IDENTIFICATION OF *BRASSICA NAPUS* L. SOURCES OF RESISTANCE AGAINST BLACKLEG (*LEPTOSPHAERIA MACULANS*)

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Blackleg, caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. et de Not. [anamorph = *Phoma lingam* (Tode: Fr.) Desm.] has become the most important disease affecting canola around the world. A study was conducted to identify sources of resistance to *L. maculans* in a collection of *B. napus* plant introduction accessions. Approximately, 5% of accessions showed highly resistance (median severity <3) reaction to the *L. maculans* PG-4 under the greenhouse conditions and three of them performed better than commercial hybrids in the field conditions. At the same time, DNA extracted and genome-wide association study (GWAS) was done for 213 and 78 accessions for PG-4 and PG-3. The 0.1 and 0.01 percentile tails of an empirical distribution, obtained from 5,000 bootstraps, was used to determine the cut off *P*-value to identify significant markers. Finally, 10 and 26 significant single nucleotide polymorphism (SNP) markers associated with resistance to PG-4 and PG-3, respectively. These markers were located across 14 chromosomes (A01, A02, A03, A04, A05, A07, A08, A10, C03, C04, C05, C07, C08 and C09) out of *B. napus* 19 chromosomes. These markers were validated under field conditions. With exploring flanking region of each significant marker eight candidate genes were identified which involved in plant defense family such as defensing and leucine-rich repeat and serine-threonine protein kinase protein. To infer the presence of R genes in commercial canola hybrids, elite *B. napus* plant introduction materials, and elite canola breeding lines, they were inoculated with different *L. maculans* races. The results showed that, resistance gene *Rlm9* was present in 18% of the genotypes evaluated; *Rlm2* and *Rlm3* were each present in 16% of them, while *LepR1*, *Rlm4* and *Rlm5* with present in 11, 5, and 5% each, respectively. However, we were not able to infer R gene(s) on 29% of the genotypes evaluated. Approximately 18% of genotypes were susceptible to all the races used. The hybrids with different R genes could use for hybrid rotation.

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DEDICATION

This dissertation is dedicated to my parents, who raised me to be the person I am today; to my lovely wife Nakisa whose encouragement, support, and understanding have made it possible for me to finish this work. Thank you all for your unconditional love that helped me succeed and instilled in me the confidence that I am capable of achieving goals that I sought for.

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CHAPTER 1. INTRODUCTION

Canola (*Brassica napus* L.) is an oilseed crop grown for its oil content. Modern canola germplasm has been bred to decrease its erucic acid and glucosinolates contents, which are toxic to human. Canola oil is the third most important vegetable oil after soybean and palm oil (FAO 2017). Canola oil is highly desirable because it has high levels of unsaturated fats and omega-3 fatty acids. The world's top producers of canola are Canada, China, India, France, Germany, Australia, Poland, United Kingdom and the United States of America (FAO 2017). In the US, North Dakota is the leading producer of canola (USDA-NASS 2017).

Blackleg is a disease caused by the ascomycetes fungus *Leptosphaeria maculans* (Desmaz) Ces. & de Not (anamorph = *Phoma lingam* (Tode:Fr.) Desmaz.) that affects canola production worldwide and is becoming the most important disease that affects canola production in North Dakota (del Rio Mendoza et al. 2012). A recent taxonomic review of the order Pleosporales using a molecular phylogenetic approach, renamed its anamorph stage, *Phoma lingam*, as *Plenodomus lingam* (Tode : Fr.) Hohn.,(de Guyter et al. 2013) but did not alter the name of the teleomorph stage. This pathogen is hemibiotrophic and its host range is limited to plants in the botanical family Brassicaceae. During the initial contact with the plant, it kills tissues forming visible lesions where it can produce pycnidia. Soon after, however, it starts growing into the stems and downwards through the vascular system without causing further visible symptoms. Once it reaches the crown area, it resumes its necrotrophic phase causing stem cankers and destroying the root tissues (Guo, 2004).

Isolates from this pathogen cause a wide range of reactions on canola plants. Early efforts to classify these isolates based on these reactions, separated them in two groups, A and B. Isolates in group A were considered more virulent and aggressive than those in group B (Howlett et al.

2001). In 1991, *L. maculans* isolates were classified into four pathogenicity groups (PG) 1, 2, 3, and 4, based on the reaction they evoked on three *B. napus* differentials, Westar, Quinta and Glacier (Mengistu et al. 1991). A fifth group, PG-T, was added in 2006 (Rimmer, 2006). An alternative classification which added "Jet Neuf" to the differential set, classified the isolates in six groups (A1-A6) (Badawy et al. 1991). More recently, the discovery and characterization of avirulence genes in *L. maculans*, *AvrLm*, confirmed the existence of physiological races of this pathogen (Balesdent et al. 2005). Following the gene-for-gene theory, the presence of major dominant resistance genes in the plant can be inferred by identifying the avirulence genes present in the pathogen (Rouxel and Balesdent 2005; Kutcher et al. 2008; Rouxel et al. 2003). The PG-4 strains become more prevalent in North Dakota, the possibilities of severe blackleg outbreaks increase (Nepal et al. 2014) and all of commercial cultivars are susceptible to it (Marino 2011). To date, 15 avirulence genes have been identified in *L. maculans* and since each race could have multiple avirulence genes, is possible to have in theory, up to 32,767 races for this pathogen. Eight avirulence genes have been cloned so far. Resistance against specific races is qualitative in nature and has proven to be very effective; however, relying almost exclusively on it is not advisable since this pathogen has shown an extraordinary ability to overcome qualitative resistance genes (Sprague et al. 2006). Quantitative resistance on the other hand, may not provide complete protection to the plants but when used in combination with qualitative resistance genes it increases their durability (Brun et al. 2010).

Genome-Wide association study (GWAS) is a powerful technique that identifies markers like single-nucleotide polymorphisms that are associated with diverse quantitative traits (Korte and Farlow, 2013) including plant diseases (Raman et al. 2016; Rahman et al. 2016) in highly genetically-diverse populations. GWAS has been used in world-wide germplasm collections like

the *B. napus* collection curated by the USDA-National Plant Germplasm System (NPGS) which are genetically diverse (Diers and Osborn, 1994) and may harbor resistance genes to many diseases that affect canola such as Sclerotinia stem rot (SSR) (Khot et al. 2011) but can also be used on collections made of breeding lines produced by different programs (Gao et al. 2016). While GWAS is a powerful tool, it is not free of limitations. The presence of significant levels of population relatedness (Kinship) due to similar genetic background could increase the possibility of obtaining false-positive results. Many statistical models such as mixed model or efficient mixed-model association (EMMA) have been developed to help identify and neutralize to a point this issue (Stich et al. 2008; Kang et al. 2008; Yu et al. 2006).

The objectives of this study were:

- Identify source(s) of resistance to *L. maculans* in a collection of *B. napus* plant introduction accessions.
- Identify and validate markers associated with resistance to *L. maculans*.
- Characterize the reaction of *B. napus* commercial hybrids, advanced breeding lines and elite plant introduction accessions to several races of *L. maculans*.

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CHAPTER 2. LITERATURE REVIEW

The Host: Canola (*Brassica napus*)

Canola (*Brassica napus*), also known as oilseed rape, belongs to the Brassicaceae family which has 338 genera and 3709 species (LeCoz and Ducombs 2006). It is an economically important oilseed around the world (Rakow 2004). Canola was developed by the natural hybridization of turnip rape (*B. rapa* 2n =2x = 20, AA) and cabbage (*B. oleracea* 2n = 2x = 18, CC) that took place between 10,000 and 100,000 years ago in southern Europe (Olsson 1960). The first use of canola has been recorded as early as 2000 BC in India and since the 13th century in Europe. The relationships among *Brassica* species were elucidated by Dr. U in 1936 (Fig. 2.1).

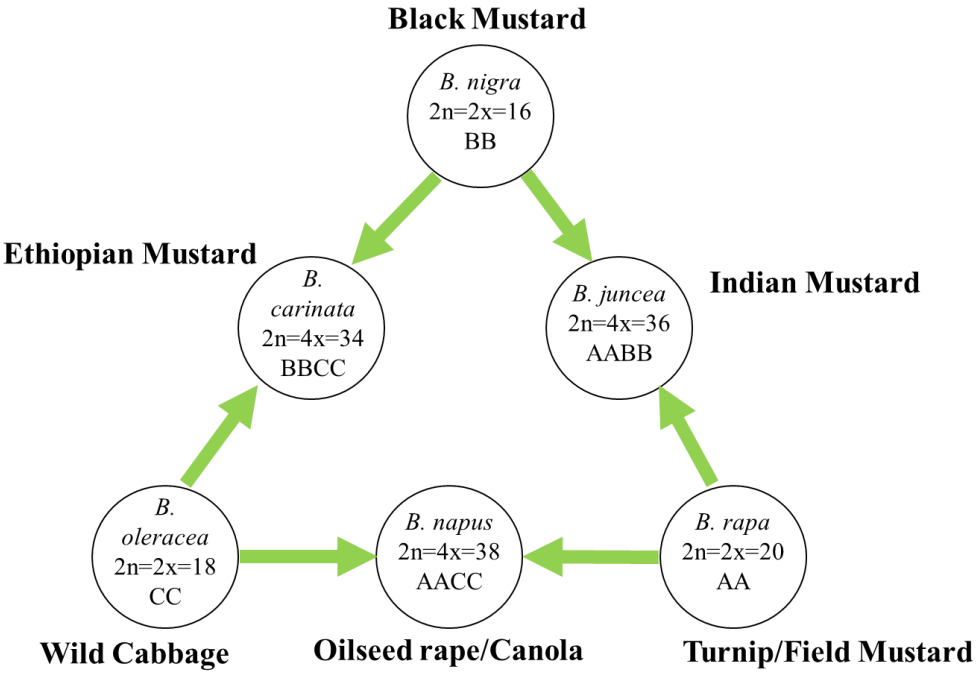

Figure 2.1. Triangle of U shows genetic relations among *Brassica* spp. (U 1935)

According to U, three diploid species *B. rapa* (A genome), *B. nigra* (B genome), and *B. oleracea* (C genome) produced, of natural hybridization *B. napus* (AC genome; *B. rapa* x *B. oleracea*), *B. juncea* (AB genome; *B. rapa* x *B. nigra*), and *B. carinata* (BC genome; *B. nigra* x *B. oleracea*). *B. napus* is mainly cultivated in major canola growing regions around the world. *B.*

rapa, one of the parents of *B. napus*, is believed to have originated from regions close to the Mediterranean Sea (Tsunoda 1980) and then moved to eastern Europe and Germany (Nishi 1980). *B. rapa* has a shorter life cycle than many other Brassicaceae (Colton and Potter 1999) but still was grown as oilseed rape in Europe in both spring and winter format. The other *B. napus* parent, *B. oleracea* L., comprises many important vegetable crops including cauliflower, broccoli, cabbages, Brussels sprouts, kohlrabi and kales (Liu et al. 2014). *B. oleracea* is found on the coasts of northern Spain, western France and southern and southwestern Britain. It is a perennial species with a strong vegetative stock, which develops over several years before it starts flowering. It has glabrous leaves which have a grayish surface. Snogerup (1980) divided this species into six groups: kales (var. acephala) which includes green kale, marrow stem kale and collards mainly used for edible forage; cabbages (var. *capitata*, *sabauda* and *bullata*) which include headed cabbages, Brussels sprouts, savoy cabbage, and others; kohlrabi (var. *gongylodes*); inflorescence kales (var. *botrytis* and *italica*) which include cauliflower, broccoli, sprouting broccoli, and others; branching bush kales (var. *fruticosa*); and Chinese kale (var. *alboglabra*).

The term "Canola" refers to Canadian oil low acid (low erucic acid and glucosinolate which are toxic to human) rape that was developed by Drs. Keith Downey and Baldur Stefansson from Agriculture and Agri-Food Canada and the University of Manitoba in 1974, using traditional plant breeding techniques. By definition, canola seeds should contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-entenyl glucosinolate, 2-hydroxy-3butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dried, oil-free solid (Brown et al. 2008).

Canola oil has become the third vegetable edible oil after soybean and palm oil. Canola equivalents that are cultivated extensively in India, China, Canada, Australia and Europe including France, Germany and Poland are known as oilseed rape 00 or double-0 rape (USDA-ERS 2017).

Canola has been planted in the US since 1988. The United States Department of Agriculture estimated at around 1.7 million acres the area planted to canola in 2016; that area produced an average yield of 1824 pounds per acre which accounts for 3 billion pounds of canola production. The harvest was valued at US$436 million and represented 87% of the United States canola production (USDA-NASS 2017).

Importance of Canola

Canola oil which is obtained by crushing canola seeds is the third most produced vegetable oil after soybean and palm oil (USDA-NASS 2017). The value of U.S. canola production was $493 million in 2016 (USDA-NASS 2017). Canola oil is used in frying and baking applications, and is an ingredient in salad dressings, margarine, and a variety of other products. Canola oil has a low percentage of saturated fat and high oleic acid; the latter makes this oil good for high temperature frying. Another application of canola oil is in biodiesel production which is mainly used in Europe. After oil extraction, the leftover canola meal is used as animal feed. The canola meal is the second largest feed after soybean meal. Canola meal has a lower protein content than soybean meal.

Canola Production

The canola production areas located far from equator that have dry condition and short growing season. Winter canola is planted from September to November in European countries, Ukraine, Russia and parts of China. In these regions, the winter temperatures are not cold enough to kill overwintering plants and when spring arrives they emerged quicker and produce 20-30 percent more yield than spring canola. Spring canola is grown in the United States, Canada, India

and parts of China and they mature approximately 85 days after planting. In 2014, Canada, China, India, Germany, and France were top five countries in canola production (Fig. 2.2) (FAOstat 2017).

The US canola production areas are mainly located in the Northern Plains. Canola production in 2016 was estimated at 3.08 billion pounds with an approximate value of $493 million of which 84% was obtained in North Dakota. The other canola-producing states were Idaho, Kansas, Minnesota, Montana, Oklahoma, Oregon, and Washington (USDA-NASS 2017). Canola in the US, is typically planted in a 2-3 years rotation with cereals like wheat and barley (Kandel et al. 2015).

