	LOC106410663	1171.588	2.020332	0.411949	9.37E-07	0.000271	381.888	276.8796	731.9644	2458.812	1503.741	1676.246
3A	106347601	LOC106347601	94.25975	2.018572	0.415149	1.16E-06	0.000321	25.4592	31.48464	55.68034	181.0697	156.7437	115.1209
3A	106452673	LOC106452673	105.2875	2.016786	0.449121	7.11E-06	0.001391	55.30791	20.37241	49.8803	125.0243	196.0858	185.0541
3A	106354293	LOC106354293	29.80095	2.011658	0.494942	4.81E-05	0.006239	14.04645	12.96426	8.120049	57.48245	47.46025	38.73226
3A	106436104	LOC106436104	436.8006	2.011301	0.477477	2.53E-05	0.003924	137.8308	109.2702	274.9217	376.51	996.0408	726.2298
3A	106419013	LOC106419013	386.2656	2.008212	0.529983	0.000151	0.014948	61.45323	117.6044	283.0417	741.5236	453.3703	660.6002
3A	106388269	LOC106388269	45.68965	2.001695	0.581729	0.00058	0.041298	21.06968	22.22445	11.60007	33.05241	113.0303	73.16093
3A	106391953	LOC106391953	115.4234	-2.04464	0.368722	2.94E-08	1.43E-05	134.3192	234.2828	189.0811	50.29714	51.20711	33.35278
3A	106364057	LOC106364057	114.4568	-2.15044	0.578557	0.000202	0.018686	151.8773	132.4207	274.9217	54.60833	13.73849	59.17428
3A	106382915	LOC106382915	124.5095	-2.15617	0.523336	3.79E-05	0.005344	249.3246	159.2752	200.6812	38.80065	19.35879	79.61631
3A	106364683	LOC106364683	31.66735	-2.16206	0.583664	0.000212	0.019325	71.11017	36.11474	47.56029	10.05943	6.869247	18.29023
3A	106428469	LOC106428469	1423.355	-2.16402	0.594413	0.000272	0.023405	1455.564	2932.702	2592.616	988.6981	417.7751	152.7772
3A	106354518	LOC106354518	30.04857	-2.17519	0.590852	0.000232	0.020593	44.77307	38.89279	62.64038	18.6818	5.620293	9.683065
3A	106380012	BNAC02G40310D	58.71824	-2.18173	0.388149	1.90E-08	1.02E-05	108.86	76.85957	102.0806	24.43004	17.48536	22.59382
3A	106420920	LOC106420920	399.7939	-2.23249	0.286834	7.07E-15	1.64E-11	812.0606	550.0552	613.6437	163.825	111.7814	147.3978
3A	106397233	LOC106397233	32.02778	-2.27415	0.59056	0.000118	0.012416	40.38355	64.82132	53.36032	18.6818	10.61611	4.303584
3A	106346300	LOC106346300	23.29998	-2.30308	0.550356	2.86E-05	0.004188	30.72662	45.37492	40.60025	5.748245	8.742678	8.607169
3A	106366733	LOC106366733	23.41351	-2.31485	0.552853	2.83E-05	0.004184	43.89517	39.81881	32.4802	10.05943	5.620293	8.607169
3A	106365724	LOC106365724	170.6086	-2.36705	0.421238	1.92E-08	1.02E-05	280.9291	336.1448	240.1215	37.36359	38.71757	90.37527
3A	106376599	LOC106376599	73.90086	-2.37091	0.565904	2.79E-05	0.004164	104.4705	152.7931	112.5207	47.42302	14.36297	11.83486
3A	106403139	LOC106403139	39.59281	-2.38541	0.523962	5.30E-06	0.00112	47.40678	92.60189	60.32037	10.05943	17.48536	9.683065
3A	106447758	LOC106447758	55.40872	-2.42407	0.56524	1.80E-05	0.002969	50.91839	146.311	82.3605	25.8671	16.2364	10.75896
3A	106442702	LOC106442702	192.12	-2.46171	0.367349	2.07E-11	2.21E-08	363.452	286.1398	328.282	48.86008	86.17782	39.80815
3A	106354180	LOC106354180	132.82	-2.48779	0.548346	5.71E-06	0.001167	215.0863	245.395	218.0813	21.55592	77.43515	19.36613
3A	106380989	LOC106380989	14.90585	-2.54696	0.732224	0.000504	0.037537	35.11613	25.00251	16.2401	2.874122	3.746862	6.455376
3A	111213136	LOC111213136	14.90585	-2.54696	0.732224	0.000504	0.037537	35.11613	25.00251	16.2401	2.874122	3.746862	6.455376
3A	106452849	LOC106452849	12.97625	-2.6868	0.725342	0.000212	0.019325	21.06968	19.4464	26.68016	4.311184	3.122385	3.227688
3A	106352779	LOC106352779	13.4122	-2.70108	0.797973	0.000712	0.047452	35.99404	19.4464	13.92008	4.311184	2.497908	4.303584

3A	106405100	LOC106405100	117.1054	-2.71217	0.494369	4.11E-08	1.89E-05	242.3013	175.9436	191.4012	15.80767	21.23222	55.9466
3A	106358204	LOC106358204	26.07483	-2.77334	0.821638	0.000737	0.048549	43.01726	41.67085	51.04031	7.185306	0.624477	12.91075
3A	106356383	LOC106356383	75.85869	-2.85077	0.370199	1.35E-14	2.69E-11	143.0982	144.4589	112.5207	14.37061	18.10983	22.59382
3A	106395191	LOC106395191	16.53388	-2.87773	0.75084	0.000127	0.01317	42.13936	22.22445	23.20014	1.437061	3.746862	6.455376
3A	106389071	LOC106389071	55.82914	-3.18716	0.463514	6.15E-12	7.60E-09	110.6158	122.2345	68.44041	12.93355	9.991632	10.75896
3A	106411440	LOC106411440	20.20358	-3.95228	1.16266	0.000675	0.045622	59.69743	29.6326	24.36015	0	0	7.531273
3A	106396576	LOC106396576	23.1836	-4.05922	0.903578	7.04E-06	0.001388	31.60452	37.04075	61.48037	5.748245	0	3.227688
3A	106349864	LOC106349864	17.55647	-4.24031	1.107041	0.000128	0.013272	14.04645	37.04075	49.8803	0	4.371339	0
3A	106359872	LOC106359872	10.15909	-4.77199	1.313329	0.00028	0.0238	18.43597	12.96426	26.68016	2.874122	0	0
3A	106429743	LOC106429743	17.55879	-4.7972	0.968593	7.32E-07	0.000216	42.13936	20.37241	39.44024	0	1.248954	2.151792
3A	106387300	LOC106387300	11.48482	-4.96925	1.30465	0.00014	0.01416	33.36033	17.59436	15.08009	2.874122	0	0
3A	106425820	LOC106425820	63.4504	-5.01178	0.565054	7.34E-19	5.10E-15	126.4181	100.9361	141.5209	2.874122	2.497908	6.455376
3A	106392049	LOC106392049	27.60291	-5.09798	1.135831	7.18E-06	0.0014	21.94758	55.56113	82.3605	5.748245	0	0
3A	106386153	LOC106386153	7.281214	-5.30722	1.473358	0.000316	0.026264	19.31387	14.8163	8.120049	1.437061	0	0
3A	106441583	LOC106441583	4.398877	-5.59597	1.632628	0.000609	0.042687	8.779033	8.33417	9.280056	0	0	0
3A	106414896	LOC106414896	4.615171	-5.66325	1.65247	0.00061	0.042702	10.53484	5.556113	11.60007	0	0	0
3A	106348403	LOC106348403	4.683492	-5.68783	1.621454	0.000452	0.034663	11.41274	7.408151	9.280056	0	0	0
3A	106428464	LOC106428464	4.824544	-5.72574	1.61854	0.000404	0.031837	8.779033	7.408151	12.76008	0	0	0
3A	106370327	LOC106370327	5.007105	-5.78401	1.640399	0.000422	0.033079	14.04645	5.556113	10.44006	0	0	0
3A	106452046	LOC106452046	5.164195	-5.82373	1.600851	0.000275	0.023579	9.656937	7.408151	13.92008	0	0	0
3A	106357111	LOC106357111	5.589305	-5.94067	1.630378	0.000269	0.023178	5.26742	16.66834	11.60007	0	0	0
3A	111203615	LOC111203615	5.755828	-5.98718	1.547307	0.000109	0.011678	12.29065	12.96426	9.280056	0	0	0
3A	106391433	LOC106391433	5.781541	-5.988	1.546104	0.000108	0.01156	9.656937	11.11223	13.92008	0	0	0
3A	106445300	LOC106445300	5.895224	-6.02216	1.578981	0.000137	0.013949	9.656937	17.59436	8.120049	0	0	0
3A	106371627	LOC106371627	6.18676	-6.09246	1.536799	7.36E-05	0.008648	15.80226	12.03825	9.280056	0	0	0
3A	106411887	LOC106411887	6.33693	-6.12339	1.560235	8.69E-05	0.009773	7.90113	18.52038	11.60007	0	0	0
3A	106445304	LOC106445304	6.43182	-6.15074	1.737186	0.000399	0.031578	14.04645	22.22445	2.320014	0	0	0
3A	106387371	LOC106387371	6.503452	-6.16458	1.52188	5.11E-05	0.006542	14.92436	14.8163	9.280056	0	0	0
3A	106453457	LOC106453457	7.813944	-6.42786	1.516575	2.25E-05	0.003555	23.70339	9.260189	13.92008	0	0	0
3A	106380183	LOC106380183	8.691847	-6.58232	1.52227	1.53E-05	0.00262	28.97081	9.260189	13.92008	0	0	0
3A	106362712	LOC106362712	8.753788	-6.59457	1.652116	6.56E-05	0.007879	17.55807	31.48464	3.480021	0	0	0

3A	106378960	LOC106378960	8.888178	-6.60462	1.498003	1.04E-05	0.001925	12.29065	12.03825	29.00018	0	0	0
3A	106372582	LOC106372582	9.090388	-6.6438	1.420694	2.92E-06	0.00069	19.31387	16.66834	18.56011	0	0	0
3A	106405519	LOC106405519	9.110351	-6.65292	1.629668	4.46E-05	0.005956	37.74984	11.11223	5.800035	0	0	0
3A	106377262	LOC106377262	9.60207	-6.71962	1.426017	2.45E-06	0.000592	18.43597	14.8163	24.36015	0	0	0
3A	111212119	LOC111212119	9.605122	-6.72753	1.481314	5.58E-06	0.001151	17.55807	29.6326	10.44006	0	0	0
3A	106347244	LOC106347244	10.46062	-6.85018	1.412351	1.23E-06	0.000338	25.4592	22.22445	15.08009	0	0	0
3A	106353961	LOC106353961	11.00029	-6.92456	1.548163	7.72E-06	0.00149	42.13936	15.74232	8.120049	0	0	0
3A	106349196	LOC106349196	12.50239	-7.10368	1.366095	1.99E-07	7.15E-05	21.94758	28.70658	24.36015	0	0	0
3A	106406011	LOC106406011	12.84455	-7.14621	1.376691	2.09E-07	7.39E-05	30.72662	27.78057	18.56011	0	0	0
3A	106361440	LOC106361440	13.9426	-7.26308	1.351468	7.69E-08	3.05E-05	28.97081	31.48464	23.20014	0	0	0
3A	106429879	LOC106429879	62.71324	-7.47058	2.152812	0.00052	0.038297	74.62178	3.704075	295.8018	0	0	2.151792
3A	106420051	LOC106420051	18.37573	-7.66091	1.539735	6.51E-07	0.000199	12.29065	80.56364	17.40011	0	0	0
3A	106371987	LOC106371987	18.71934	-7.68818	1.384799	2.83E-08	1.39E-05	59.69743	21.29843	31.32019	0	0	0
3A	106445897	LOC106445897	20.3568	-7.81069	1.557462	5.30E-07	0.000168	73.74388	42.59687	5.800035	0	0	0
3A	106383142	LOC106383142	21.61981	-7.89309	1.507241	1.63E-07	5.94E-05	8.779033	76.85957	44.08027	0	0	0
3A	106430929	LOC106430929	28.70516	-8.30134	1.470778	1.66E-08	9.13E-06	11.41274	88.89781	71.92044	0	0	0
3A	106413873	LOC106413873	40.21583	-8.78788	1.305745	1.69E-11	1.85E-08	95.69146	43.52289	102.0806	0	0	0
3A	106437571	LOC106437571	40.59383	-8.80054	1.288584	8.51E-12	9.86E-09	50.04049	92.60189	100.9206	0	0	0
3A	106396518	LOC106396518	44.63073	-8.9385	1.247639	7.82E-13	1.09E-09	89.54614	79.63762	98.6006	0	0	0
3A	106402120	LOC106402120	46.08086	-8.98354	1.253878	7.80E-13	1.09E-09	83.40082	80.56364	112.5207	0	0	0
3A	106441123	LOC106441123	52.4019	-9.16958	1.344882	9.22E-12	1.05E-08	38.62775	138.9028	136.8808	0	0	0
3A	111215132	LOC111215132	53.90611	-9.21043	1.24249	1.24E-13	1.96E-10	93.05775	107.4182	122.9607	0	0	0
3A	106406418	LOC106406418	67.81395	-9.54459	1.468162	7.98E-11	7.15E-08	247.5687	140.7549	18.56011	0	0	0
3A	106372721	LOC106372721	72.05517	-9.63215	1.237813	7.16E-15	1.64E-11	154.511	162.9793	114.8407	0	0	0
3A	106387660	LOC106387660	74.34437	-9.67519	1.243999	7.40E-15	1.64E-11	112.3716	180.5737	153.1209	0	0	0
3A	106381075	LOC106381075	80.06164	-9.78235	1.274295	1.63E-14	3.03E-11	209.8189	90.74985	179.8011	0	0	0
3A	106369077	LOC106369077	106.5691	-10.1952	1.233761	1.41E-16	5.62E-13	253.7141	162.9793	222.7213	0	0	0
3A	106410475	LOC106410475	124.9937	-10.4246	1.217621	1.11E-17	5.63E-14	223.8654	254.6552	271.4416	0	0	0
3A	111212112	LOC111212112	216.8528	-11.221	1.265036	7.31E-19	5.10E-15	539.9106	536.1649	225.0414	0	0	0
3D	106369190	LOC106369190	97.25101	10.12462	1.217195	8.95E-17	2.51E-12	0	0	0	152.7393	206.514	224.2528
3D	106415389	LOC106360375	62.9644	9.495581	1.300777	2.88E-13	1.80E-09	0	0	0	135.0304	49.03529	193.7207

3D	106387670	LOC106387670	60.912	9.44902	1.221578	1.03E-14	9.68E-11	0	0	0	116.2147	119.7593	129.4981
3D	106407096	LOC106434345	52.50336	9.235799	1.242519	1.06E-13	7.45E-10	0	0	0	104.0398	132.0181	78.96225
3D	106345856	LOC106345856	36.09498	8.694207	1.328569	5.99E-11	1.77E-07	0	0	0	99.61257	89.58371	27.37358
3D	111203285	LOC111203285	35.10634	8.654857	1.275916	1.18E-11	4.40E-08	0	0	0	45.37906	72.60995	92.64904
3D	106359764	LOC106359764	31.36817	8.490878	1.256217	1.39E-11	4.88E-08	0	0	0	65.30157	56.57918	66.32829
3D	106367691	LOC106367691	25.47231	8.192764	1.279134	1.50E-10	4.03E-07	0	0	0	44.27225	62.2371	46.32452
3D	106354500	LOC106354500	383.9397	7.892726	1.147687	6.11E-12	2.45E-08	0	7.679775	2.036354	1318.206	445.0896	530.6263
3D	106418557	LOC106418557	69.45993	7.769955	1.081237	6.66E-13	3.75E-09	0	0	2.036354	119.5351	114.1014	181.0868
3D	111202344	LOC111202344	16.58259	7.574531	1.396251	5.80E-08	0.000109	0	0	0	13.28168	46.20633	40.00754
3D	106438935	BNAC03G32930D	15.86811	7.510746	1.343394	2.26E-08	4.54E-05	0	0	0	26.56335	43.37737	25.26792
3D	111215159	LOC106391890	15.52445	7.477764	1.32646	1.73E-08	3.73E-05	0	0	0	28.77696	34.8905	29.47924
3D	106381160	LOC106381160	15.00119	7.427953	1.506799	8.24E-07	0.001119	0	0	0	6.640838	25.46063	57.90565
3D	106373193	LOC106373193	13.91822	7.320924	1.350919	5.99E-08	0.000109	0	0	0	25.45655	34.8905	23.16226
3D	106424765	LOC106424765	12.27542	7.141416	1.784988	6.31E-05	0.033167	0	0	0	1.106806	64.12307	8.42264
3D	106451235	LOC106451233	106.8983	7.08052	1.165795	1.25E-09	3.06E-06	1.741067	1.919944	1.018177	366.3529	1.885973	268.4717
3D	106440958	LOC106440960	11.07284	6.988551	1.480823	2.37E-06	0.00251	0	0	0	12.17487	13.20181	41.06037
3D	106405157	LOC106405157	10.32629	6.888513	1.611794	1.92E-05	0.014545	0	0	0	4.427225	12.25882	45.27169
3D	106349195	LOC106452310	9.85259	6.8197	1.408223	1.28E-06	0.001565	0	0	0	19.92251	16.03077	23.16226
3D	106348764	LOC106410382	9.639874	6.788784	1.423752	1.86E-06	0.002132	0	0	0	24.34974	19.80271	13.68679
3D	106387342	LOC106387342	9.543656	6.771453	1.510246	7.34E-06	0.006346	0	0	0	35.4178	11.31584	10.5283
3D	106450864	LOC106450934	8.59496	6.620741	1.473244	6.99E-06	0.006141	0	0	0	22.13613	9.429864	20.00377
3D	106360585	LOC106425230	7.611675	6.450441	1.556082	3.39E-05	0.020966	0	0	0	5.534032	16.97375	23.16226
3D	106377392	LOC106377392	7.572089	6.440554	1.461928	1.06E-05	0.008496	0	0	0	15.49529	14.1448	15.79245
3D	111202271	LOC111202271	7.351322	6.398994	1.475142	1.44E-05	0.011392	0	0	0	12.17487	15.08778	16.84528
3D	111198424	LOC111198424	7.278408	6.386143	1.492054	1.87E-05	0.014387	0	0	0	9.961257	17.91674	15.79245
3D	106394966	BNAC05G09720D	6.904792	6.304144	1.586333	7.07E-05	0.035534	0	0	0	18.81571	4.714932	17.89811
3D	106367736	LOC106367736	6.797919	6.286999	1.514903	3.32E-05	0.02076	0	0	0	8.85445	15.08778	16.84528
3D	111204221	LOC111204221	6.44666	6.210733	1.515726	4.18E-05	0.025244	0	0	0	11.06806	16.03077	11.58113
3D	106346659	LOC111216268	10.59658	5.055365	1.280924	7.93E-05	0.038485	1.741067	0	0	15.49529	17.91674	28.42641
3D	106395264	LOC106395264	21.60823	2.905508	0.636767	5.04E-06	0.004809	4.352667	3.839888	7.127238	52.0199	25.46063	36.84905
3D	106419005	LOC106419005	22.78965	2.551467	0.598978	2.05E-05	0.014946	4.352667	11.51966	4.072708	40.95183	35.83348	40.00754