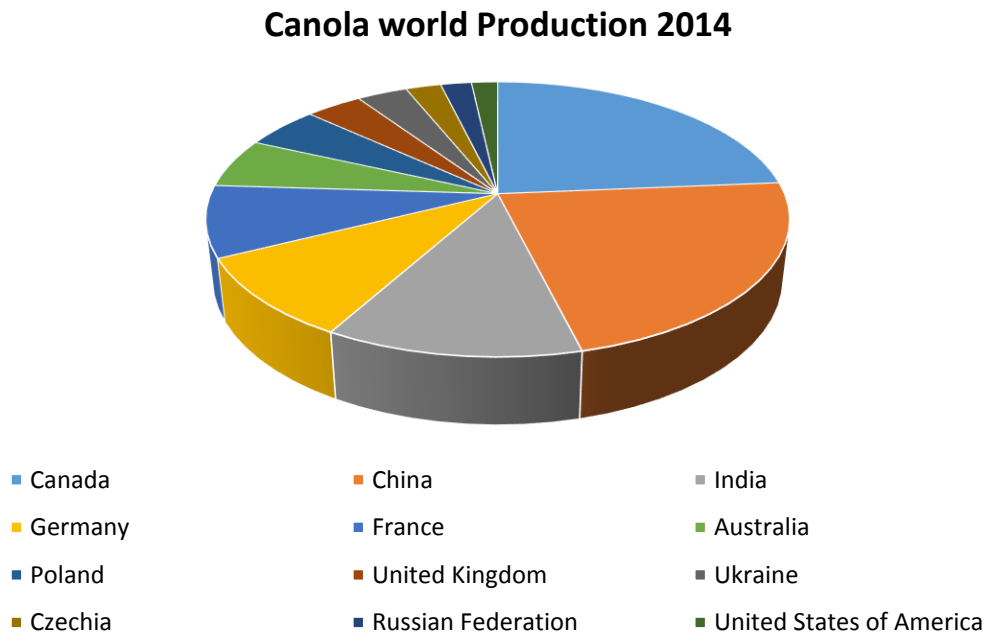

Figure 2.2. Global canola production in 2014 (FAO 2017).

Canola (B. napus) Genome

The canola genome was produced by the duplication of chromosomes after a natural hybridization of the C subgenome from *B. oleraceae* (525.8 Mb) and the A subgenome from *B.*

rapa (314.2 Mb) to get together. The genome assembly contains 34.8% of transposable elements (TEs) and 101,040 genes. Also, 34,255 and 38,661 orthologous gene pairs were found in the A and C genomes, respectively. More analysis showed that 96% of genes are expressed in leaves, roots or both (Chalhoub et al. 2014). Moreover, 425 nucleotide binding site leucine-rich repeat (NBS-LRR) which encode resistance genes (153 on A genome and 224 on C genome) located in *B. napus* genome (Chalhoub et al. 2014).

Diseases

Diseases are a major limitation to canola production worldwide. In North Dakota, the most important disease affecting canola production is blackleg. Other economically important diseases are Sclerotinia stem rot, which is caused by *Sclerotinia sclerotiorum*; and clubroot, which is caused by *Plasmodiophora brassicae*.

The plant develops cankers on stem crown from infected leaves and eventually causes yield reduction because of infection by *Leptosphaeria maculans*. The yield losses of this disease have been observed up to 45 % (del Rio et al. 2012). Lamey (1995) reported severe disease caused by PG- 2. Most of canola cultivars at that time (2002) were resistant or moderately resistant to PG-2 (Berglund 2003). In 2003, PG-3, 4 and T were detected in canola residues from two North Dakota counties in 2003 (Bradley et al. 2005; Chen and Fernando 2006) and all of cultivars were susceptible to them at that time.

Blackleg was described for the first time as a saprophytic organism growing on dead red cabbage tissues and was named *Sphaeria lingam* by Tode (1791). Fifty-eight years later, Desmazieres (1849) found the same fungus from living *Brassica oleracea* plants and reassigned it to the genus *Phoma*. Since then, the name *Phoma lingam* (Tode ex Fr.) Desm. (1849) has been

changed to *Phoma brassicae*, *Phoma oleracea*, *Phoma napobrassicae*, *Plenodomus lingam* and other genus and species (Boerema 1976).

Epidemiology of Blackleg

Blackleg disease is polycyclic (Hua et al. 2007). The ascospores are primary sources of inoculum, however, epidemics can be initiated by conidia (Molina et al. 2017). Ascospores are released after rain events and can remain around 3-4 months (Paul and Rawlinson 1992). Ascospore germination occurs within 4 hours at 12-20°C. Under optimum temperature and wetness conditions, one or two ascospores are enough to start an epidemic (Wood 1977). However, the time between ascospore germination and lesion formation varies from cultivar to cultivar and based on life stage of plants (Poisson and Peres 1999). At the beginning, lesions appear as pale green spots around 1-2 cm in diameter, gradually turning pale brown and produce pycnidia on lesion surface. Eventually, the center of lesion may break or fall out completely (Ansan-Melayah et al. 1997). The conidia produced in these pycnidia are the secondary inoculum although the lesions they cause do not affect yield significantly (Hall 1992). Pseudothecia also develop in plant stubble until release time. They need their mating type to form pseudothecia. In earlier times, infected seeds were an important way to spread the pathogen; when it occurred, the pathogen could be found as dormant mycelium in seed coats or even in the embryo (Jacobson and Williams 1971). The incidence of disease is correlated with incidence of infected seed at the harvesting (Hall et al. 1996). Nowadays, however, seed transmission is relatively not important due to the extensive use of certified pathogen-free seeds.

Factors such as virulence profile of a population, climate, cultivar, cultural practices that affect severity of *L. maculans* epidemics. The pathogen population within a region could consist of isolates that range from highly aggressive to weak. Climate factors such as temperature and

rainfall affect not only inoculum survival, pseudothecia maturation, timing of ascospore release and host resistance (Huang et al. 2007) but also the rate of residue degradation (Barbetti and Khangura 1997). Expression of symptoms seems to be more evident at temperatures below 10 °C tend to mask symptom expression (Rimmer et al. 2007). Temperature affects symptoms expression with lesions developing faster and more visibly at higher temperatures.

The Pathogen: *Leptosphaeria maculans*

Leptosphaeria maculans (Desm.) Ces. & de Not [anamorph = *Phoma lingam* (Tode: Fr.) Desm.] is a hemibiotrophic and heterothallic fungus belongs to the phylum Ascomycota. However, the proposed name for asexual stage this fungus after revision of one name for each fungus is *Plenodomus lingam* (Tode : Fr.) Hohn.,(de Guyter et al. 2013).

Classification of *L. maculans*

Kingdom: Fungi

Phylum: Ascomycota

Class: Dothideomycetes

Order: Pleosporales

Family: Leptosphaeriaceae

Genus: *Leptosphaeria* (*Plenodomus*)

Species: *maculans* (Desm.) Ces. & De Not. (*lingam*)

Taxonomy and Nomenclature

L. maculans belongs to Leptosphaeria species complex, which infect crucifer plants. Based on molecular data, this species has been divided into two subspecies, *L. maculans* ‘brassicae’ from *Brassica* sp. and *L. maculans* ‘lepidii’ from *Lepidium* sp. *L. biglobosa* was considered earlier as a

less virulent form of *L. maculans* (Rouxel and Balesdent 2005). Whereas *L. biglobosa* has been divided to five subspecies including *L. biglobosa* 'brassicae', *L. biglobosa* 'canadensis', *L. biglobosa* 'thlaspii', *L. biglobosa* 'australensis' and *L. biglobosa* 'occiaustralensis'.

Morphological Features of L. maculans

This fungus is a saprophyte which produces ascospores and releases them during the spring. These ascospores are the primary source of inoculum. they are produced in groups of eight and are cylindrical to ellipsoidal in shape with rounded ends and measure 35-70 x 5-8 μm). Ascospores are produced in bitunicate asci (80-125 x 15-22 μm) that grow in biseriate fashion within pseudothecia. Pseudothecia measure 300-500 μm in diameter (Williams 1992). The pycnidia (fIG. 2.3) are asexual fruiting bodies and are black, globose to subglobose in shape (250-600μm), and have an ostiole through which conidia are extruded when mature. Conidia are single-celled, hyaline and cylindrical (4-5 ×1.5-2 μm) and are embedded in a hygroscopic, gelatinous matrix (Vakili Zarj et al. 2017).

Life Cycle of L. maculans

The primary inoculum for infection are ascospores in Australia and Europe while pycnidiospores are considered the primary inoculum in the United States and Canada. This fungus is heterothallic and needs two different mating types to be present for sexual reproduction to happen (Williams 1992). Ascospores, which are formed in pseudothecia on stems infected during previous growing seasons, are released between May and August (Hall 1992). Ascospore discharges are affected by weather conditions and can last for 3-4 months or longer but their release peaks one or two months after its onset (McGee 1977; Thürwächter et al. 1999; Khangura et al. 2001). The fungus penetrates through stomata or wounds made by flea beetles or other factors. After initial infection of the leaves, which results in extensive necrosis, the pathogen colonizes

intercellular spaces between mesophyll cells and then grows asymptomatically down the petiole in xylem vessels or between cells of the xylem parenchyma and cortex. While this occurs, pycnidia may be produced on the dead tissue. Pycnidia act as secondary sources of inoculum and pycnidiospores spread easily in moist weather condition (Hammond et al. 1985; Hammond and Lewis 1987). Finally, once the fungus reaches the crown region, it kills cells of stem cortex which makes cankers. These cankers can completely girdle the base of the stem causing the dead of the plant. Also, lesions may form on pods; when this happens, the fungus can colonize the seeds (McGee 1977).

Figure 2.3. *Leptosphaeria maculans* conidia on leaf surface

Variation of L. maculans Virulence

Several classifications have been used to characterize *L. maculans*' virulence profile. One of the earliest classifications divided *L. maculans* into two pathotypes: groups A and B. Isolates from group A were virulent and able to develop canker on *B. napus* stems and produce a non-host-specific toxin, sirodesmin PL, in culture filtrate while isolates from group B were avirulent and did not produce sirodesmin PL (Shoemaker and Brun 2001). Then, pathogenicity groups (PG) were created based on the reaction of a set of three *B. napus* differentials: Westar, with no resistance genes; Glacier, with *Rlm2* resistance gene; and Quinta, with *Rlm1* and *Rlm3* resistance genes (Mengistu et al. 1991). In this way, all differentials were susceptible to isolates from PG-4

(Table 2.1). The reaction of these differentials to isolates from other PGs is shown in table 2. 1. A fourth PG, T, was added to reflect the resistant reaction of Quinta and susceptible reaction of Glacier to some isolates (Rimmer 2006). Another classification that used these three differentials and replaced Westar by a winter type *B. napus* cv., Lirabon and adding "Jet Neuf", which carries resistance gene *Rlm4*, classified the isolates into six groups (A1 to A6) (Badawy et al. 1991) (Table 2.1). The specific interaction of each group could be clearly explained using gene-for-gene theory and they could consider as six races of *L. maculans*, which are different in their *Avr* gene combination (Rouxel et al. 2003).

Table 2.1

Reaction of differential set to different pathogenicity groups (PGs) of Leptosphaeria maculans adopted from Rouxel et al. 2003 with modification

PG		Differential Sets			
Mengitsu et al. 1991	Badawy et al. 1991	Westar Lirabon (None)	Glacier (*Rlm2*)	Quinta (*Rlm1,3*)	Jet Neuf (*Rlm4*)
PG-4	A1	S[a]	S	S	S
	A5	S	S	S	R
PG-3	A2	S	S	R	S
	A6	S	S	R	R
PG-2	A4	S	R [b]	R	S
	A3	S	R	R	R

[a] Susceptible reaction
[b] Resistant reaction

Sixteen avirulence genes have been identified in *L. maculans*, so far. Of these, eight genes including *AvrLm1* (Gout et al. 2006), *AvrLm2* (Ghanbarnia et al. 2015), *AvrLm3* (Plissonnean et al. 2016), *AvrLm5* (*AvrLmJ1*) (Van de Wouw et al. 2014), *AvrLm4-7* (Parlange et al. 2009),

AvrLm6 (Fudal et al. 2007) and *AvrLm11* (Balesdent et al. 2013) have been cloned. These avirulence genes have been classified in two clusters. The first cluster covers *AvrLm1*, *AvrLm2* and *AvrLm6* (Balesdent et al. 2002) and second one including *AvrLm3*, *AvrLm4*, *AvrLm7*, *AvrLm9* and *AvrLepR1* (Balesdent et al. 2005; Ghanbarnia et al. 2012).

Blackleg Management

Genetic Resistance

Two types of resistance to *L. maculans* have been identified in *B. napus* (Rimmer and van den Berg 1992). The first type is qualitative or race-specific resistance which is controlled by one major gene. Genes that provide this type of protection activate plant defenses after recognizing effectors produced by corresponding avirulence gene in the pathogen. However, the interactions among these genes is still not clearly understood. For example, *L. maculans* isolates containing *AvrLm1* interacts with *Rlm1* and *LepR3*; to explain this, Larkan et al. (2013) suggested the possibility that "redundant phenotypes in the gene for gene interaction" exist. A similar dual interaction has been observed with *AvrLm4-7* which interacts with *Rlm4* and *Rlm7* (Parlange et al. 2009). The second type of resistance is quantitative, race-non-specific or polygenic resistance which can be more easily detected at the adult-plant stage. Durable resistance is final goal of most of breeding programs. Theoretically, cultivars with quantitative resistance should have longer shelf-life than cultivars with race-specific resistance because the former allows the pathogen to infect and reproduce without exerting too much selection pressure to change for specific virulence profiles (Marcroft et al. 2004). The quantitative resistance which is controlled by multiple genes and is sensitive to environmental conditions and it is difficult to infer if the pathogen strain is different in its degree of pathogenicity on the same cultivar in different environmental conditions. As with qualitative resistance, quantitative resistance can also be overcome by the pathogen, this

process is known as “erosion” rather than “break-down” and in general occurs at a much slower rate than the breakdown of qualitative genes.