3D	106359817	LOC106359817	35.73276	2.476782	0.534956	3.66E-06	0.003809	17.41067	3.839888	11.19995	66.40838	56.57918	58.95848
3D	106361053	LOC106361053	199.9407	2.436008	0.45438	8.27E-08	0.000145	43.52667	25.91924	118.1085	294.4105	355.5059	362.1735
3D	106436838	LOC106436838	52.30412	2.30631	0.539947	1.94E-05	0.014545	24.37494	22.07935	6.109061	50.91309	110.3294	100.0189
3D	106376947	LOC106376947	58.56475	2.200272	0.541729	4.87E-05	0.028206	22.63387	28.79916	11.19995	79.69005	49.03529	160.0302
3D	106360549	LOC106360549	76.73885	2.028174	0.484095	2.79E-05	0.018266	22.63387	16.31952	51.92702	139.4576	76.3819	153.7132
3D	106347833	LOC106347833	96.43695	2.013564	0.423856	2.03E-06	0.002281	53.97307	42.23876	18.32718	105.1466	190.4833	168.4528
3D	106361139	LOC106361139	36.68632	2.010172	0.501416	6.10E-05	0.032342	19.15174	18.23947	6.109061	66.40838	48.09231	62.11697
3D	106414699	LOC106451978	213.6005	-2.0093	0.409554	9.29E-07	0.001215	174.1067	279.3518	573.2336	99.61257	85.81176	69.48678
3D	106446549	LOC106446549	51.82681	-2.5165	0.554196	5.60E-06	0.005165	39.17401	107.5169	118.1085	8.85445	14.1448	23.16226
3D	106446549	LOC106446547	51.82681	-2.5165	0.554196	5.60E-06	0.005165	39.17401	107.5169	118.1085	8.85445	14.1448	23.16226
3D	111215514	LOC111215514	15.4505	-4.40858	1.110472	7.19E-05	0.035758	12.18747	34.55899	41.74525	0	0	4.21132
3D	106356645	LOC106354859	6.968336	-6.19775	1.570566	7.94E-05	0.038485	8.705334	24.95927	8.145415	0	0	0
3D	106375609	LOC111197742	29.66351	-6.42068	1.558144	3.78E-05	0.02308	72.25428	103.677	0	1.106806	0.942986	0
3D	106375677	LOC106375677	45.28872	-6.44034	1.307219	8.36E-07	0.001119	123.6157	143.9958	1.018177	1.106806	0.942986	1.05283
3D	111207780	LOC106399970	10.05575	-6.724	1.473649	5.05E-06	0.004809	11.31693	14.39958	34.61801	0	0	0
3D	106430449	LOC106430449	11.6064	-6.93596	1.466669	2.26E-06	0.002478	36.5624	8.639747	24.43625	0	0	0
3D	106407096	LOC106407096	64.03423	-6.94591	0.893302	7.51E-15	8.45E-11	108.8167	143.9958	128.2903	1.106806	0.942986	1.05283
3D	106422663	LOC106391874	12.15784	-7.00277	1.381635	4.01E-07	0.000609	27.85707	28.79916	16.29083	0	0	0
3D	106348764	LOC106348764	13.04063	-7.1028	1.349708	1.42E-07	0.000235	26.98654	27.83918	23.41807	0	0	0
3D	111201380	LOC111201380	14.06207	-7.20921	1.357386	1.09E-07	0.000186	19.15174	32.63904	32.58166	0	0	0
3D	106445227	LOC106445227	14.20541	-7.22779	1.430358	4.35E-07	0.000627	35.69187	39.35885	10.18177	0	0	0
3D	106387670	LOC106411808	62.07983	-7.49405	1.075965	3.29E-12	1.42E-08	144.5086	101.757	124.2176	0	0.942986	1.05283
3D	106421938	LOC106421938	25.37105	-8.06038	1.307703	7.10E-10	1.82E-06	36.5624	40.31882	75.34509	0	0	0
3D	106418133	LOC106418133	25.36064	-8.06293	1.338313	1.69E-09	3.97E-06	73.12481	23.03933	55.99973	0	0	0
3D	106438040	LOC106420565	29.80853	-8.29559	1.288452	1.21E-10	3.39E-07	56.58467	82.55758	39.7089	0	0	0
3D	111203285	LOC106396787	30.86011	-8.34591	1.25808	3.27E-11	1.02E-07	69.64268	59.51826	55.99973	0	0	0
3D	106393794	LOC106393812	39.77957	-8.7127	1.293499	1.63E-11	5.39E-08	120.1336	43.19874	75.34509	0	0	0
3D	106395617	LOC106395617	46.83068	-8.94782	1.25042	8.32E-13	4.25E-09	104.464	111.3567	65.16332	0	0	0
3D	106446276	LOC106446276	56.03695	-9.20409	1.291223	1.02E-12	4.76E-09	48.74987	128.6362	158.8356	0	0	0
3D	106372614	LOC106390598	64.68438	-9.41277	2.231241	2.46E-05	0.016528	168.8835	213.1138	6.109061	0	0	0
3D	106421365	LOC106421365	89.26001	-9.87761	1.302323	3.33E-14	2.68E-10	159.3076	310.0709	66.1815	0	0	0

3D	106367073	LOC111200314	92.58742	-9.92882	1.241456	1.27E-15	1.78E-11	122.7452	167.0351	265.7442	0	0	0
3D	106411466	LOC106453109	96.12683	-9.98485	1.232872	5.55E-16	1.04E-11	202.8343	247.6727	126.2539	0	0	0
3D	106422506	BNAC05G35540D	253.6482	-11.3844	1.195647	1.71E-21	9.59E-17	559.753	487.6657	474.4704	0	0	0
3H	106431125	LOC106447544	287.9354	11.60488	1.295375	3.29E-19	5.68E-15	0	0	0	1063.866	388.1934	275.5529
3H	106371451	LOC111211703	171.2588	10.85522	1.204804	2.06E-19	5.35E-15	0	0	0	356.2137	345.1746	326.1647
3H	106354725	LOC111212112	149.6138	10.66064	1.304793	3.07E-16	1.77E-12	0	0	0	169.9894	170.0267	557.6667
3H	106349452	LOC106349452	110.6991	10.22517	1.23679	1.37E-16	1.18E-12	0	0	0	216.7842	290.889	156.5216
3H	111204564	LOC106410708	108.3592	10.19545	1.242108	2.25E-16	1.46E-12	0	0	0	259.7591	138.2747	252.1216
3H	106429960	LOC106367228	98.75105	10.06045	1.22269	1.90E-16	1.41E-12	0	0	0	188.1343	236.6034	167.7686
3H	106367051	LOC106367051	88.14409	9.896667	1.220049	4.99E-16	2.35E-12	0	0	0	178.5844	195.6331	154.6471
3H	106368761	LOC106368761	60.52057	9.354903	1.229204	2.73E-14	1.01E-10	0	0	0	130.8345	108.5712	123.7176
3H	106367225	LOC106429879	103.5083	9.164395	1.213975	4.38E-14	1.34E-10	1.011752	0	0	203.4143	226.3608	190.2627
3H	111212474	LOC106388769	51.231	9.114837	1.265345	5.87E-13	1.27E-09	0	0	0	138.4745	68.62522	100.2863
3H	106401888	LOC106391230	50.59984	9.095989	1.238469	2.06E-13	4.87E-10	0	0	0	109.8246	107.547	86.22745
3H	106416296	LOC106416296	49.70518	9.070455	1.234133	1.99E-13	4.87E-10	0	0	0	101.2296	101.4014	95.6
3H	106366797	LOC106410946	48.85567	9.045671	1.391173	7.92E-11	1.32E-07	0	0	0	22.91992	113.6925	156.5216
3H	106388044	LOC106447175	41.32898	8.80462	1.245118	1.53E-12	2.95E-09	0	0	0	90.72467	74.77076	82.47843
3H	106401488	LOC106401488	38.81962	8.713975	1.249091	3.03E-12	5.62E-09	0	0	0	68.75975	78.86779	85.2902
3H	106446276	LOC106446276	29.3308	8.310441	1.475607	1.78E-08	1.85E-05	0	0	0	97.40965	9.218313	69.35686
3H	106402194	LOC106368238	26.32575	8.154576	1.29866	3.40E-10	5.04E-07	0	0	0	45.83984	39.94602	72.16863
3H	106425359	LOC106437389	25.39179	8.101571	1.276143	2.17E-10	3.42E-07	0	0	0	45.83984	51.21285	55.29804
3H	106367691	LOC106349316	21.6256	7.869231	1.307086	1.74E-09	2.26E-06	0	0	0	49.65982	49.16434	30.92941
3H	106418665	LOC106348205	18.99495	7.681292	1.339294	9.73E-09	1.08E-05	0	0	0	29.60489	55.30988	29.0549
3H	111212055	LOC111212055	17.84649	7.590917	1.410631	7.40E-08	6.85E-05	0	0	0	24.82991	63.50394	18.7451
3H	106428789	LOC106428790	15.15944	7.356691	1.435267	2.96E-07	0.000248	0	0	0	44.88484	34.82474	11.24706
3H	106440113	LOC106440112	14.66898	7.308917	1.365685	8.71E-08	7.92E-05	0	0	0	19.09993	38.92177	29.99216
3H	106416299	LOC106354317	14.46239	7.290875	1.491166	1.01E-06	0.000729	0	0	0	14.32495	14.3396	58.1098
3H	106372327	LOC106372327	14.03607	7.246735	1.355209	8.93E-08	7.98E-05	0	0	0	36.28987	23.55791	24.36863
3H	106397804	LOC106397804	12.84048	7.11954	1.518309	2.74E-06	0.001767	0	0	0	32.46988	6.145542	38.42745
3H	106449492	LOC106449492	11.73586	6.987038	1.39748	5.74E-07	0.000451	0	0	0	26.7399	28.6792	14.99608
3H	106360791	LOC106360791	21.89773	6.916037	1.493117	3.62E-06	0.002185	1.011752	0	0	12.41496	20.48514	97.47451