Durable Resistance

Durable genetic resistance can be obtained through different strategies. One of them is pyramiding resistance genes. This can be achieved through combination of major genes in a background containing a high level of quantitative resistance in a single cultivar (Rimmer 2006). Marker-assisted selection using molecular markers tightly linked to resistance genes will be necessary to reach this goal (McDonald and Linde 2002). Having a strong quantitative-resistance background in cultivars will reduce yield losses even when the qualitative resistance genes are overcome by the pathogen. Another strategy is to rotate major resistance genes by planting mixture of cultivars with different resistance genes. For this strategy to work, however, we must generate information on the resistance genes present in commercial hybrids. To date, eighteen resistance genes have been identified in canola, which include *Rlm1-11*, *RlmS, LepR1-4, BLMR1-2*. Of these, two genes *Rlm2* (Larkan et al. 2015) and *LepR3* (Larkan et al. 2013), have been cloned. The genes *Rlm5* and *Rlm6* have been identified on the B genome of *B. juncea* cvs. Picra and Aurea (Balesdent et al. 2002); *Rlm1* in *B. napus* cv. Quinta (Ansan-Melayah et al. 1998); *Rlm4* in *B. napus* cv. Jet Neuf (Balesdent et al. 2001); *Rlm2* (Ansan-Melayah et al. 1998) and *Rlm3* (Balesdent et al. 2002) in *B. napus* cv. Glacier; *Rlm7* in non-commercial lines (Balesdent et al. 2002); *Rlm9* in *B. napus* cv. Darmor (Delourme et al. 2004); *Rlm8* (Balesdent et al. 2002) and *Rlm11* (Balesdent et al. 2013) in a *B. rapa*; *Rlm10* in *B. nigra* (Chevre et al. 1996); *LepR1* and *LepR2* (Yu et al. 2005), *LepR4* (Yu et al. 2013) and *RlmS* (Van de Wouw et al. 2009) in *B. rapa* subsp. *sylvestris;* and *LepR3* (Yu et al. 2007) and*BLMR1* and *BLMR-2* (Long et al. 2011) in *B. napus* cv. Surpass400. Five of these genes including *Rlm1, Rlm3, Rlm4, Rlm7* and *Rlm9* located on chromosome A07 (Delourme et al.

2004), *LepR1* on A02 (Rimmer 2006), *LepR4* on A06 (Yu et al. 2013), *LepR3* and *Rlm2* on A10 (Larkan et al. 2015) which control resistance to *L. maculans*.

Cultural Management

Application of disease resistance is the major management strategy for blackleg disease whenever available; however, other cultural practices such as rotation with cereals for 2-3 years, use of disease-free seeds and fungicide seed treatment, and foliar fungicide application are also used to augment the effective disease management (Markell et al. 2008).

Identification of Markers Associated With Disease Resistance

The identification of markers associated with disease resistance can facilitate the transfer of quantitative resistance genes into modern breeding lines. Two approaches are used to identify markers, association mapping and quantitative trait loci mapping.

Genome-wide association study (GWAS) is a test for statistical association between genotypes based on single nucleotide polymorphism (SNPs) and phenotypic reactions based on a trait of interest, i.e. resistance to a plant disease. Linkage disequilibrium (LD) is the non-random association between alleles at different loci, which is created by evolutionary forces such as mutation, drift and selection and is broken down by recombination (Visscher et al. 2012). Generally, physically close loci show stronger LD than those located far from each other on a chromosome. Larger populations will show lower LD for a given distance. The strength of the association between alleles at two loci strongly depends on their allele frequencies; in this way, a rare variant with frequency <0.01 will be in low LD with its neighbor common variant, but SNP with more than 0.05 allele frequency will be common (Moghaddam et al. 2016). Therefore, GWAS has enough power to detect associations with causal SNP that are common in the population. In general, conducting GWAS on large populations give results that are more reliable. However, for

some traits good results can be achieved with less than 100 individuals (Atwell et al. 2010). Similarly, conducting GWAS on a rather genetically diverse population, i.e. a population composed by individuals collected from different geographical regions, increases the reliability of GWAS results to a point (Li et al. 2010). What this means is, geographically distant populations could also increase the probability of identifying non-causative markers as better descriptors of the phenotype than causative ones (Platt et al. 2010). This issue, however, can be solved by including competing variants as cofactors within a mixed model setting (Segura et al. 2012). In this way, causative SNP will be included as cofactors. Another way to minimize the negative effect of heterogeneous populations is to increase the size of said populations. Confounding due to relatedness can also reduce the power of GWAS (Vilhjálmsson and Nordborg 2013). This happens when two related individuals share casual and non-casual alleles, which leads to LD between them. These artificial associations have been called synthetic associations, are considered a natural consequence of the linkage and error structure of the data, and may still appear as a significant SNP even when using large size populations (Korte and Farlow 2013). This problem could be solved by using mixed models that account for phenotypic covariance that is due to kinship (Yu et al. 2006). Nevertheless, one should always keep in mind that even the most significant SNP might not necessarily be a causative SNP but a synthetic association.

In the process of conducting GWAS, thresholds (i.e. cut-off *P* values) are typically used to identify markers that show statistically significant associations with trait of interest. One approach to identify the appropriate *P* value to use is the Bonferroni correction threshold. This correction will adjust *P* values because of the increased risk of a type I error when making multiple statistical tests (Armstrong 2014). The ratio of false positive to false negative association, which is called false discovery rate (FDR), can be used.

QTL mapping is a powerful method used to identify genomic regions, which co-segregate with a given trait in recombinant inbred line (RIL) families, but has two drawbacks. First, the limitation in lower allelic diversity imposed by the two parents results in low-resolution mapping (Borevitz and Nordborg 2003); on the other hand, that limitation allows a more accurate determination of the QTL (Balasubramanian et al. 2009).

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CHAPTER 3. IDENTIFICATION OF SOURCES OF RESISTANCE TO BLACKLEG (*LEPTOSPHAERIA MACULANS*) IN A *BRASSICA NAPUS* L. GERMPLASM COLLECTION[a]

Abstract

The increased prevalence of strains of pathogenicity group 4 of *Leptosphaeria maculans*, causal agent of blackleg, represents a serious threat to the canola (*Brassica napus* L.) industry in North Dakota, state that contributes > 85% of the U.S. canola production. The objective of this study was to identify sources of resistance to PG-4 in a collection of 559 *B. napus* plant introduction materials (PIs). Replicated trials were conducted twice in greenhouse using a mixture of five PG-4 isolates to evaluate the reaction at seedling stage; then an elite group of PIs was evaluated in replicated field trials between 2014 and 2016 at Langdon, ND using a combination of lab-produced inoculum and blackleg-infested canola residues. Combined analysis of field data indicated three PIs had on average 73 to 80% less ($P < 0.05$) internal stem tissue discoloration than the commercial hybrids used as the controls. These PIs could be used in canola breeding programs as good sources of resistance against PG-4; in addition, efforts should be made to characterize the resistance they carry.

Introduction

Blackleg, caused by the fungus *Leptosphaeria maculans* (Desm.) Ces. et de Not. [anamorph = *Phoma lingam* (Tode: Fr.) Desm.] has become the most important disease affecting canola production in the U. S. (Fig. 3.1). In North Dakota, the largest canola producer in the country (USDA-NASS 2015), the disease was first observed in the early 1990s (Lamey 1995). At

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that time, the most prevalent *L. maculans* strains were classified as pathogenicity group (PG) 2; strains belonging to this group are incapable of affecting plants carrying resistance genes *Rlm1 Rlm2* or *Rlm3* (Ansan-Melayah et al. 1998; Balesdent et al. 2002). The introduction of cultivars carrying these resistance genes reduced the severity and importance of this disease in the following decade (Bradley and Lamey 2005). Since then, however, the disease has steadily increased in severity and prevalence powered by the spread of strains belonging to PG-4 (del Rio Mendoza et al. 2012).

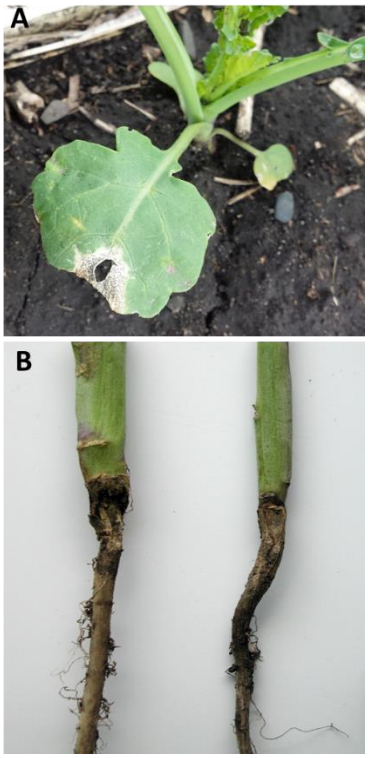

Figure 3.1. Typical blackleg symptoms caused by *Leptosphaeria maculans* on canola (*Brassica napus*) plants with lesions on A) leaves; and B) stems.

Identifying sources of resistance against PG-4 is necessary and urgent. A study published in 2010 suggested that most canola cultivars currently in use in North Dakota are susceptible to strains of PG-4. (Marino and del Rio 2010), which can overcome resistance genes *Rlm1*, *Rlm2*, and *Rlm3*. As PG-4 strains become more ubiquitous in North Dakota, the possibilities of severe

blackleg outbreaks increase. An end-of-season survey conducted in 2014 on 114 canola fields in North Dakota revealed that blackleg-symptomatic plants were present in 96%, with several fields having incidences greater than 30% (del Río Mendoza, unpublished data).

Many efforts have been put forth to identify sources of resistance against blackleg and to transfer that resistance into canola cultivars. These resistance genes have been identified in different species of Brassicaceae such as *B. juncea* (Balesdent et al. 2002), *B. napus* (Ansan-Melayah et al. 1998; Balesdent et al. 2001; Delourme et al. 2004) *B. rapa* (Balesdent et al. 2013; Yu et al. 2005; Yu et al. 2013) and *B. nigra* (Chevre et al. 1996). In *B. napus,* major resistance genes have been located on either the A or B genomes (Raman et al. 2013), but successful efforts also have been carried to transfer resistance from related species, e.g. genes *LepR1*, *LepR2*, and *LepR4* were transferred to *B. napus* from *B. rapa* subsp. *sylvestris* (Yu et al. 2005; Yu et al. 2013). To date, there is no evidence to indicate whether these three genes are similar or different from *Rlm1, Rlm2* and *Rlm4*, respectively.

The objective of this study was to identify *B. napus* germplasm that could be used as sources of resistance against PG-4 strains of *L. maculans*.

Material and Methods

Plant Materials

Greenhouse screenings characterized the reaction of 559 *B. napus* plant introductions (PI) at the seedling stage. Seeds, obtained from the North Central Regional PI Station of the U.S. National Plant Germplasm System located in Ames, IA (Appendix A).

Greenhouse Screening

PIs were planted in batches containing 29 entries at a time and cv. Westar served as the susceptible control. Westar is no longer a commercial cultivar and has been used as standard

susceptible control because it does not carry known blackleg resistance genes (Balesdent et al. 2005). Entries in each batch were evaluated using a randomized complete block design (RCBD) with three replicates. Seeds were placed in individual plastic cells in trays (44 x 57 x 50 mm) filled with soilless potting mix (PRO-MIXR BX, Premier Tech Horticulture, Quakertown, PA) and kept in greenhouse room at 20 $\pm$ 2^{o} C with 16 h light daily supplemented with 600-watt high pressure sodium lamps (P.L. Light Systems, Inc., Beamsville, Ontario, Canada). Seedlings were inoculated, as described below, 10 days after planting at the cotyledon stage. A spore suspension containing equal amounts of spores from five PG-4 isolates was used as inoculum. These five *L. maculans* isolates were collected in North Dakota and selected for their high aggressiveness (Franceschi and del Rio 2014; Franceschi 2015).

Inoculum Preparation

Inoculum of each *L. maculans* isolate was produced by culturing in separate dishes containing V8 agar medium as described by Nepal et al. (2014). After harvest, spore concentrations of each isolate were estimated with help of a hemocytometer and adjusted to 10^7 spores/ml. Then equal volumes of each suspension were combined for inoculations. During inoculation, the center of each cotyledon leaf was lightly pricked once with sharp forceps and a 10µl droplet of the spore suspension was deposited on the wound. The inoculated seedlings were incubated in cool mist chambers at 20°C and 98% humidity in dark for 24 h and then returned to the greenhouse room.

Data Collection and Analyses

The reaction of cotyledon leaves was recorded ten days after inoculation based on the 0- 9 scale of Williams and Delwiche (1979) where 0-3 represents a resistant reaction and 7 to 9 shows susceptibility (Fig. 3.2. A). The Levene's test for homogeneity of variances was conducted on the reaction of cv. Westar across batches and trials to determine whether batches and trials could be

combined for analysis. Upon confirmation, the median severity per replication for each PI was calculated using PROC MEANS of SAS v. 9.4 (SAS Institute, Cary, NC) and ranked with respect to other accessions using PROC RANK. Ranks closest to one are associated with most resistant reactions. ANOVA-type statistics analysis was conducted on the ranks using PROC MIXED as described by Shah and Madden (2004). To calculate each PI's relative effect and 95% confidence interval, the ranks were analyzed using the SAS macro *LD_CI.sas* from Brunner (2002).

Figure 3.2. Blackleg severity scales used to evaluate damage at A) at seedling stage on cotyledon leaves and (B) at adult plant stage as percentage of internal tissues in the crown region of the stems.

Field Evaluations

Field plots were established on May 15, 2014; May 24, 2015; and May 19, 2016 at the Langdon Research Extension Center of North Dakota State University in Langdon, ND. A total of 24 entries including 16 PG- 4-resistant, five PG-4-susceptible PIs as determined by greenhouse evaluations, cv. Westar as the standard susceptible control and two commercial hybrids were planted by hand in each year in single 3 ft. long rows, following a RCBD with four replications. Each block consisted of six tiers of four entries and two border rows. Rows were separated 18 cm. At least four canker-bearing canola stems collected from commercial fields established in the previous growing seasons were deposited in each tier to provide inoculum for the plants. In addition, seedlings were sprayed at least three times between the cotyledon and three-leaf stages with a 10^7 spores/ml spore suspension of the five isolates used in greenhouse screenings. The spore

suspensions were prepared as described earlier and were delivered using a backpack sprayer until runoff. Disease severity was recorded when plants reached physiological maturity by cutting the stems at the crown and estimating the percentage of internal tissue discoloration (Fig. 3.2. B). Levene's test of homogeneity of variances was conducted using PROC GLM of SAS to determine whether data for three years could be combined for analysis. Upon confirmation of the homogeneity of variances ($P > 0.05$), a combined analysis was performed using PROC MIXED of SAS with years and replications considered random variables and entries considered fixed variables. Pairwise comparisons between least square means of PIs were estimated using the option "pdiff" in the lsmeans statement and its output analyzed using the SAS macro "*pdmix800.sas*" (Saxton 1998) to separate PIs using a letter system.