3H	106348764	LOC106410382	9.773498	6.723111	1.434112	2.76E-06	0.001767	0	0	0	12.41496	25.60643	20.61961
3H	106371532	LOC106400624	9.386759	6.665848	1.492898	8.01E-06	0.004563	0	0	0	30.55989	16.38811	9.372549
3H	106397232	LOC106397232	8.014369	6.436337	1.50417	1.88E-05	0.009275	0	0	0	8.594969	23.55791	15.93333
3H	106368687	LOC106415700	7.267954	6.294454	1.600159	8.37E-05	0.033131	0	0	0	5.729979	26.63068	11.24706
3H	106422534	LOC106422534	6.805755	6.201827	1.593724	9.97E-05	0.037873	0	0	0	22.91992	12.29108	5.623529
3H	106446503	LOC106446503	6.515517	6.141297	1.607635	0.000133	0.047077	0	0	0	12.41496	5.121285	21.55686
3H	106451236	LOC106451234	5.798102	5.971525	1.547698	0.000114	0.041127	0	0	0	9.549966	10.24257	14.99608
3H	106361605	LOC106375953	62.9524	5.730735	1.061849	6.78E-08	6.51E-05	2.023503	2.738864	2.199045	253.0741	6.145542	111.5333
3H	106375552	LOC106375500	7.966396	5.435762	1.428226	0.000141	0.049179	1.011752	0	0	17.18994	17.41237	12.18431
3H	106353789	LOC106353789	14.09174	5.340089	1.397134	0.000132	0.046999	1.011752	0	1.099523	29.60489	4.097028	48.73725
3H	106394749	LOC106394749	14.39988	4.792939	1.090176	1.10E-05	0.005945	1.011752	0.912955	1.099523	19.09993	17.41237	46.86275
3H	106381118	LOC106381118	23.5102	4.782578	1.075235	8.67E-06	0.004836	3.035255	1.825909	0	8.594969	78.86779	48.73725
3H	106388327	LOC106388327	113.1033	4.63904	0.81899	1.48E-08	1.60E-05	10.11752	7.303636	8.796181	517.6081	97.30442	37.4902
3H	106381160	LOC106381160	44.23423	4.170059	0.723365	8.18E-09	9.42E-06	6.070509	4.564773	3.298568	95.49966	26.63068	129.3412
3H	106427885	LOC106427885	19.83009	3.294613	0.690927	1.86E-06	0.001284	4.047006	3.651818	3.298568	49.65982	24.58217	33.74118
3H	106413711	LOC106413711	21.66141	3.242071	0.812266	6.57E-05	0.027703	6.070509	0	6.597136	42.01985	25.60643	49.67451
3H	111210294	LOC111210294	29.30337	3.183251	0.716832	8.97E-06	0.004948	10.11752	0.912955	6.597136	65.89476	60.43117	31.86667
3H	106368948	LOC106368948	34.93048	3.151653	0.811952	0.000104	0.038833	5.058758	13.69432	2.199045	116.5096	21.5094	50.61176
3H	106424238	LOC106424238	126.756	2.982315	0.69371	1.72E-05	0.008638	63.74035	6.390682	15.39332	136.5645	215.094	323.3529
3H	106392052	LOC106434136	28.55355	2.973968	0.776326	0.000128	0.04569	12.14102	1.825909	5.497613	40.10986	24.58217	87.16471
3H	106445852	LOC106361121	33.36831	2.81646	0.554342	3.76E-07	0.00031	12.14102	8.216591	4.398091	40.10986	70.67374	64.67059
3H	106366164	BNAA09G06210D	24.74882	2.762741	0.713087	0.000107	0.039139	12.14102	3.651818	3.298568	51.56981	22.53366	55.29804
3H	106381656	LOC106360826	18.95966	2.722686	0.64675	2.56E-05	0.012392	3.035255	6.390682	5.497613	42.97485	28.6792	27.18039
3H	106395264	LOC106395264	30.97803	2.700509	0.557068	1.25E-06	0.000875	7.082261	10.95545	6.597136	38.19986	47.11582	75.91765
3H	106376947	LOC106376947	58.92959	2.59948	0.682584	0.00014	0.049045	26.30554	12.78136	10.99523	193.8643	32.77623	76.8549
3H	106359517	LOC106359517	22.91464	2.558454	0.550027	3.30E-06	0.002059	5.058758	8.216591	6.597136	39.15486	40.97028	37.4902
3H	106388579	LOC106438781	81.04606	2.439141	0.490305	6.53E-07	0.000506	14.16452	45.64773	15.39332	147.0695	117.7896	146.2118
3H	106361053	LOC106361053	125.5291	2.28135	0.317205	6.38E-13	1.32E-09	41.48181	41.99591	45.08043	151.8445	225.3366	247.4353
3H	106416269	LOC106416269	62.73829	2.275846	0.4887	3.21E-06	0.00203	18.21153	14.60727	31.88616	93.58966	151.59	66.5451
3H	106443917	LOC106443917	37.34051	2.206854	0.425653	2.16E-07	0.000187	14.16452	14.60727	10.99523	57.29979	60.43117	66.5451
3H	106360694	LOC106360694	79.24243	2.182636	0.401642	5.50E-08	5.49E-05	36.42306	34.69227	14.29379	118.4196	141.3475	130.2784

3H	106361629	LOC106361629	128.9068	2.165297	0.410235	1.30E-07	0.000115	52.61108	40.17	48.379	281.724	110.6198	239.9373
3H	106447056	LOC106447056	293.1729	2.150412	0.396738	5.95E-08	5.83E-05	66.7756	149.7245	106.6537	601.6478	287.8162	546.4196
3H	106437473	LOC106347117	64.07176	2.080646	0.523211	6.99E-05	0.02877	16.18803	44.73477	12.09475	117.4646	109.5955	84.35294
3H	106405957	LOC106405957	97.29289	-2.02781	0.331481	9.51E-10	1.33E-06	118.3749	168.8966	181.4212	39.15486	44.04305	31.86667
3H	106454118	LOC106396863	46.95909	-2.29807	0.444775	2.38E-07	0.000202	91.05764	91.29545	51.67756	17.18994	17.41237	13.12157
3H	106405957	LOC106403221	46.44886	-2.39449	0.570187	2.68E-05	0.012732	44.51707	110.4675	79.16563	6.684976	15.36386	22.49412
3H	106418228	LOC106418228	72.92242	-2.50219	0.510181	9.37E-07	0.000684	117.3632	62.08091	192.4165	19.09993	29.70345	16.87059
3H	106370921	LOC106370921	44.15209	-2.78445	0.593509	2.71E-06	0.001767	102.1869	73.94932	54.97613	5.729979	21.5094	6.560784
3H	106413723	LOC106413723	38.23832	-4.64995	0.695739	2.33E-11	4.17E-08	52.61108	76.68818	91.26038	0	5.121285	3.74902
3H	106444185	LOC106444185	18.96944	-4.80658	1.098245	1.21E-05	0.006381	41.48181	58.42909	9.895704	0	3.072771	0.937255
3H	106446088	LOC106446088	21.48617	-4.98744	1.154889	1.57E-05	0.008095	16.18803	92.20841	16.49284	0.954997	3.072771	0
3H	106433989	LOC106433989	19.76034	-5.28968	1.050223	4.74E-07	0.000384	36.42306	27.38864	51.67756	0	3.072771	0
3H	111215764	LOC111215764	100.3655	-5.42693	0.585358	1.84E-20	9.55E-16	108.2574	254.7143	225.4021	4.774983	7.169799	1.87451
3H	111202271	LOC111202271	6.116809	-6.10208	1.606415	0.000146	0.049996	9.105764	20.99795	6.597136	0	0	0
3H	106383381	LOC106383381	7.421204	-6.37449	1.476335	1.58E-05	0.008095	12.14102	13.69432	18.69188	0	0	0
3H	106381316	LOC106381316	7.859377	-6.45856	1.490816	1.48E-05	0.007734	9.105764	18.25909	19.79141	0	0	0
3H	106348764	LOC106348764	8.689712	-6.6037	1.425283	3.60E-06	0.002185	17.19978	17.34614	17.59236	0	0	0
3H	106416340	LOC106416339	10.11654	-6.82736	1.510816	6.21E-06	0.003625	19.22328	33.77932	7.696659	0	0	0
3H	106425863	LOC106425863	24.34892	-7.12045	1.282437	2.82E-08	2.87E-05	54.63458	52.03841	38.48329	0	0	0.937255
3H	106419446	LOC106419446	28.0807	-7.32915	1.359585	7.02E-08	6.62E-05	37.43481	100.425	29.68711	0	0	0.937255
3H	106405964	LOC106405964	16.86479	-7.56192	1.340275	1.68E-08	1.78E-05	42.49357	35.60523	23.08998	0	0	0
3H	106411439	LOC106411439	19.13442	-7.73989	1.349582	9.75E-09	1.08E-05	27.31729	29.21454	58.2747	0	0	0
3H	106367691	LOC106367691	21.76581	-7.92932	1.294565	9.06E-10	1.31E-06	49.57583	44.73477	36.28425	0	0	0
3H	106371517	LOC106371517	30.59705	-8.41795	1.304986	1.11E-10	1.81E-07	40.47006	52.95136	90.16086	0	0	0
3H	106411442	LOC106411442	32.12596	-8.49192	1.273087	2.55E-11	4.41E-08	66.7756	77.60113	48.379	0	0	0
3H	106404375	LOC106404375	33.24368	-8.54183	1.351553	2.62E-10	3.99E-07	70.82261	102.2509	26.38854	0	0	0
3H	106401888	LOC106401888	50.82689	-9.1531	1.239625	1.54E-13	3.99E-10	96.1164	118.6841	90.16086	0	0	0
3H	106345789	LOC106345789	52.48475	-9.19935	1.295129	1.22E-12	2.44E-09	150.751	111.3805	52.77709	0	0	0
3H	111211109	LOC106347618	53.6203	-9.2303	1.238063	8.96E-14	2.45E-10	101.1752	125.9877	94.55895	0	0	0
3H	106422522	LOC106360545	59.34381	-9.37579	2.124012	1.01E-05	0.005536	95.10465	251.0625	9.895704	0	0	0
3H	106433976	LOC106433976	85.8783	-9.9076	1.350202	2.17E-13	4.89E-10	201.3386	45.64773	268.2835	0	0	0

3H	106367225	LOC106381822	88.80994	-9.95665	1.222794	3.87E-16	2.01E-12	205.3856	151.5504	175.9236	0	0	0
3H	106429960	LOC106367230	91.4838	-9.99868	1.234665	5.57E-16	2.41E-12	190.2093	133.2914	225.4021	0	0	0
3H	106446870	LOC106446870	92.86588	-10.0219	1.241224	6.79E-16	2.71E-12	124.4454	228.2386	204.5112	0	0	0
3H	111204650	LOC111204650	95.19612	-10.0575	1.327407	3.54E-14	1.17E-10	358.1601	130.5525	82.4642	0	0	0
3H	106380926	LOC106396518	100.5478	-10.1353	1.355678	7.65E-14	2.20E-10	65.76385	140.595	396.9277	0	0	0
3H	111211015	LOC106430858	146.5281	-10.6793	1.796216	2.76E-09	3.41E-06	99.15165	126.9007	653.1165	0	0	0
3H	106371451	LOC106371451	165.3908	-10.8542	1.234863	1.50E-18	1.94E-14	211.4561	391.6575	389.231	0	0	0
3H	106425457	LOC106437465	172.9925	-10.9191	1.246019	1.90E-18	1.97E-14	197.2916	432.7404	407.9229	0	0	0

Note: R = resistant; S = susceptible; R1-3 and S1-3 represent replicates 1-3 of R and S bulks.

Table S4.4. GO terms significantly (FDR < 0.05) enriched with differentially expressed genes identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Pathotype	GO Term	Pathway	Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Genes
3A	GO:0009744	Response to sucrose	0.042054835	1	3	461.3699	BnaAnng38970D
3A	GO:0034285	Response to disaccharide	0.042054835	1	3	461.3699	BnaAnng38970D
3A	GO:0015802	Basic amino acid transport	0.044236717	1	5	276.8219	BnaA06g35110D
3A	GO:0015808	L-alanine transport	0.044236717	1	5	276.8219	BnaA06g35110D
3A	GO:0015809	Arginine transport	0.044236717	1	5	276.8219	BnaA06g35110D
3A	GO:0015812	Gamma-aminobutyric acid transport	0.044236717	1	5	276.8219	BnaA06g35110D
3A	GO:0032328	Alanine transport	0.044236717	1	5	276.8219	BnaA06g35110D
3A	GO:1903826	Arginine transmembrane transport	0.044236717	1	5	276.8219	BnaA06g35110D
3A	GO:1990822	Basic amino acid transmembrane transport	0.044236717	1	5	276.8219	BnaA06g35110D
3A	GO:0006026	Aminoglycan catabolic process	0.032526595	2	60	46.13699	BnaA09g15440D BnaC03g24270D
3A	GO:0006030	Chitin metabolic process	0.032526595	2	60	46.13699	BnaA09g15440D BnaC03g24270D
3A	GO:0006032	Chitin catabolic process	0.032526595	2	60	46.13699	BnaA09g15440D BnaC03g24270D
3A	GO:0046348	Amino sugar catabolic process	0.032526595	2	60	46.13699	BnaA09g15440D BnaC03g24270D
3A	GO:1901072	Glucosamine-containing compound catabolic process	0.032526595	2	60	46.13699	BnaA09g15440D BnaC03g24270D
3A	GO:0006022	Aminoglycan metabolic process	0.032526595	2	63	43.93999	BnaA09g15440D BnaC03g24270D
3A	GO:0016998	Cell wall macromolecule catabolic process	0.032970637	2	71	38.989	BnaA09g15440D BnaC03g24270D
3A	GO:1901071	Glucosamine-containing compound metabolic process	0.032970637	2	72	38.44749	BnaA09g15440D BnaC03g24270D
3A	GO:0006040	Amino sugar metabolic process	0.042054835	2	87	31.81861	BnaA09g15440D BnaC03g24270D
3A	GO:0006952	Defense response	0.032526595	5	897	7.715215	BnaCnng78360D BnaCnng51700D BnaA01g31910D BnaA01g13350D BnaA03g38630D
3D	GO:0019509	L-methionine salvage from methylthioadenosine	0.026744204	1	12	647.6923	BnaA01g19100D
3D	GO:0043102	Amino acid salvage	0.026744204	1	12	647.6923	BnaA01g19100D
3D	GO:0071267	L-methionine salvage	0.026744204	1	12	647.6923	BnaA01g19100D
3D	GO:0071265	L-methionine biosynthetic process	0.028407282	1	17	457.1946	BnaA01g19100D
3D	GO:0006122	Mitochondrial electron transport, ubiquinol to cytochrome c	0.039277701	1	32	242.8846	BnaA08g12190D
3D	GO:0051017	Actin filament bundle assembly	0.039277701	1	50	155.4462	BnaC08g14110D
3D	GO:0061572	Actin filament bundle organization	0.039277701	1	50	155.4462	BnaC08g14110D
3D	GO:0009086	Methionine biosynthetic process	0.039277701	1	52	149.4675	BnaA01g19100D