Results

Greenhouse Screening

Thirty countries were represented in the *B. napus* collection. The largest contributor to the collection, by far, was South Korea with approximately 48% of accessions originating from that country, followed by Germany and Japan with 11 and 10% of accessions, respectively. The PIs reaction to inoculation had an approximate normal distribution (Fig. 3.3) with an overall mean severity of 4.83 and a median severity of five. Approximately 30% of accessions were considered resistant, among which 29 PIs had medians < 3, an indication of strong hypersensitive reactions (Table 3.1). The group of resistant accessions had a median severity of 3 with a mean of 2.6 and a standard deviation of 0.7. South Korea contributed approximately 57% of these accessions while Germany and Japan contributed approximately 10% each. On the other extreme, the group of accessions considered susceptible had a medina severity of 7 with a mean of 7.6 and a standard deviation of 0.9. As with the previous group, South Korea was the largest contributor with

approximately 38% of accessions in this group followed by Poland with 11% and Germany with 8% (Appendix A).

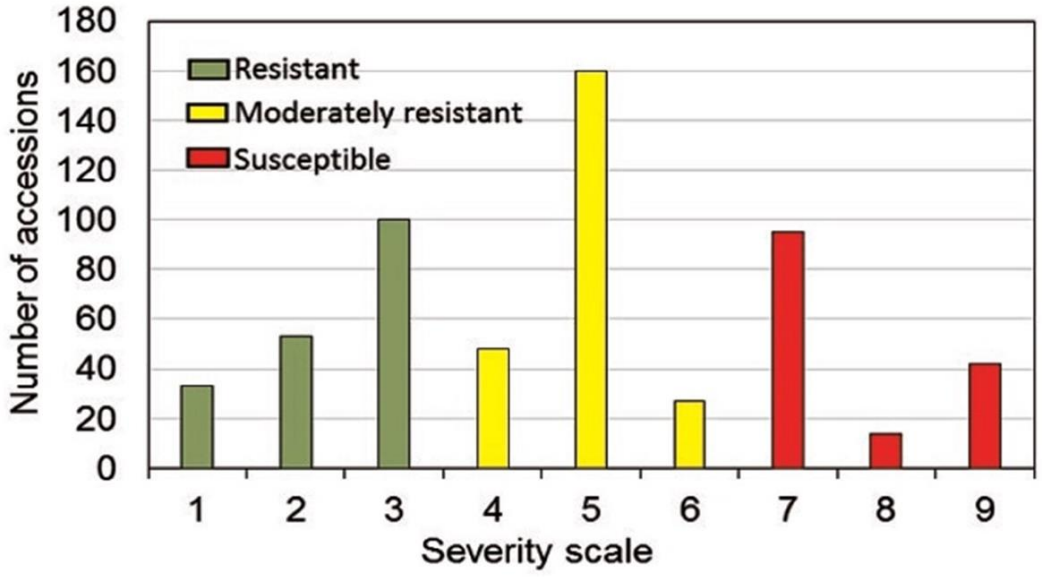

Figure 3.3. Frequency distribution of the reaction of 559 *Brassica napus* plant introduction materials to inoculations with a mixture of five pathogenicity group 4 strains of *Leptosphaeria maculans* in greenhouse trials. Reactions evaluated at seedling stage using the 0-9 severity scale of Williams and Delwiche (1979) where 0-3 are resistant reactions and 7-9 are susceptible reactions.

Table 3.1

Reaction of seedlings of 29 Brassica napus plant introduction materials to inoculations with a mixture of PG-4 isolates of Leptosphaeria maculans inoculated in greenhouse conditions

Accession name	Median[a]	Mean Ranks	Treatment relative effect [b]	
			Mean	95% Confidence Interval
Mokpo 2	1	11	0.02	0.01 - 0.05
Iwao natane	1	16	0.03	0.02 - 0.06
Gebr Dippes	1	56	0.11	0.06 - 0.22
Giant rape	1.5	8	0.04	0.02 - 0.10
Gylle	1.5	148	0.30	0.15 - 0.51
Wira	2	32	0.07	0.05 - 0.10
Kinki 21	2	55	0.10	0.07 - 0.13
Wichita	2	48	0.10	0.05 - 0.17
Dong Hae 16	2	54	0.11	0.07 - 0.17
Mokpo #3	2	55	0.11	0.05 - 0.22
Su weon cheg	2	54	0.11	0.06 - 0.22
Aomori-1	2	60	0.12	0.06 - 0.22
Hobson	2	64	0.12	0.06 - 0.22
Jet Neuf	2	80	0.15	0.09 - 0.24
Liropa	2	70	0.15	0.09 - 0.25
PI 169080	2	87	0.16	0.06 - 0.39
Armander	2	71	0.16	0.06 - 0.37
Abilene	2	80	0.18	0.04 - 0.55
France 8	2	94	0.20	0.06 - 0.49
KS 1701	2	137	0.26	0.08 - 0.60
Dong Hae 20	2	161	0.30	0.14 - 0.54
Norin 35	2	155	0.33	0.15 - 0.59
Synra	2	152	0.33	0.11 - 0.67
Ames 6073	2	162	0.38	0.05 - 0.88
Kuju 40	2.5	74	0.13	0.12 - 0.14
77-71	2.5	74	0.15	0.08 - 0.25
Hamburg	2.5	86	0.18	0.08 - 0.35
Iwashiro-natane	2.5	150	0.30	0.11 - 0.61
Dong Buk	2.5	150	0.33	0.12 - 0.63

a Medians based on the reactions on two cotyledons per plant; ten plants per replication; three replications per trial and two trials. Reactions rated according to the 0-9 scale of Williams and Delwiche.

b Mean relative effects closer to 0 are considered more resistant.

Field Evaluations

Field trials showed significant differences ($P \leq 0.05$) among PIs (Table 3.2) but not all entries performed as expected from their behavior in greenhouse trials. Disease severity ranged from 6 to 68% with the Westar, the susceptible control having a mean severity of 61%. Thirteen of the 16 PIs considered resistant in greenhouse trials had significantly less ($P = 0.05$) disease severity than Westar; however, the remaining three, Colt, Gido and Eckendorfer Mali, behaved statistically as susceptible as Westar and were more susceptible than the PIs Liglandor and CR167/65a. The latter two were considered susceptible ($P = 0.05$) in the greenhouse trials.

The two commercial hybrids used as control had on average 27 and 37% disease severity. The average disease severities of both commercial controls were statistically lower ($P = 0.05$) than that of Westar but greater than that of Sumner, Aomori-1 and CR165/76a. Three additional accessions, CR167/65a, Oleifera, and Bolko, had on average less disease severity ($P = 0.05$) than one of the commercial controls but not the other.

Disease incidence ranged from 31% to 94% with Westar, the susceptible control having a mean incidence of 92% (Table 3.2). Accessions Sumner, Aomori and CR165/76a had average incidences < 42% that were statistically smaller ($P = 0.05$) than the average incidence of both commercial controls (Table 3.2). The commercial controls had mean incidences of 61 and 72%, respectively. Accessions CR167/65a, Oleifera, and Bolko, had on average less disease incidence ($P = 0.05$) than one of the commercial controls but not the other.

Table 3.2

Reaction of elite Brassica napus plant introduction materials to Leptosphaeria maculans evaluated in field trials conducted at Langdon, ND in 2014-2016

		Treatment relative effect [b]		Severity (%)[c]		Incidence (%)	
Accessions	Median	Mean	95% CI	Mean	Letter Group	Mean	Letter Group
Colt	3	0.19	0.14-0.26	68	A	94	A
Westar [a]	7	0.82	0.80-0.84	61	AB	92	AB
Ujfertodi	5	0.48	0.21-0.75	55	ABC	84	ABCDE
Ames 26653	7	0.72	0.50-0.87	47	BCD	86	AB
Gido	3	0.14	0.07-0.25	47	BCDE	88	AB
Titus	7	0.68	0.46-0.83	46	BCDEF	82	ABCD
Eckendorfer Mali	3	0.27	0.12-0.48	43	BCDEF	81	ABC
Rico	3	0.18	0.13-0.26	42	CDEFG	84	AB
Laura	3	0.22	0.18-0.26	40	CDEFG	73	BCDEF
Integra 7121R [a]	-	-	-	37	CDEFG	72	BCDE
Legend	3	0.24	0.13-0.39	34	CDEFGH	82	ABC
Target	3	0.24	0.23-0.25	31	CDEFGHIJKL	83	ABCDEF
Su weon cheg	2	0.11	0.06-0.22	31	CDEFGHI	63	CDEF
N001-28-246-5-4	3	0.18	0.13-0.26	28	EFGHIJ	56	DEFG
Integra 7150R [a]	-	-	-	27	FGHIJ	61	CDEF
77-71	2.5	0.15	0.08-0.25	27	DEFGHIJK	55	EFGH
Nabo	3	0.24	0.13-0.39	24	FGHIJKL	63	DEFG
Liglandor	9	0.94	0.87-0.97	22	GHIJKL	59	DEFG
CR 167/65a	7	0.77	0.67-0.85	17	HIJKL	51	FGH
Oleifera	3	0.23	0.12-0.40	14	IJKL	54	EFG
Bolko	3	0.17	0.12-0.24	11	JKL	35	GH
Sumner	3	0.28	0.14-0.48	8	KL	36	GH
Aomori-1	2	0.12	0.06-0.22	8	KL	42	GH
CR 165/76a	3	0.38	0.20-0.60	6	L	31	H

a Westar= standard susceptible control; Integra 7121R and 7150R = commercial hybrids used as controls. [b]Treatment relative effects not calculated for hybrids (-); CI= confidence interval. [c] Severity expressed as percentage of discolored internal stem tissues at crown. Means in a column with the same letter are not significantly different ($P = 0.05$) per Fisher's protected least significant difference test.

Discussion

The frequency of accessions that showed high levels of resistance to infection in greenhouse assays by isolates of PG-4 was unexpectedly high. Roughly, 30% of accessions were resistant to inoculations at the seedling stage. Of these, at least 29 had median scores below 3, an indication that hypersensitive responses were involved and therefore, major resistance genes were present in these accessions (Balesdent et al. 2001). Since all accessions were inoculated with a mixture of five isolates, it is not possible to identify individual genes and that should be part of the next step of research for this project.

The slight inconsistency in performance under field conditions of certain accessions deemed resistant in greenhouse trials could be explained in part by the nature of the resistance present in them. Resistance to blackleg can be qualitative or quantitative and the genes controlling them are "generally distinct" (Delourme et al. 2006). The former, also known as race-specific or vertical resistance is typically evaluated on seedlings in greenhouse conditions while the latter, also known as polygenic or partial resistance, is evaluated on adult plants under field conditions. By supplementing the fields with blackleg-infested canola residues, we exposed these plants to strains other than the ones used in the greenhouse screenings. While no efforts were made to identify the strains of blackleg present on the residues or in infected PIs, previous studies determined that approximately 22% of isolates retrieved from commercial canola residues do not fit the profile of any PG (Nepal et al. 2014). Some studies showed the correlation between canola and *L. maculans* is strong but in some other is poor or no correlation; (Newman and Bailey 1987) and some other studies no correlation found between seedling and adult stage (Helms and Cruickshank 1979). It seems like, the relationship between seedling and adult stage depends on plant stage, plant genotype and inoculation method. Among the PIs whose reaction was consistent

in greenhouse and field trials, CR 165/76a, Aomori-1 and Sumner had on average 73 to 80% less disease severity and 40 to 49% less incidence than both commercial controls while Bolko and Oleifera had on average 66% less severity and 38% less incidence than one of the commercial controls and had a statistically similar reaction than the other.

The sources of resistance identified in this study could be a valuable tool for canola breeding programs. The top three PIs presented a consistent resistant reaction to exposure to lab and field-produced inoculum. They were collected from different countries, which increases the possibility that the genetic basis of their resistance to blackleg may be different from one another. Moreover, they do not require vernalization to flower, which will facilitate the development of breeding populations adapted to the growing conditions of the region.

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CHAPTER 4. IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO BLACKLEG

Abstract

Blackleg, caused by *Leptosphaeria maculans,* is a serious threat to canola (*Brassica napus*) production in North Dakota, the largest producer of canola in the United States. Genome-wide association study (GWAS) was conducted on sets of 213 and 78 *B. napus* accessions inoculated with mixtures of five *L. maculans* isolates from pathogenicity groups (PG) 4 and 3, respectively, to identify markers associated with resistance to this disease. Phenotypic data was obtained by evaluating reaction of plants at the seedling stage. Genotypic data was obtained using a genotype-by sequence procedure that produced 37,734 single nucleotide polymorphisms. Three markers located on chromosomes A03, C03 and C08 were significantly (P <0.00004) associated with resistance to PG-4. These markers explained on average approximately 10% of the phenotypic variation. Six markers located on chromosomes A02, A07, C05, and C08 were significantly (P < 0.00034) associated with resistance to PG-3. These markers explained on average 20-23% of the phenotypic variation observed. Seven additional markers for PG-4 (P < 0.00023) and 20 for PG-3 (P < 0.0013) that were identified on four other chromosomes explained on average 8-9% of the phenotypic variation. Similarly, 20 additional markers for PG-3 were identified on ten other chromosomes; these markers explained on average 14-19% of the phenotypic variation. A BLAST search within 105 kpb of these markers identified 17 candidate genes involved in plant defense system. These markers could help transfer blackleg resistance into modern breeding lines.