3D	GO:0006555	Methionine metabolic process	0.039277701	1	53	146.6473	BnaA01g19100D
3D	GO:0003333	Amino acid transmembrane transport	0.039831711	1	79	98.38364	BnaA05g32770D
3D	GO:0042775	Mitochondrial ATP synthesis coupled electron transport	0.039831711	1	89	87.3293	BnaA08g12190D
3D	GO:0019646	Aerobic electron transport chain	0.039831711	1	90	86.35897	BnaA08g12190D
3D	GO:0000097	Sulfur amino acid biosynthetic process	0.039831711	1	103	75.4593	BnaA01g19100D
3D	GO:0009067	Aspartate family amino acid biosynthetic process	0.039831711	1	103	75.4593	BnaA01g19100D
3D	GO:1905039	Carboxylic acid transmembrane transport	0.039831711	1	105	74.02198	BnaA05g32770D
3D	GO:1903825	Organic acid transmembrane transport	0.039831711	1	106	73.32366	BnaA05g32770D
3D	GO:0042773	ATP synthesis coupled electron transport	0.039831711	1	111	70.02079	BnaA08g12190D
3D	GO:0000096	Sulfur amino acid metabolic process	0.039831711	1	118	65.86701	BnaA01g19100D
3D	GO:0043094	Cellular metabolic compound salvage	0.039831711	1	120	64.76923	BnaA01g19100D
3D	GO:0009066	Aspartate family amino acid metabolic process	0.039831711	1	123	63.18949	BnaA01g19100D
3D	GO:0006119	Oxidative phosphorylation	0.039831711	1	128	60.72115	BnaA08g12190D
3D	GO:0022904	Respiratory electron transport chain	0.039831711	1	132	58.88112	BnaA08g12190D
3H	GO:0034414	TRNA 3 -trailer cleavage, endonucleolytic	0.009162855	1	3	1981.176	BnaC05g37130D
3H	GO:0072684	Mitochondrial tRNA 3 -trailer cleavage, endonucleolytic	0.009162855	1	3	1981.176	BnaC05g37130D
3H	GO:1905267	Endonucleolytic cleavage involved in tRNA processing	0.009162855	1	3	1981.176	BnaC05g37130D
3H	GO:0098734	Macromolecule depalmitoylation	0.013096261	1	7	849.0756	BnaC06g42800D
3H	GO:0042779	TRNA 3 -trailer cleavage	0.013601685	1	9	660.3922	BnaC05g37130D
3H	GO:0042780	TRNA 3 -end processing	0.018407995	1	16	371.4706	BnaC05g37130D
3H	GO:0000963	Mitochondrial RNA processing	0.018940396	1	19	312.8173	BnaC05g37130D
3H	GO:0036092	Phosphatidylinositol-3-phosphate biosynthetic process	0.021586734	1	24	247.6471	BnaA07g10360D
3H	GO:0046686	Response to cadmium ion	0.021586734	1	26	228.5973	BnaC01g39170D
3H	GO:0046855	Inositol phosphate dephosphorylation	0.025252877	1	36	165.098	BnaC09g30760D
3H	GO:0071545	Inositol phosphate catabolic process	0.025252877	1	36	165.098	BnaC09g30760D
3H	GO:0046838	Phosphorylated carbohydrate dephosphorylation	0.025252877	1	39	152.3982	BnaC09g30760D
3H	GO:0000103	Sulfate assimilation	0.025252877	1	43	138.2216	BnaC09g30760D
3H	GO:0046854	Phosphatidylinositol phosphate biosynthetic process	0.009162855	2	89	133.5625	BnaC09g30760D BnaA07g10360D
3H	GO:0000463	Maturation of LSU-rRNA from tricistronic rRNA transcript (SSU-rRNA, 5.8S rRNA, LSU-rRNA)	0.025252877	1	48	123.8235	BnaA06g35050D
3H	GO:0010038	Response to metal ion	0.025252877	1	53	112.1421	BnaC01g39170D
3H	GO:0000959	Mitochondrial RNA metabolic process	0.025252877	1	57	104.2724	BnaC05g37130D

3H	GO:0046174	Polyol catabolic process	0.025252877	1	58	102.4746	BnaC09g30760D
3H	GO:0006026	Aminoglycan catabolic process	0.025252877	1	60	99.05882	BnaC03g24270D
3H	GO:0006030	Chitin metabolic process	0.025252877	1	60	99.05882	BnaC03g24270D
3H	GO:0006032	Chitin catabolic process	0.025252877	1	60	99.05882	BnaC03g24270D
3H	GO:0046348	Amino sugar catabolic process	0.025252877	1	60	99.05882	BnaC03g24270D
3H	GO:1901072	Glucosamine-containing compound catabolic process	0.025252877	1	60	99.05882	BnaC03g24270D
3H	GO:0043628	NcRNA 3 -end processing	0.025252877	1	61	97.43491	BnaC05g37130D
3H	GO:0046164	Alcohol catabolic process	0.025252877	1	61	97.43491	BnaC09g30760D
3H	GO:0006022	Aminoglycan metabolic process	0.02537194	1	63	94.34174	BnaC03g24270D
3H	GO:1901616	Organic hydroxy compound catabolic process	0.025591024	1	67	88.70939	BnaC09g30760D
3H	GO:0016998	Cell wall macromolecule catabolic process	0.026148959	1	71	83.71168	BnaC03g24270D
3H	GO:1901071	Glucosamine-containing compound metabolic process	0.026148959	1	72	82.54902	BnaC03g24270D
3H	GO:0098732	Macromolecule deacylation	0.026231282	1	74	80.31797	BnaC06g42800D
3H	GO:0006040	Amino sugar metabolic process	0.028753948	1	87	68.31643	BnaC03g24270D
3H	GO:0043647	Inositol phosphate metabolic process	0.028753948	1	87	68.31643	BnaC09g30760D
3H	GO:0046856	Phosphatidylinositol dephosphorylation	0.029415069	1	92	64.60358	BnaA07g10360D
3H	GO:0046839	Phospholipid dephosphorylation	0.029415069	1	93	63.90892	BnaA07g10360D
3H	GO:0006661	Phosphatidylinositol biosynthetic process	0.009162855	2	199	59.73396	BnaC09g30760D BnaA07g10360D
3H	GO:0140053	Mitochondrial gene expression	0.031567109	1	102	58.2699	BnaC05g37130D
3H	GO:0000470	Maturation of LSU-rRNA	0.032071829	1	108	55.03268	BnaA06g35050D
3H	GO:0046434	Organophosphate catabolic process	0.036055584	1	124	47.93169	BnaC09g30760D
3H	GO:0009737	Response to abscisic acid	0.039122303	1	140	42.45378	BnaCnng27950D
3H	GO:0097305	Response to alcohol	0.039122303	1	140	42.45378	BnaCnng27950D
3H	GO:0046474	Glycerophospholipid biosynthetic process	0.013096261	2	290	40.98986	BnaC09g30760D BnaA07g10360D
3H	GO:0046488	Phosphatidylinositol metabolic process	0.013096261	2	300	39.62353	BnaC09g30760D BnaA07g10360D
3H	GO:0031123	RNA 3 -end processing	0.042190937	1	154	38.59435	BnaC05g37130D
3H	GO:0045017	Glycerolipid biosynthetic process	0.013601685	2	345	34.45524	BnaC09g30760D BnaA07g10360D
3H	GO:1901136	Carbohydrate derivative catabolic process	0.046935514	1	178	33.39061	BnaC03g24270D
3H	GO:0019751	Polyol metabolic process	0.048326357	1	190	31.28173	BnaC09g30760D
3H	GO:0008654	Phospholipid biosynthetic process	0.015657267	2	389	30.55799	BnaC09g30760D BnaA07g10360D
3H	GO:0030258	Lipid modification	0.04996822	1	200	29.71765	BnaA07g10360D

3H	GO:0006650	Glycerophospholipid metabolic process	0.016839337	2	422	28.16839	BnaC09g30760D BnaA07g10360D
3H	GO:0046486	Glycerolipid metabolic process	0.018876312	2	484	24.56004	BnaC09g30760D BnaA07g10360D
3H	GO:0006644	Phospholipid metabolic process	0.021586734	2	581	20.45965	BnaC09g30760D BnaA07g10360D
3H	GO:0034470	NcRNA processing	0.025252877	2	822	14.46114	BnaA06g35050D BnaC05g37130D
3H	GO:0006790	Sulfur compound metabolic process	0.025252877	2	839	14.16813	BnaC09g30760D BnaC01g39170D
3H	GO:0016311	Dephosphorylation	0.025252877	2	909	13.07707	BnaC09g30760D BnaA07g10360D
3H	GO:0090407	Organophosphate biosynthetic process	0.025410067	2	946	12.5656	BnaC09g30760D BnaA07g10360D
3H	GO:0034660	NcRNA metabolic process	0.028753948	2	1082	10.98619	BnaA06g35050D BnaC05g37130D
3H	GO:0008610	Lipid biosynthetic process	0.032071829	2	1236	9.617362	BnaC09g30760D BnaA07g10360D
3H	GO:0044255	Cellular lipid metabolic process	0.04222966	2	1508	7.882665	BnaC09g30760D BnaA07g10360D
3H	GO:0019637	Organophosphate metabolic process	0.046937305	2	1628	7.301633	BnaC09g30760D BnaA07g10360D

Table S4.5. KEGG pathways significantly (FDR < 0.05) enriched with differentially expressed genes identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H.

Pathotype	KEGG ID	Pathway	Enrichment FDR	Number of Genes	Pathway Genes	Fold Enrichment	Genes
3A	bna00196	Photosynthesis	0.027557573	1	15	92.27397	BnaA07g07560D
3A	bna00052	Galactose metabolism	0.0379214	1	23	60.17868	BnaC05g09640D
3A	bna00480	Glutathione metabolism	0.006323165	2	61	45.38064	BnaC01g39170D BnaA03g14140D
3A	bna00520	Amino sugar and nucleotide sugar metabolism	0.006323165	2	62	44.6487	BnaA09g15440D BnaC03g24270D
3A	bna04130	SNARE interactions in vesicular transport	0.047810688	1	32	43.25342	BnaA08g25060D
3A	bna00260	Glycine, serine and threonine metabolism	0.047884159	1	35	39.54599	BnaA03g50990D
3A	bna00240	Pyrimidine metabolism	0.049130079	1	39	35.48999	BnaA09g54360D
3A	bna00330	Arginine and proline metabolism	0.049130079	1	42	32.95499	BnaA05g05760D
3A	bna04141	Protein processing in endoplasmic reticulum	0.01007829	2	102	27.1394	BnaC01g20320D BnaC03g28860D
3A	bna04626	Plant-pathogen interaction	0.01007829	2	104	26.61749	BnaA09g44420D BnaA03g38630D
3A	bna01230	Biosynthesis of amino acids	0.015863243	2	142	19.4945	BnaA03g50990D BnaA05g05760D
3A	bna01100	Metabolic pathways	1.46E-09	12	1189	13.96915	BnaC01g39170D BnaA06g01500D BnaC05g43940D BnaC05g09640D BnaA09g08530D BnaA09g15440D BnaA09g54360D BnaA07g07560D BnaC03g24270D BnaA03g50990D BnaA05g05760D BnaA03g14140D
3A	bna03010	Ribosome	0.027557573	2	210	13.182	BnaA04g21290D BnaA08g08550D
3A	bna01110	Biosynthesis of secondary metabolites	0.006323165	4	621	8.91536	BnaA06g01500D BnaC05g09640D BnaA03g50990D BnaA05g05760D
3D	bna03010	Ribosome	1.99E-05	3	210	111.033	BnaA09g38730D BnaA07g32060D BnaC01g04410D
3D	bna00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0.003771842	1	11	706.5734	BnaA08g11640D
3D	bna00941	Flavonoid biosynthesis	0.004370351	1	17	457.1946	BnaA08g11640D
3D	bna00270	Cysteine and methionine metabolism	0.013319824	1	69	112.6421	BnaA01g19100D
3D	bna00940	Phenylpropanoid biosynthesis	0.013319824	1	78	99.64497	BnaA08g11640D
3D	bna00190	Oxidative phosphorylation	0.015199198	1	104	74.73373	BnaA08g12190D
3D	bna01100	Metabolic pathways	0.001702576	3	1189	19.61053	BnaA08g11640D BnaA08g12190D BnaA01g19100D
3H	bna01100	Metabolic pathways	5.93E-09	7	1189	34.99134	BnaC09g30760D BnaA08g11640D BnaC01g39170D BnaA07g10360D BnaC06g42800D BnaA01g17980D BnaC03g24270D

3H	bna00945	Stilbenoid, diarylheptanoid and gingerol biosynthesis	0.006780721	1	11	540.3209	BnaA08g11640D
3H	bna00941	Flavonoid biosynthesis	0.00785574	1	17	349.6194	BnaA08g11640D
3H	bna00562	Inositol phosphate metabolism	0.009604884	1	26	228.5973	BnaA07g10360D
3H	bna01212	Fatty acid metabolism	0.011331176	1	38	156.4087	BnaC06g42800D
3H	bna00230	Purine metabolism	0.011331176	1	43	138.2216	BnaA01g17980D
3H	bna00480	Glutathione metabolism	0.01268823	1	61	97.43491	BnaC01g39170D
3H	bna00520	Amino sugar and nucleotide sugar metabolism	0.01268823	1	62	95.86338	BnaC03g24270D
3H	bna00940	Phenylpropanoid biosynthesis	0.014348187	1	78	76.1991	BnaA08g11640D
3H	bna03010	Ribosome	0.003150275	2	210	56.60504	BnaA06g35050D BnaA10g11350D

Table S4.6. QTLs and single nucleotide polymorphisms (SNPs) identified from resistant and susceptible bulks of F_2 plants inoculated with *Plasmodiophora brassicae* pathotypes 3A, 3D and 3H. QTLs are distinguished using different colours, whereas multiple nomenclatures marked with the same colour represent the same QTL identified from bulks of plants inoculated with different pathotypes.

Patho type	CHR	QTL	Type	start	end	length	Number of SNPs	Peak ΔSNP	CHR_POS	REF	ALT	ΔSNP	Gene ID	Gene Name
3D	**A01**	*BnaA1P3D.CRX1.1*	**Minor**	**14059868**	**14060851**	983	8	**0.290**	A01_14059868	G	A	0.322917	106361131	cilia- and flagella-associated protein 251
									A01_14059889	T	C	0.336691	106361131	cilia- and flagella-associated protein 251
									A01_14060296	A	G	0.529978	106361131	cilia- and flagella-associated protein 251
									A01_14060344	T	C	0.479668	106361131	cilia- and flagella-associated protein 251
									A01_14060350	A	C	0.44295	106361131	cilia- and flagella-associated protein 251
									A01_14060386	C	A	0.465459	106361131	cilia- and flagella-associated protein 251
									A01_14060389	C	A	0.447659	106361131	cilia- and flagella-associated protein 251
									A01_14060851	C	A	0.344068	106361131	cilia- and flagella-associated protein 251
3A	**A05**	***BnaA5P3A.CRX1.1***	**Major**	**12272166**	**12272166**	0	1	**0.385**	A05_12272166	A	C	0.3891	106362025	hsp70-Hsp90 organizing protein 3
3D	**A05**	*BnaA5P3D.CRX1.1*	**Minor**	**13623271**	**13623271**	0	1	**0.270**	A05_13623271	A	G	0.278006	106411554	proteasome subunit alpha type-4-A-like
3D	**A08**	***BnaA8P3D.CRX1.1***	**Major**	**7443712**	**7443835**	123	4	**0.346**	A08_7443712	A	C	0.335069	106396583	enoyl-CoA delta isomerase 2, peroxisomal
									A08_7443718	C	G	0.333032	106396583	enoyl-CoA delta isomerase 2, peroxisomal
									A08_7443781	G	A	0.382622	106396583	enoyl-CoA delta isomerase 2, peroxisomal
									A08_7443835	G	A	0.327308	106396583	enoyl-CoA delta isomerase 2, peroxisomal
3D	**A08**	***BnaA8P3D.RCr91.2***	**Major**	**10275090**	**11076201**	801111	3	**0.343**	A08_10275090	C	T	0.428556	106418916	transcription initiation factor TFIID subunit 1
									A08_11076144	C	A	0.499209	106418959	peptidyl-prolyl cis-trans isomerase CYP28, chloroplastic-like
									A08_11076201	T	C	0.431037	106418960	peptidyl-prolyl cis-trans isomerase CYP28, chloroplastic-like
3H	**A08**	***BnaA8P3H.RCr91.2***	**Major**	**11310428**	**12506566**	1196138	8	**0.426**	A08_11310428	C	T	0.143813	106390924	selenium-binding protein 1

									A08_11512210	C	T	0.525974	106397231	UDP-glycosyltransferase 75C1-like
									A08_11512231	G	A	0.532271	106397231	UDP-glycosyltransferase 75C1-like
									A08_11512264	G	A	0.529789	106397231	UDP-glycosyltransferase 75C1-like
									A08_11512516	C	G	0.506316	106397231	UDP-glycosyltransferase 75C1-like
									A08_12506476	T	G	0.294049	106422372	5-amino-6-(5-phospho-D-ribitylamino)uracil phosphatase, chloroplastic-like
									A08_12506482	A	T	0.289254	106422372	5-amino-6-(5-phospho-D-ribitylamino)uracil phosphatase, chloroplastic-like
									A08_12506566	G	C	0.334804	106422372	5-amino-6-(5-phospho-D-ribitylamino)uracil phosphatase, chloroplastic-like
3H	**A08**	***BnaA8P3H.Crr11.3***	**Major**	**15908289**	**16050956**	142667	6	**0.356**	A08_15908289	C	T	0.416168	106361033	ethylene-responsive transcription factor ERF109
									A08_16016873	C	T	0.383318	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016897	T	G	0.426056	106361045	UDP-glucosyl transferase 73B2-like
									A08_16040507	C	G	0.238857	106361049	polyubiquitin 11
									A08_16040588	A	G	0.348649	106361049	polyubiquitin 11
									A08_16050956	G	T	0.323655	106361048	polyadenylate-binding protein 2
3D	**A08**	***BnaA8P3D.Crr11.3***	**Major**	**16016864**	**16050956**	34092	12	**0.338**	A08_16016864	C	A	0.32254	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016867	T	A	0.313691	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016873	C	T	0.314286	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016882	T	C	0.268013	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016885	C	T	0.275083	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016888	T	C	0.299111	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016897	T	G	0.296263	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016909	G	A	0.369062	106361045	UDP-glucosyl transferase 73B2-like
									A08_16016912	C	G	0.358393	106361045	UDP-glucosyl transferase 73B2-like
									A08_16040507	C	G	0.433877	106361049	polyubiquitin 11
									A08_16040624	C	T	0.480007	106361049	polyubiquitin 11
									A08_16050956	G	T	0.416092	106361048	polyadenylate-binding protein 2