Introduction

Canola (*Brassica napus* L., genome AACC, 2n = 4x = 38) is the product of a natural hybridization and genome doubling between turnip rape (*B. rapa* 2n =2x = 20, AA) and cabbage

(*B. oleracea* 2n = 2x = 18, CC) that occurred around 10,000- 100,000 years ago in southern Europe (Olsson, 1960). Canola is cultivated because of its oil, which is a good source of omega-3 and unsaturated fats, and is the third most important edible vegetable oil in the world after palm and soybean oil (USDA-ERS 2017). Canola contributes around 14% of the world's edible oil production (USDA-ERS 2016). The state of North Dakota is the largest producer of canola in the United States. In 2016, North Dakota produced more than 2.6 billion pounds valued at US$436 million. This represented 87% of the United States canola production (USDA-NASS 2016).

Blackleg disease, which is caused by the hemi biotrophic fungal pathogen *Leptosphaeria maculans* (Desm.) Ces. et de Not. is a major threat for canola production worldwide. It can affect canola plants at all stages of growth but infections that take place between plant emergence and the sixth leaf growth stage cause the most severe yield losses (Khangura and Barbetti 2005). Yield losses attributed to this disease are estimated more than $900 million per growing season, worldwide, in particular in Europe, North America and Australia (Fitt et al. 2008). Blackleg is quickly becoming the most important disease affecting canola production in North Dakota. The first blackleg epidemic in the state occurred in 1991 (Lamey 1995) and was caused by isolates belonging to PG-2. Isolates from this group were most prevalent in the region during the 1990s. In 2003, strains of PG-3 and PG-4 were detected in the region (Bradley et al. 2005) and since then, they have quickly replaced PG-2 as the most prevalent groups (Nepal et al. 2014). End of season field surveys conducted in 2016 revealed that blackleg is present in most canola producing fields in North Dakota.

While genetic resistance is the most effective, environmental friendly and cost-effective strategy to manage this disease (Salisbury et al. 1995; Sprague et al. 2006), its efficacy and stability are usually hindered by the ability of the pathogen to change its virulence profile. Earlier

classification established four pathogenicity groups (PG) according to the virulence of *L. maculans* isolates on three *B. napus* differentials (Mengistu et al. 1991). In recent years, however, 16 avirulence genes (*AvrLm*) have been identified in the pathogen. These genes interact with corresponding genes in the plant that control resistance. The PG classification is based on reaction of *B. napus* cvs. Westar, which does not carry known blackleg-resistance genes, Quinta, which has resistance genes *Rlm1* and *Rlm4*; and Glacier, which has resistance genes *Rlm2* and *Rlm3* to the pathogen. Isolates from PG-4 can infect all three differentials, which means they do not have genes *AvrLm1*, *AvrLm2* or *AvrLm3*. On the other hand, isolates classified as PG-3 infect Glacier but not Quinta, which means they carry *AvrLm1*. *L. maculans* isolates can carry multiple avirulence genes (Balesdent et al. 2005). Their work showed European isolates from PG-4 typically carry *AvrLm* genes 5, 6, 7 and/or 8. Our work suggests PG-4 isolates typically carry *AvrLm* genes 6, 4-7, and 11 (Mansouripour et al. 2016).

Genome-wide association study (GWAS) has opened a new window to identify the genetic basis of phenotypic variation (Burghardt et al. 2017). GWAS could resolve allelic associations for traits based on the marker density, experimental population size and statistical models and has been widely used in canola (Hassan et al. 2008; Zou et al. 2010; Lu et al. 2016; Rezaeizad et al. 2011). The power of GWAS has been used to associate single nucleotide polymorphisms (SNPs) typically obtained through plant DNA sequencing and phenotypic response of plants to inoculation with the pathogen for a number of plant diseases including blackleg of canola (Rahman et al. 2016; Raman et al. 2016), leaf and stripe rust of winter wheat (Kertho et al. 2015), *Phytophthora sojae* in soybean (Schneider et al. 2016), and *Fusarium* head blight in Durum wheat (Ghavami et al. 2011) among others. The ability of GWAS to identify multiple marker alleles at the same time in populations with wider genetic background and the likelihood of obtaining a finer resolution

mapping thanks to the use of larger populations makes GWAS the choice tool to identify markers in worldwide germplasm collections (Kraakman et al. 2006).

The objective of this study was to identify SNP markers associated with resistance to PG-3 and PG-4 of *L. maculans* in a worldwide collection of *B. napus* germplasm.

Material and Methods

A panel of 213 *B. napus* accessions from a germplasm collection curated by the US National Plant Germplasm System in Ames, IA were used in this study. The panel which was composed of 120 winter-, 55 spring-, and 33 semi-winter-type lines and five rutabaga lines was inoculated with a mixture of five PG-4 strains of *L. maculans* (Appendix B). A subset of this panel, composed of 53 winter-, 13 semi-winter- and 12 spring-type lines were inoculated with five strains of PG-3 (Appendix C). All inoculations were conducted when seedlings were at the cotyledon stage in replicated trials conducted twice as described by Mansouripour and del Río Mendoza (2017). Briefly, the inoculation trials were conducted in batches, containing 29 entries along with cv. Westar as a susceptible control. The batches were conducted in a randomized complete block design (RCBD) with three replicates and ten seedlings per replication. The study was conducted two times. For each accession, the cotyledon leaves of 12-days old seedlings were lightly pricked once with sharp tweezers and 10 µl of a mixture of equal proportions of spores of five isolates each at a concentration of 10^7 spores/ml were deposited on the wounds. Twelve days after inoculation, reaction to the pathogen was evaluated using the 0 to 9 severity scale developed by Williams and Delwiche (1979). The plant materials for PG-4 were originated from 20 countries located in four continents (Appendix B). The plant materials for PG-3 came from eight countries located in Europe and Asia (Appendix C).

Statistical Analyses of Phenotyping Data

Levene's test for homogeneity of variances was conducted on the performance of the control cultivar Westar to determine whether the batches and trials could be combined for analysis. Upon confirmation of homogeneity of variances, median severities representing 120 disease assessments per accession were calculated using PROC MEANS of SAS 9.4 and used in the association analysis.

DNA Extraction and SNP Genotyping

Plant leaf samples from two to three plants per accession were collected 12 days after inoculation and stored in a vial. The samples were placed in a freezer (-80C) immediately and were lyophilized later for DNA extraction. DNA was extracted using Qiagen DNeasy Kit (Qiagen, CA, US). The quality of the DNA samples was measured using ND-1000 spectrophotometer (Nanodrop, Wilmington, DE) and its concentration adjusted to 50 ng/µl before sending it to the Institute of Genomic Diversity (IGD) at Cornell University for genotyping. At the IGD, samples were digested with ApeKI to create Genotype by Sequencing (GBS) libraries with 96 unique barcodes as described by Elshire et al. (2011). The samples were sequenced—100-bp single end—using an Illumina GAII sequencer.

Association Analysis

The association analysis was performed with TASSEL 5.0 (Bradbury et al. 2007) using information from 213 accessions genotyped with 37,734 markers. Each model SNPs showing minor allele frequencies (MAF) < 0.05 were removed prior to data analysis. Four models were used to analyze the data (Table 4.1). For models that included kinship, the Centered-IBS method with max alleles of six was selected for calculation of kinship matrix. For mixed models that included principal component (PC), number of PC was selected based on population structure and

scores that explained 25% of cumulative variation. The model selected to describe the association had the best fitness on QQ-plot and the smaller mean of the squared differences (MSD) between expected and observed *P*-values. The expected P-values were obtained dividing the rank of an observed *P*-value by the total number of markers (Stitch et al. 2008). The 0.01 and 0.1 percentile tails of an empirical distribution, obtained from 5,000 bootstraps, was used to determine the cut off *P*-value to identify significant markers as described by Mamidi et al. (2014). Selected markers were drawn on a physical chromosome map using Mapchart. The phenotypic variation explained by significant markers in the best model was calculated based on the likelihood-ratio-based R^2 (R^2 LR) (Sun et al. 2010) using the genABLE package in R (Aulchenko et al. 2007).

Table 4.1

Models used for association mapping and their respective statistics

Models	Statistical model	Description
Naïve	$Y = X\alpha + \mathcal{E}$	Y is related to Marker without correction factors.
PCA	$Y = X\alpha + P\beta + \mathcal{E}$	Y is related to Marker with correction factor for PCA.
Kinship (K)	$Y = X\alpha + K\gamma + \mathcal{E}$	Y is related to Marker with correction factor for Kinship.
PCA + K	$Y = X\alpha + P\beta + K\gamma + \mathcal{E}$	Y is related to Marker with correction factor for PCA and Kinship.

Marker Validation

The three significant markers which linked to the resistance to blackleg disease were used to identify PIs resistant to this disease. A total 11 PIs were checked at the field in RCBD with four replications were inoculated and scored based on stem discoloration percentage. The sequences of these PIs, which already sequenced before, were available to determine which markers present in each PI.

Genome Positions of Trait Related Candidate Genes

The Basic local alignment search tool (BLAST) was used to mine the TAIR database (https://www.arabidopsis.org/Blast/index.jsp) in search of nucleotide sequences genes located within 105 kb flanking regions of each significant marker. All positions with Bit-score ≥ 300 and a BLAST identity≥95% and E value 0 were selected. The Bit-score measures sequence similarity independent of query sequence length and database size and is normalized based on the raw pairwise alignment score, The E score represents the number of hits that could be expected due to chance when searching a data base. Of the genes present in these regions, those whose putative functions have been related to plant defense by other researchers were selected.

Phylogenetic Analysis

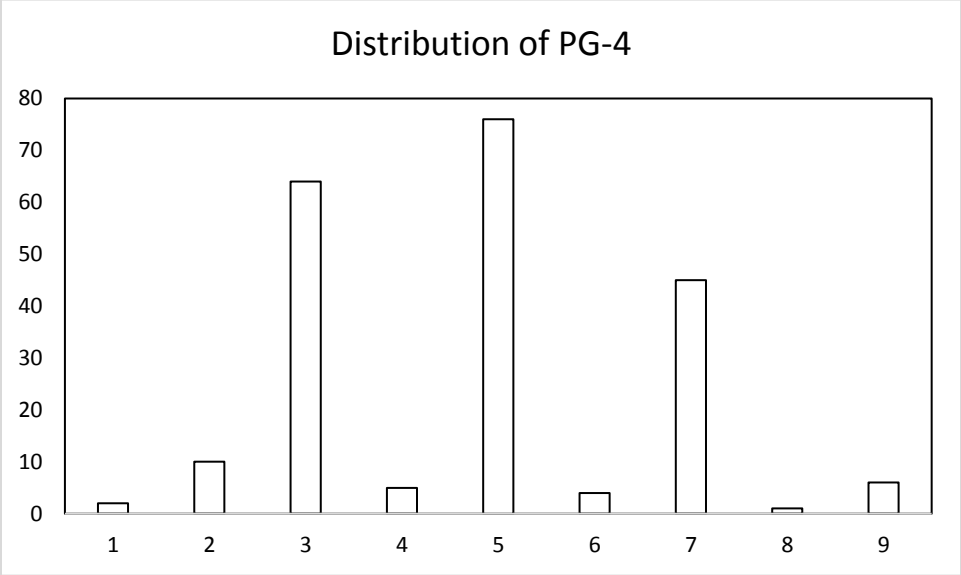
The phylogenetic analysis of 213 *B. napus* accessions (Appendix B) using SNP markers were performed using Splits tree4 software (Version 4.14.5) (Huson and Bryant 2005) using unweighted pair group method with arithmetic mean (UPGMA) with EqualAngle splits method. A phylogenetic network representing possible evolutionary relationships among accessions was calculated.

Results

Phenotyping

The reaction of accessions to inoculations with isolates from PG-3 and PG-4 were not normally distributed (Fig 4.1.A and B). Approximately 24% and 36% of accessions had resistant reactions (≤ 3 in the severity scale) to PG-3 and PG-4, respectively. In addition, 77% of winter type and 67% of spring type were resistant to *L. maculans* (≤ 5 in the severity scale) (Table 4.2).

A



B

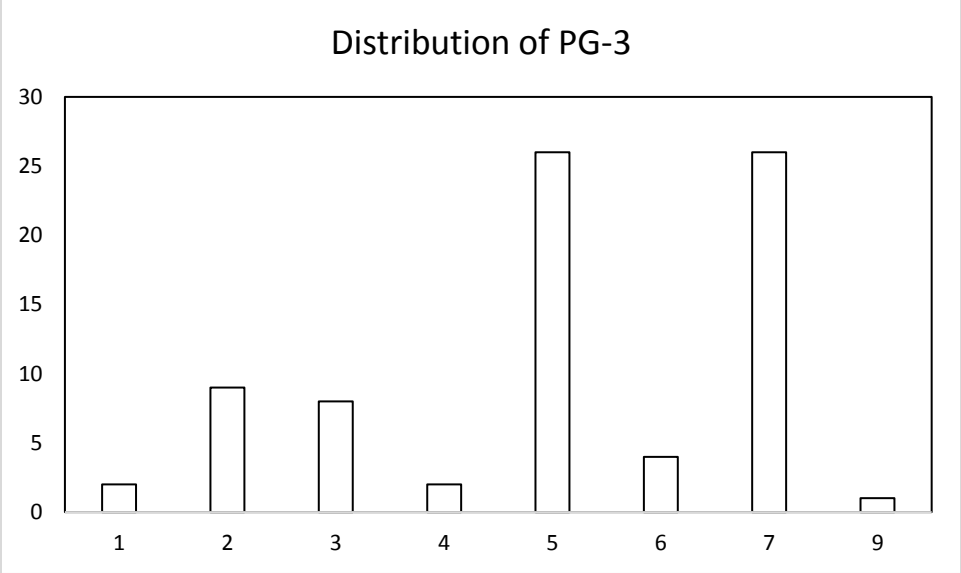


Figure 4.1. Frequency distribution of the reaction of 213 and 78 *Brassica napus* accessions from a worldwide collection to inoculations with a mixture of strain of *Leptosphaeria maculans* PG-3 (B) and PG-4 (A) in greenhouse trials.

Table 4.2

Summary of accession frequency and range of median for each origin and growth habit

Country originated/obtained from	Growth habit			Range of median severity		
	Spring	Semi-winter	Winter	Spring	Semi-winter	Winter
Albania	0	0	1	-[a]	-	5
Canada	7	0	0	5-8	-	-
China	1	1	1	5	5	7
Czechoslovakia	1	0	1	7	-	7
France	5	0	7	3-5	-	2-5
Germany	7	4	31	3-5	3-5	2-9
Hungary	1	0	3	7	-	3-5
Mongolia	1	0	0	7	-	-
Netherlands	0	0	1	-	-	3
New Zealand	0	0	3	-	-	6-7
Poland	2	2	8	5-7	5-7	3-7
Romania	0	0	1	-	-	5
Russia	3	0	2	7-9	-	5
Serbia	0	0	1	-	-	9
South Korea	16	25	42	3-7	2-9	1-7
Sweden	3	0	5	5	-	5-7
Taiwan	1	0	0	3	-	-
Turkey	1	0	0	5	-	-
Ukraine	2	0	2	7	-	5
United States	4	1	10	3-7	3	2-7
Unknown	0	0	6	-	-	2-5

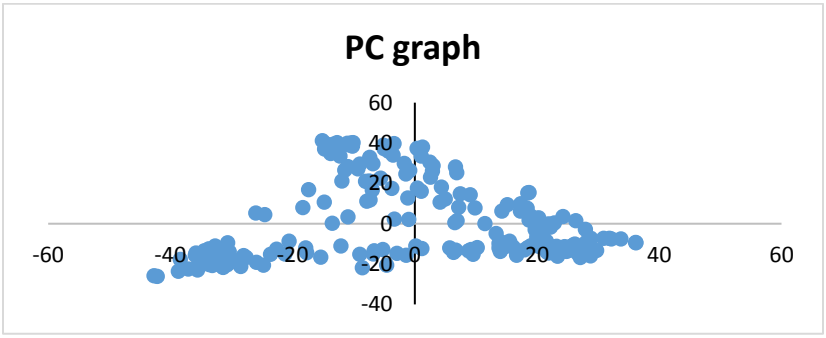
[a] Range of median for each growth habit of each country based on the reactions on two cotyledons per plant; ten plants per replication; three replications per trial and two trials. Reactions rated according to the 0-9 scale of Williams and Delwiche (1979).

Markers

For PG-4, 4,468 markers (~12%) were discarded prior to the analysis because they had MAF >0.05. When analyzing the accessions for principal components three PCs explained 25% of cumulative variation. The first two PCs could not to be clustered into separate groups (Fig 4.2), which means the population is mixed and does not have an explainable structure. The model controlling PCA scores and kinship was considered the best with an MSD = 0.0002 (Table 4.3)

and QQ-plots showing tight association between predicted and observed quantiles (Fig 4.3A and B). Three markers, each of them explaining 10% of the phenotypic variation, were significant at the 0.01 percentile tail (P < 3.88E-05) of the empirical distribution, and were located on chromosomes A03, C03 and C08 (Fig 4.4.A and B; Table 4.4; Fig 4.5). Seven other markers, each of them explaining 8-9% of the phenotypic variation, were significant at the 0.01 percentile tail (P < 2.30E-04) of the empirical distribution and were located on chromosomes A04, C05, C07 and C09 (Fig 4.4.A, B; Table 4.4; Fig 4.5).

A



B

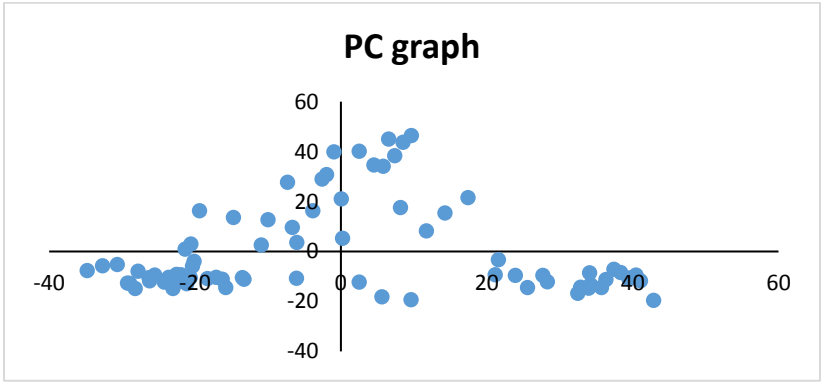
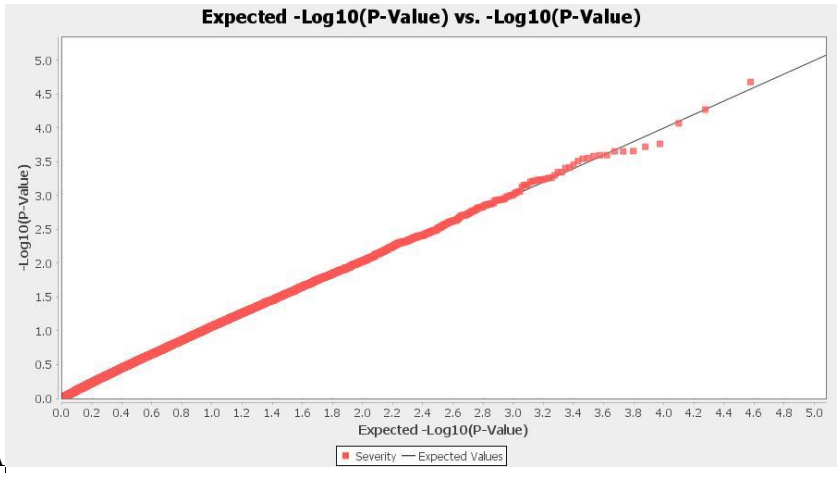


Figure 4.2. Distribution of genotypes based on the first two principal components for PG-4 (A) and PG-3 (B).

A



B

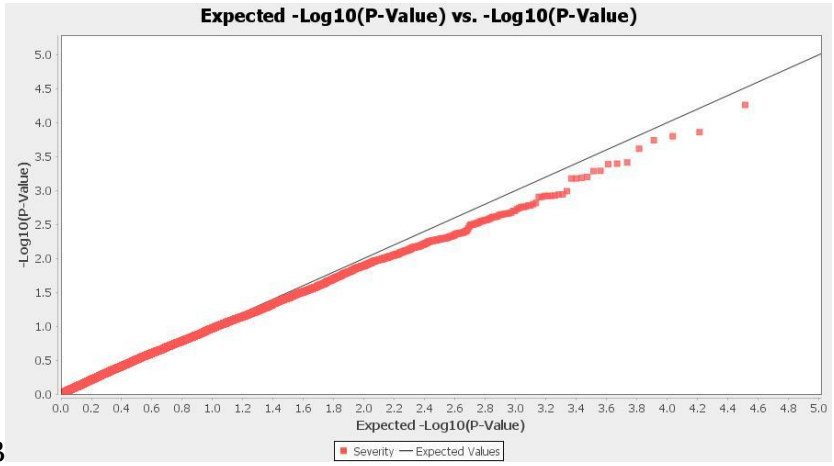


Figure 4.3. QQ plot for the best model for data to fit for PG-4 (MLM: PCA+K) (A) and PG-3 (GLM: PCA) (B) association analysis.

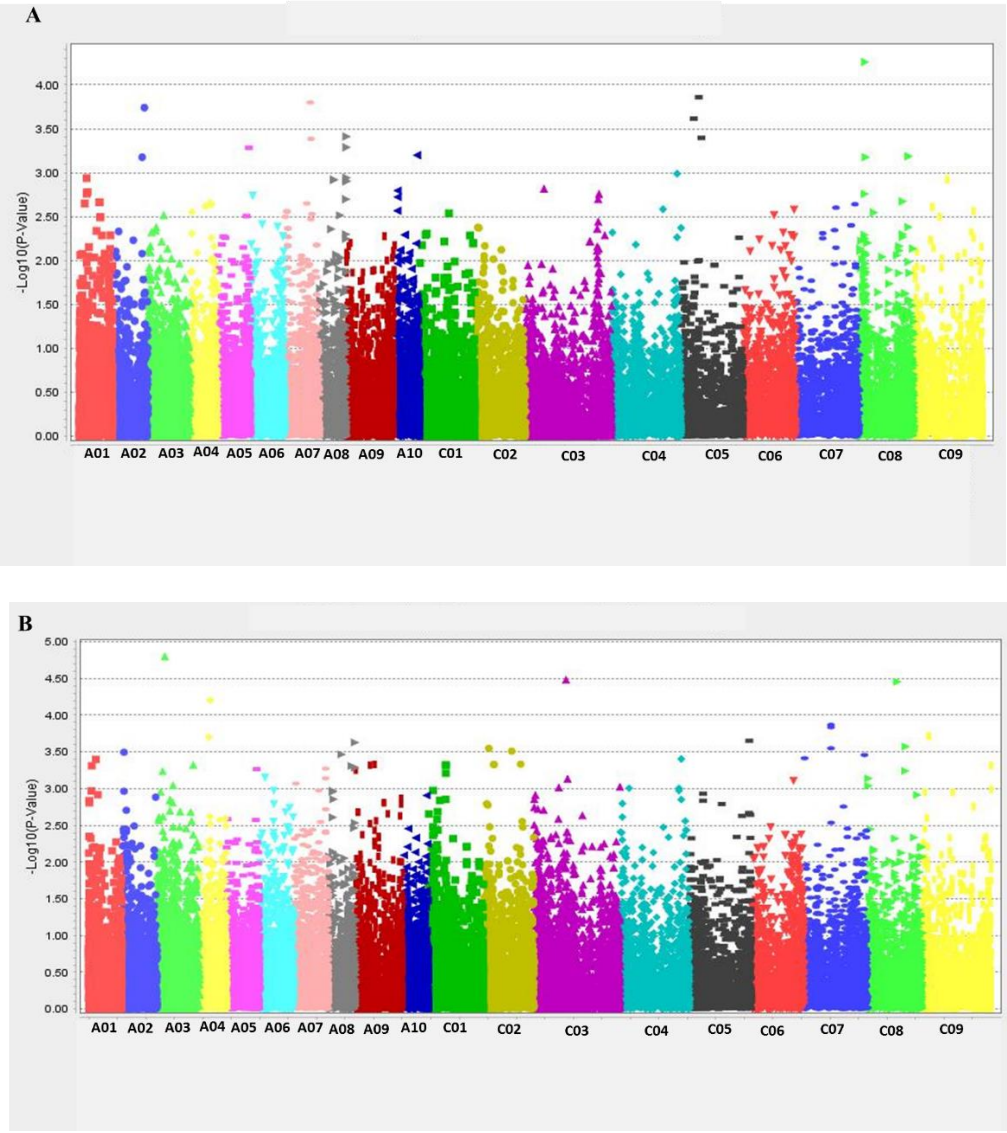


Figure 4.4. Manhattan plot for genome wide association study across 19 chromosomes of *Brassica napus* associated with resistance to *Leptosphaeria maculans*, PG-3 (A) and PG-4 (B) using single nucleotide polymorphism (SNP) markers. The y-axis shows negative base-10 logarithm of the association *p*-value for each SNP.

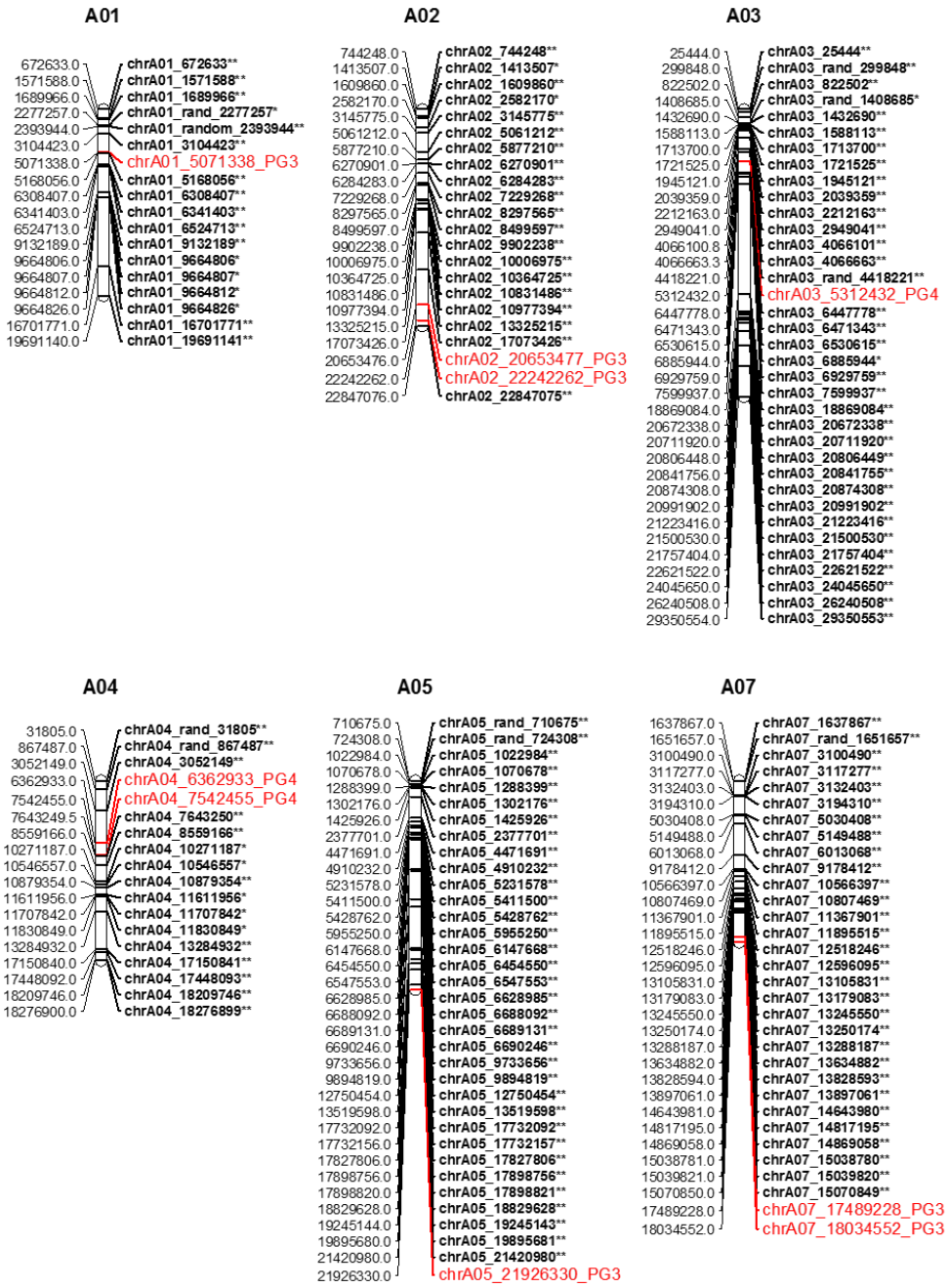


Figure 4.5. Physical map of *B. napus* chromosomes representing in red the location of Single nucleotide polymorphism markers associated with resistance to *Leptosphaeria maculans* in this study. * and ** represent markers identified by Rahman et al (2016) and Raman et al. (2016), respectively. The chromosome map was constructed using the Mapchart 2.2 program. Marker locations are expressed in base pairs (bp) and chromosome numbers are located at the top of each chromosome.