3D	**A08**	***BnaA8P3D.qBrCR381.4***	**Major**	**17871036**	**20312633**	2441597	39	**0.386**	A08_17871036	A	G	0.142409	106361356	probable beta-1,3-galactosyltransferase 4
									A08_17871142	G	A	-0.14903	106361356	probable beta-1,3-galactosyltransferase 4
									A08_17871156	T	A	0.18913	106361356	probable beta-1,3-galactosyltransferase 4
									A08_17871895	G	T	-0.07335	106361356	probable beta-1,3-galactosyltransferase 4
									A08_17882811	C	T	0.260847	106361356	probable beta-1,3-galactosyltransferase 4
									A08_18204273	G	C	0.416327	106361316	31 kDa ribonucleoprotein, chloroplastic
									A08_18281611	G	A	0.658778	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281614	G	A	0.645953	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281650	C	A	0.604275	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281659	A	C	0.611093	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281677	A	C	0.585807	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18286203	A	G	0.413374	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
									A08_18286206	G	T	0.418836	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
									A08_18286249	G	A	0.471861	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
									A08_18286401	G	A	0.464912	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
									A08_18346735	G	C	0.33628	106361295	aconitate hydratase 1
									A08_18441680	C	A	0.511803	106361282	phosphoserine aminotransferase 1, chloroplastic
									A08_18441743	T	C	0.407673	106361282	phosphoserine aminotransferase 1, chloroplastic
									A08_18441755	A	G	0.417602	106361282	phosphoserine aminotransferase 1, chloroplastic
									A08_18506249	C	T	-0.0277	106361274	50S ribosomal protein L25-like
									A08_18839333	A	G	0.53037	106405582	protein PLASTID MOVEMENT IMPAIRED 1
									A08_18839639	A	G	0.461583	106405582	protein PLASTID MOVEMENT IMPAIRED 1
									A08_18839835	A	C	0.470653	106405582	protein PLASTID MOVEMENT IMPAIRED 1
									A08_18839860	T	A	0.44824	106405582	protein PLASTID MOVEMENT IMPAIRED 1

									A08_18839873	C	T	0.427838	106405582	protein PLASTID MOVEMENT IMPAIRED 1
									A08_18872711	G	T	0.301851	106361258	bZIP transcription factor 60
									A08_18872843	A	G	0.291383	106361258	bZIP transcription factor 60
									A08_19040806	C	G	0.125156	106451550	GLABROUS1 enhancer-binding protein-like 1
									A08_19040824	G	C	0.142857	106451550	GLABROUS1 enhancer-binding protein-like 1
									A08_19040897	T	G	0.148792	106451550	GLABROUS1 enhancer-binding protein-like 1
									A08_19040960	A	G	0.114629	106451550	GLABROUS1 enhancer-binding protein-like 1
									A08_19366524	C	G	0.058586	106384864	uncharacterized LOC106384864
									A08_19366530	A	G	0.089843	106384865	uncharacterized LOC106384865
									A08_20016622	T	A	0.571968	106361389	NAD(P)H-quinone oxidoreductase subunit M, chloroplastic
									A08_20016645	T	G	0.542171	106361389	NAD(P)H-quinone oxidoreductase subunit M, chloroplastic
									A08_20016697	T	C	0.599501	106361389	NAD(P)H-quinone oxidoreductase subunit M, chloroplastic
									A08_20016914	G	A	0.305847	106361389	NAD(P)H-quinone oxidoreductase subunit M, chloroplastic
									A08_20101875	C	T	0.427928	106361389	NAD(P)H-quinone oxidoreductase subunit M, chloroplastic
									A08_20312633	T	A	0.319406	106361360	apoptotic chromatin condensation inducer in the nucleus
3H	**A08**	*BnaA8P3H.qBrCR381.4*	**Minor**	**18281327**	**18872996**	591669	34	**0.276**	A08_18281327	A	G	0.130488	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281569	C	G	0.233291	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281611	G	A	0.269875	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281614	G	A	0.262769	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281650	C	A	0.31722	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281659	A	C	0.31837	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281677	A	C	0.346226	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281716	G	A	0.643411	106361308	heat shock 70 kDa protein 6, chloroplastic-like
									A08_18281737	C	G	0.714286	106361308	heat shock 70 kDa protein 6, chloroplastic-like

A08_18281740	G	T	0.714286	106361308	heat shock 70 kDa protein 6, chloroplastic-like
A08_18281776	G	A	0.334326	106361308	heat shock 70 kDa protein 6, chloroplastic-like
A08_18281984	A	G	0.272727	106361308	heat shock 70 kDa protein 6, chloroplastic-like
A08_18286185	G	A	0.237711	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
A08_18286192	G	T	-0.25354	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
A08_18286200	A	T	0.251089	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
A08_18286203	A	G	0.259507	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
A08_18286206	G	T	0.258586	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
A08_18286249	G	A	0.233528	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
A08_18286401	G	A	0.19342	106361304	3-oxo-Delta(4,5)-steroid 5-beta-reductase-like
A08_18441680	C	A	0.257531	106361282	phosphoserine aminotransferase 1, chloroplastic
A08_18441743	T	C	0.31138	106361282	phosphoserine aminotransferase 1, chloroplastic
A08_18441755	A	G	0.26431	106361282	phosphoserine aminotransferase 1, chloroplastic
A08_18839165	T	C	0.198926	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18839297	C	T	0.318931	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18839333	A	G	0.318501	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18839639	A	G	0.3564	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18839835	A	C	0.361314	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18839860	T	A	0.361672	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18839873	C	T	0.357247	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18840122	G	C	0.288243	106405582	protein PLASTID MOVEMENT IMPAIRED 1
A08_18840155	C	G	0.265288	106405582	protein PLASTID MOVEMENT IMPAIRED 1

									A08_18840182	G	A	0.266355	106405582	protein PLASTID MOVEMENT IMPAIRED 1
									A08_18872711	G	T	0.318923	106361258	bZIP transcription factor 60
									A08_18872996	G	A	0.337833	106361258	bZIP transcription factor 60
3H	**C01**	*BnaC1P3H.CRX1.1*	**Minor**	**17677705**	**17678086**	381	4	**0.293**	C01_17677705	T	C	0.47906	106376056	GLABROUS1 enhancer-binding protein-like
									C01_17677753	A	G	0.422169	106376056	GLABROUS1 enhancer-binding protein-like
									C01_17677778	C	A	0.473746	106376056	GLABROUS1 enhancer-binding protein-like
									C01_17678086	G	T	0.444454	106376056	GLABROUS1 enhancer-binding protein-like
3H	**C01**	***BnaC1P3H.CRX1.2***	**Major**	**26237303**	**26648486**	411183	4	**0.322**	C01_26237303	T	A	0.314815	106349084	polyubiquitin 11
									C01_26237923	A	G	0.309984	106349084	polyubiquitin 11
									C01_26237959	C	T	0.306119	106349084	polyubiquitin 11
									C01_26648486	A	G	0.335025	106349049	uncharacterized LOC106349049
3H	**C01**	*BnaC1P3H.CRX1.3*	**Minor**	**42700048**	**42700048**	0	1	**0.293**	C01_42700048	T	C	0.401852	111202359	60S ribosomal protein L27-3
3D	**C07**	*BnaC7P3D.CRX1.1*	**Minor**	**34501975**	**34509210**	7235	2	**0.277**	C07_34501975	C	T	0.190992	106418155	serine/threonine-protein kinase prp4
									C07_34509210	C	A	0.39529	106418157	uncharacterized LOC106418157
3A	**C07**	***BnaC7P3A.CRX1.0***	**Major**	**45670744**	**46185588**	514844	5	**0.530**	C07_45670744	T	G	-0.03105	106406248	60S ribosomal protein L26-1
									C07_45670795	A	G	-0.03431	106406248	60S ribosomal protein L26-1
									C07_46185519	A	C	0.78169	111208271	alpha-humulene/(-)-(E)-beta-caryophyllene synthase-like
									C07_46185523	T	G	0.786207	111208271	alpha-humulene/(-)-(E)-beta-caryophyllene synthase-like
									C07_46185588	C	T	0.745098	111208271	alpha-humulene/(-)-(E)-beta-caryophyllene synthase-like
3D	**C08**	*BnaC8P3D.CRX1.1*	**Minor**	**6001383**	**6001383**	0	1	**0.274**	C08_6001383	T	C	0.274324	NA	
3D	**C09**	*BnaC9P3D.CRX1.1*	**Minor**	**31610782**	**31611253**	471	6	**0.234**	C09_31610782	A	T	0.272549	106418720	vicilin-like seed storage protein At2g18540
									C09_31610837	A	C	0.282667	106418720	vicilin-like seed storage protein At2g18540
									C09_31610859	G	C	0.274913	106418720	vicilin-like seed storage protein At2g18540
									C09_31610878	A	G	0.204412	106418720	vicilin-like seed storage protein At2g18540
									C09_31611051	T	C	0.167072	106418720	vicilin-like seed storage protein At2g18540
									C09_31611253	G	C	0.118079	106418720	vicilin-like seed storage protein At2g18540

3H	**C09**	*BnaC9P3H.CRX1.1*	**Minor**	**31610782**	**32261061**	650279	8	**0.261**	C09_31610782	A	T	0.213363	106418720	vicilin-like seed storage protein At2g18540
									C09_31610837	A	C	0.230161	106418720	vicilin-like seed storage protein At2g18540
									C09_31610859	G	C	0.258899	106418720	vicilin-like seed storage protein At2g18540
									C09_31610878	A	G	0.289958	106418720	vicilin-like seed storage protein At2g18540
									C09_31610911	G	C	0.320291	106418720	vicilin-like seed storage protein At2g18540
									C09_31611051	T	C	0.313389	106418720	vicilin-like seed storage protein At2g18540
									C09_31611253	G	C	0.253349	106418720	vicilin-like seed storage protein At2g18540
									C09_32261061	T	C	0.34833	106392952	uncharacterized LOC106392952

Note: CHR = chromosome; POS = position; REF = the allele in the reference genome at the locus of a SNP. ALT = any other allele found at the locus of a SNP.