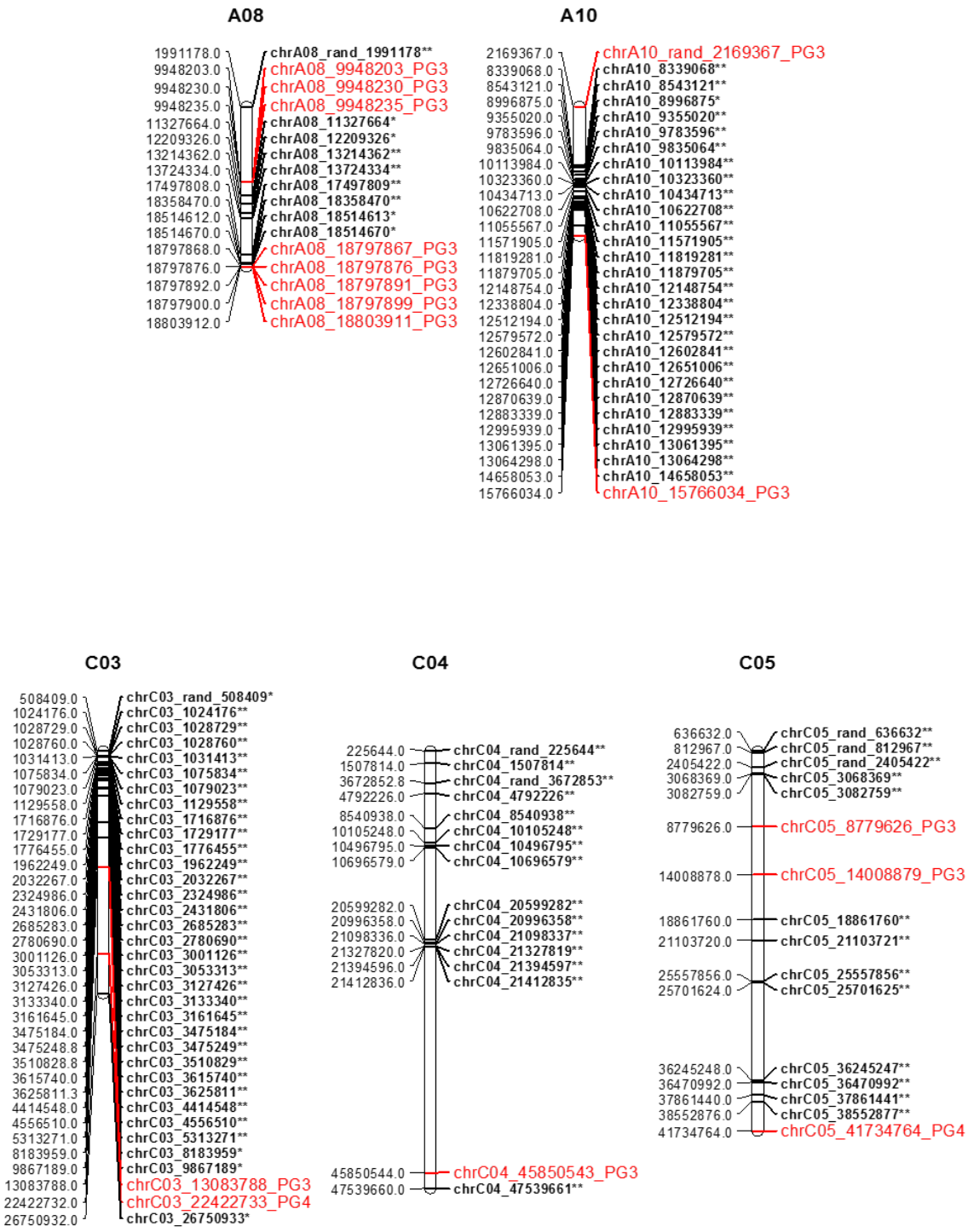


Figure 4.5. Physical map of *B. napus* chromosomes representing in red the location of Single nucleotide polymorphism markers associated with resistance to *Leptosphaeria maculans* in this study (continued). * and ** represent markers identified by Rahman et al (2016) and Raman et al. (2016), respectively. The chromosome map was constructed using the Mapchart 2.2 program. Marker locations are expressed in base pairs (bp) and chromosome numbers are located at the top of each chromosome.

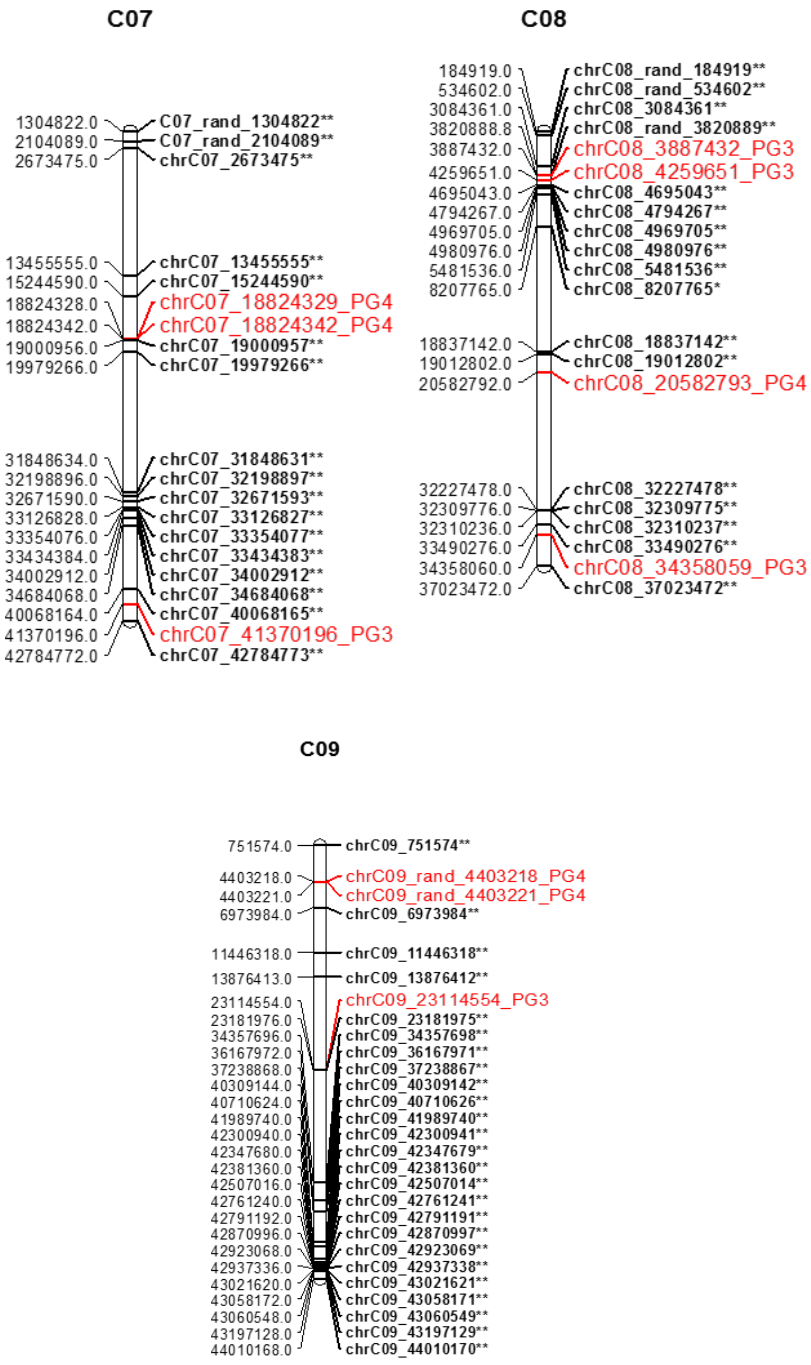


Figure 4.5. Physical map of *B. napus* chromosomes representing in red the location of Single nucleotide polymorphism markers associated with resistance to *Leptosphaeria maculans* in this study (continued). * and ** represent markers identified by Rahman et al (2016) and Raman et al. (2016), respectively. The chromosome map was constructed using the Mapchart 2.2 program. Marker locations are expressed in base pairs (bp) and chromosome numbers are located at the top of each chromosome.

Table 4.3

Test statistics for four models used to detect marker-trait associations for resistance to PG-3 and PG-4 of Leptosphaeria maculans

PG-3		PG-4	
Model	MSD[a]	Model	MSD[a]
Naïve	0.0005	K	0.002
K	0.004	Naive	0.0009
PCA	0.0002	PCA	0.002
PCA + K	0.003	PCA + K	0.0002

[a] Mean square difference.

For PG-3, 32,527 of the 37,734 markers were retained for analysis after meeting the >0.05 MAF threshold. Three principal components that explained 25 % of cumulative variation were used to control for population structure. The first two PCs could not to be clustered into separate groups (Fig. 4.2. B). The general linear model (GLM) which controls PCA was the best model (Fig 4.3. B). Six markers, at 0.01 percentile tail (P < 3.44E-04), and 20 markers at 0.1 percentile tail (P < 1.32E-03) of the empirical distribution were considered significant (Fig 4.2. B; Table 4.4). Of total markers for PG-3 and PG-4, 19 were located throughout the ten chromosomes of the A-genome and 17 were in C-genome's chromosomes (Table 4.4). These markers explained between 8 and 23% of the phenotypic variation. These markers were located on chromosomes A02, A07, C05 (Three markers) and C08 (Table 4.4). The R-square explained by these markers were 20-23%. Twenty additional markers located on chromosomes A01, A02, A05, A07, A08, A10, C03, C04, C07, C08 and C09 which explained 16-19% of phenotypic variation.

Table 4.4

Significant markers associated with resistance to Leptosphaeria maculans

Marker	Chromosome	Position	Pathogenicity Group (PG)	*P*-Value	R-Square
chrA02_22242262	A02	22,242,262	3	1.70E-04	21
chrA03_5312432	A03	5,312,432	4	1.60E-05	10
chrA07_17489228	A07	17,489,228	3	1.51E-04	21
chrC03_22422733	C03	22,422,733	4	5.64E-05	10
chrC05_8779626	C05	8,779,626	3	4.05E-04	20
chrC05_12177862	C05	12,177,862	3	1.53E-04	21
chrC05_14008879	C05	14,008,879	3	2.26E-04	20
chrC08_3887432	C08	3,887,432	3	5.64E-05	23
chrC08_20582793	C08	20,582,793	4	3.50E-05	10
chrA04_7542455	A04	7,542,455	4	6.26E-05	9
chrC07_18824329	C07	18,824,329	4	1.36E-04	8
chrC07_18824342	C07	18,824,342	4	1.46E-04	8
chrC09_rand_4403218	C09_rand	4,403,218	4	1.93E-04	8
chrC09_rand_4403221	C09_rand	4,403,221	4	1.93E-04	8
chrA04_6362933	A04	6,362,933	4	1.99E-04	8
chrC05_41734764	C05	41,734,764	4	2.24E-04	8
chrA08_18797867	A08	18,797,867	3	4.24E-04	19
chrC08_34358059	C08	34,358,059	3	4.31E-04	19
chrA07_18034552	A07	18,034,552	3	4.53E-04	19
chrA05_21926330	A05	21,926,330	3	5.52E-04	18
chrA08_18797876	A08	18,797,876	3	5.80E-04	18
chrA02_20653477	A02	20,653,477	3	6.90E-04	14
chrA10_15766034	A10	15,766,034	3	7.11E-04	18
chrC08_4259651	C08	4,259,651	3	7.47E-04	18
chrA01_5071338	A01	5,071,338	3	9.13E-04	14
chrC04_45850543	C04	45,850,543	3	0.00103	17
chrC07_41370196	C07	41,370,196	3	0.00103	17
chrA08_18803911	A08	18,803,911	3	0.00112	17
chrA10_rand_2169367	A10_rand	2,169,367	3	0.00114	17
chrC09_23114554	C09	23,114,554	3	0.00125	17
chrC03_13083788	C03	13,083,788	3	0.00128	17
chrA08_9948203	A08	9,948,203	3	0.00129	17
chrA08_9948230	A08	9,948,230	3	0.00129	17
chrA08_9948235	A08	9,948,235	3	0.00129	17
chrA08_18797891	A08	18,797,891	3	0.0013	17
chrA08_18797899	A08	18,797,899	3	0.0013	17

Candidate Genes

The significant marker for PG-4 on chromosomes C03 and C08 at 22.42 and 20.58 Mbp and PG-3 on chromosomes A07, C05 and C08 at 17.48, 8.77 and 3.88 Mbp were close to eight genes involved in plant defense family such as defensing and leucine-rich repeat and serine-threonine protein kinase protein (Table 4.5). PG-4 markers in chromosomes C03 and C08 were near genes BnaC03g37070D, BnaC03g37160D, BnaC08g16670D and BnaC08g16850D, respectively. These genes have been associated with defense response and signal transduction (Table 4.5). Similarly, four PG-3 markers, one in C08, two in A07 and one in C05, were near genes BnaC08g03770D, BnaA07g23000D, BnaA07g23030D and BnaC05g14810D that have been associated with defense responses too. Genes BnaA07g23000D and BnaA07g23030D are close to a single PG-3 marker. Similarly, genes BnaC08g16670D and BnC08g16850D are near PG-4 marker.

Table 4.5

Brassica napus Candidate genes associated with resistance to Leptosphaeria maculans

Brassica Gene Model	Marker	PG	Gene Starts	Gene Ends	Marker distance from gene(kb)	Function	Reference
BnaC03g37070D	chr C03_22422733	PG-4	22429506	22430398	7	Defense response	Sels et al. 2008
BnaC03g37160D	chr C03_22422733	PG-4	22478041	22480532	55	Leucine-rich repeat protein that mediates protein interactions, in signal transduction	You et al. 2010
BnaC08g16670D	chr C08_20582793	PG-4	20604503	20606027	22	signal transducer activity, defense response	
BnaC08g16850D	chr C08_20582793	PG-4	20685045	20687250	102	Involved in defense response to bacteria, fungi and other organisms	Ohata et al. 2013
BnaC08g03770D	chr C08_3887432	PG-3	3843296	3843945	44	Defense response to fungus	
BnaA07g23000D	chr A07_17489228	PG-3	17398503	17399429	91	Play a role in plant defense.	
BnaA07g23030D	chr A07_17489228	PG-3	17407929	17408486	81	Defense response to fungus.	
BnaC05g14810D	chr C05_8779626	PG-3	8732759	8734515	47	Repressor of jasmonate responses, regulation of defense response.	Chung and Howe 2009

Marker Validation

The reaction of each genotype was predicted based on presence of three significant markers for PG-4 presented in each of them, in the field condition. Westar used as susceptible control for disease reading in the field. As predicted, three resistant genotype had all three markers present and one, which showed moderately resistant, had one of the markers, and seven other genotypes, which had not any of these markers, showed susceptible reaction in the field (Table 4.6).

Table 4.6

Validation of the PG-4 markers in plants, which tested under field condition

	Markers				
Plant name	chrA03_5312432	chrA04_6362933	chrC08_20582793	Genotype based predicted phenotype	Disease reaction based phenotype
Oleifera	P	P	P	Resistance	R
Bolko	P	P	P	Resistance	R
Sumner	P	P	P	Resistance	R
Eckendorfer Mali	A	P	P	Intermediate	MR
Gido	A	A	A	Susceptible	S
Laura	A	A	A	Susceptible	S
Rico	A	A	A	Susceptible	S
Nabo	A	A	A	Susceptible	S
Titus	A	A	A	Susceptible	S
Legend	A	A	A	Susceptible	S
Colt	A	A	A	Susceptible	S

P=Present; A= Absent; R=Resistant; S=Susceptible; MR= Moderately resistance

Phylogenetic Analysis

The Splits Tree graph showed clear separation of 213 *B. napus* accessions into three distinct splits, however, there is no explanation for this clustering based on country of origin, growth habit and reaction to blackleg disease (Fig. 4.6).

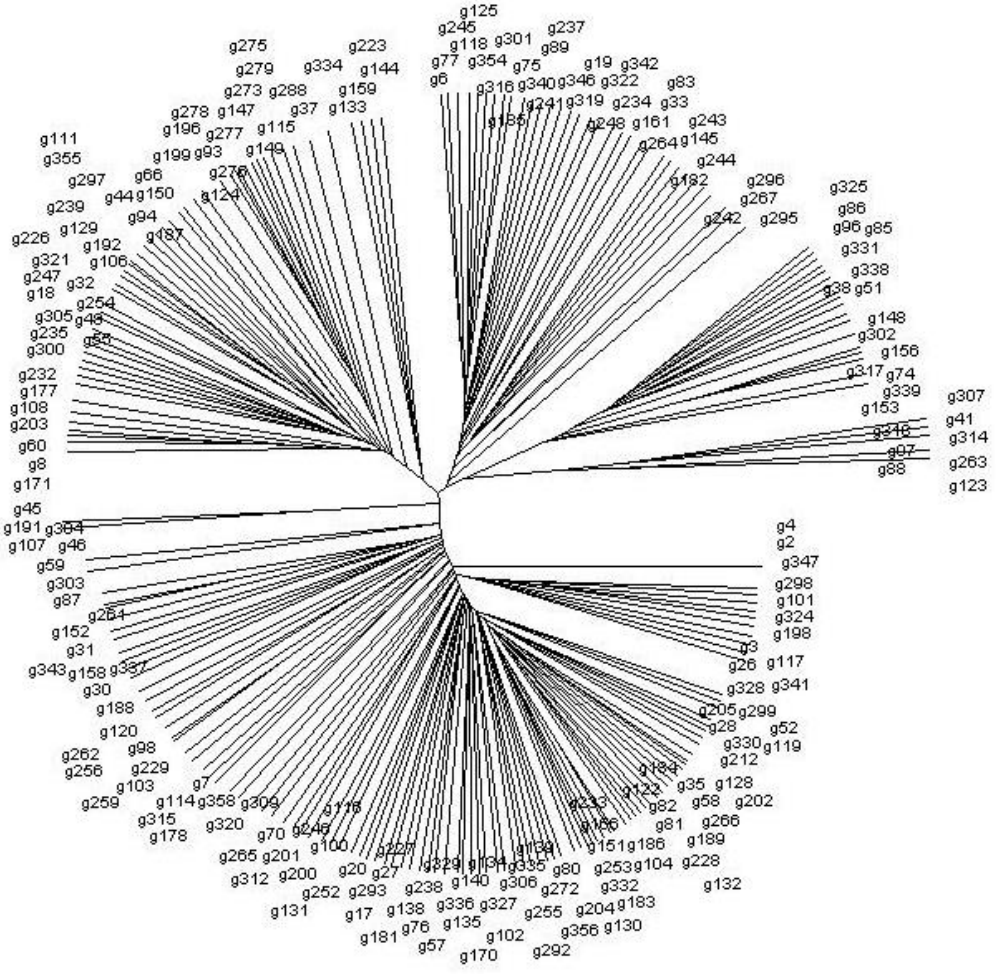


Figure 4.6. Phylogenetic analyses of population of 213 *Brassica napus* accessions using unweighted pair group method with arithmetic mean (UPGMA).

Discussion

Identification of durable resistance for canola is critical and necessary because of the destructive nature of blackleg. While qualitative resistance to blackleg can be more effective, it is typically not durable. Quantitative resistance on the other hand, may be less effective but could last longer. Numerous markers and QTL for resistance to blackleg have been found distributed on most of the chromosomes of *B. napus* (Raman et al. 2012; Raman et al. 2016; Rahman et al. 2016). In our study, markers were distributed almost equally well between the A and C genomes;

however, within the A genome, approximately 40% of them were in A08. In contrast, in the C genome, chromosomes C05 and C08 carried 25% of markers each.

An accurate assessment of the phenotyping response is very important for identification of markers associated with resistance to *L. maculans*. In this study, plants were inoculated at cotyledon stage and the median reaction of each genotype was calculated using 120 observations obtained from sets of 60 plants distributed in replicated studies conducted twice. Association studies in other pathosystems have been based on the reaction of fewer plants (Kertho et al. 2015). The first three categories in the severity scale used describe hypersensitive reactions that are typical of gene-for-gene interactions; however, for the association study median values were used rather than converting them into binary data (Bansal et al. 1994). Further, the cotyledon inoculation is the standard method used to evaluate resistance to *L. maculans* and has been shown to correlate well with adult plant reactions in the field (Li et al. 2006; Mansouripour and del Rio 2017).

Genome-wide association study is a good strategy for understanding the occurrence of phenotypic variation and its relationship with specific genetic sequences (Aranzana et al. 2005). The development of new technologies such as genotype by sequencing (GBS) and high-throughput single nucleotide polymorphism (SNP) genotyping platforms are facilitating the use of high resolution GWAS in canola (Wei et al. 2016; Qian et al. 2014). The efficiency of this tool is affected by population size, genetic diversity of the population, accuracy of phenotypic evaluation and marker density (Liu et al. 2016; Hong and Park 2012). A recent review of the use of association mapping in plants indicated that populations' sizes ranging from 57 to > 500 are being used in GWAS studies that identified markers associated with different plant traits including disease resistance (Zhu et al. 2008). In the present study, a panel of 213 genotypes were evaluated for their

reaction to inoculation with PG-4 of *L. maculans* while a subset of 78 genotypes were evaluated for their reaction to PG-3.

The identification of numerous markers associated with resistance to blackleg has been published in recent years (Raman et al. 2016; Rahman et al. 2016; Jestin et al. 2011). In this study, we report 36 significant SNP markers associated with blackleg resistance in canola These markers were located on chromosomes A01, A02, A03, A04, A05, A07, A08, A10, C03, C04, C05, C07, C08, and C09.

We explored genes around the significant markers to find genes that may be potentially involved in resistance to blackleg disease. The region of 105 kb on both sides of each marker were selected because of low LD of the population, to search for genes, which may be involved in resistance. Most of the candidate genes found were involved in plant defense response or belong to leucine-rich repeat (LRR) protein family (Marone et al. 2013). This family has many proteins, which act in plant defense system against all kinds of pathogens such as fungi, Oomycetes, nematodes, bacteria, and viruses. For instance, Mi-1 protein in tomato against *Meloidogyne* sp. (Milligan et al. 1998), I2 protein in tomato against *Fusarium oxysporum* (Simons et al. 1998), RPP8 in *Arabidopsis* against *Peronospora parasitica* (Van Der Biezen and Jones 1998), RPS5 in *Arabidopsis* against *Pseudomonas syringae* (Warren et al. 1998) and N in tobacco against Tobacco mosaic virus(TMV). Therefore, it is most likely one of these candidate genes for resistance to blackleg be from this family.

Three markers including chrA03_5312432, chrA04_6362933, and chrC08_4259651, which contribute, 10, 8 and 18% to the phenotype variation, were used for validation using data collected in a field trial. Because these markers were present in all of resistance and absented in all of susceptible genotypes tested in the field. Therefore, these markers for resistance genes were

validated. These markers can be used as a tool to prevent from extensive selection in breeding programs and remove any susceptible entry from segregating population for blackleg disease.

Altogether, this paper provides information that expands our understanding of the genes affecting resistance to blackleg in canola. The markers identified in this study, however, will have to be validated before being used in a marker-assisted selection program.

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CHAPTER 5. CHARACTERIZATION OF REACTION OF ELITE CANOLA/RAPESEED TO *LEPTOSPHAERIA MACULANS*

Abstract

Blackleg disease, caused by the fungus *Leptosphaeria maculans*, is a major disease of canola (*Brassica napus*) worldwide. A set of six *L. maculans* isolates was used to characterize seedling resistance in 24 commercial hybrids, five breeding lines and nine blackleg resistant plant introductions (PIs). All plant genotypes were inoculated at the seedling stage in greenhouse and were rated ten days after inoculation based on 0-9 scale. The results showed that resistance gene *Rlm9* was present in 18% of the genotypes evaluated; *Rlm2* and *Rlm3* were each present in 16% of them, while *LepR1, Rlm4* and *Rlm5* were present in 11, 5, and 5%, respectively. However, the presence of R genes could not be inferred on 29% of the genotypes evaluated. Approximately 18% of the genotypes were susceptible to all the races used. Reaction of these plant genotypes to three North Dakota races of blackleg were also evaluated. The characterized hybrids could be used in a hybrid rotation with different resistance gene program, in order to prevent from any resistance break. Moreover, the resistance sources in breeding lines and PIs could be used in breeding programs. Two-thirds of the commercial hybrids were susceptible to all three ND isolates, an indication that progress needs to be made to prevent severe epidemics in the near future. Also, 60% of the breeding lines were resistant to ND races, this is a good sign. Finally, additional races should be used to characterize reaction of germplasm.

Introduction

Leptosphaeria maculans, the causal agent of blackleg of canola is a serious threat to canola industry around the world (Fitt et al. 2006). Blackleg affects canola plants at all growth stages but infections that take place when plants are younger than the 5-6 leaf stage because the most severe

yield losses. Economic losses of more than $900 million in Europe, North America and Australia have been attributed to this disease (West et al. 2001; Howlett 2004). Therefore, management of this disease should be considered as a major objective of canola breeding programs. While planting resistant varieties is the most effective, environmental friendly and cost-effective strategy to manage this disease (Salisbury et al. 1995; Sprague et al. 2006), breeding durable resistance is a challenge as the pathogen has the ability to quickly change the virulence profile of its populations in response to selection pressure exerted by major resistance genes in plants (Sprague et al. 2006).

Early attempts to characterize the virulence of *L. maculans* isolates on canola plants resulted in the creation of the first differential set that was composed by three cultivars, Westar (susceptible, spring type), Quinta and Glacier (winter types) (Koch et al. 1991; Mengistu et al. 1991). Using this differential set, *L. maculans* isolates were classified into four pathogenicity groups (PG) 1, 2, 3 and 4 (Table 5.1). In the same year, Badawy et al. (1991) replaced Westar with the winter-type cultivar Lirabon and added Jet Neuf to the set of differentials. Isolates evaluated on this new set were divided into six pathogenicity groups that were called A1-A6 (Badawy et al. 1991; Kuswinanti et al. 1995; Ansan-Melayah et al. 1998). *L. maculans* races follow gene for gene interactions and each PG could have multiple races (Dilmaghani et al. 2009). The formation of races in *L. maculans* is accomplished by the presence in the pathogen of different avirulence genes (*AvrLm*), the first of which was identified in 1995 by Ansan-Melayah and collaborators (Ansan-Melayah et al. 1995). To date, 16 avirulence genes have been identified; of these, seven have been cloned, *AvrLm1* (Gout et al. 2006), *AvrLm2* (Ghanbarnia et al. 2015), *AvrLm3* (Plissonneau et al. 2016), *AvrLm5/AvrLmJ1* (Van de Wouw et al. 2014), *AvrLm4-7* (Parlange et al. 2009), *AvrLm6* (Fudal et al. 2007) and *AvrLm11* (Balesdent et al. 2013).

Table 5.1

Reaction[a] of differential B. napus cultivars to inoculation with different pathogenicity groups (PGs) of Leptosphaeria maculans. (Adopted from Rouxel et al. 2003 with modifications).

Virulence groups		*B. napus* differentials[b]			
Mengistu et al. 1991	Badawy et al. 1991	Westar Lirabon (None)	Glacier (*Rlm2*)	Quinta (*Rlm1*,*Rlm3*)	Jet Neuf (*Rlm4*)
PG-4	A1	S[a]	S	S	S
	A5	S	S	S	R
PG-3	A2	S	S	R	S
	A6	S	S	R	R
PG-2	A4	S	R	R	S
	A3	S	R	R	R
PG-1	-	R	R	R	-

[a] S= susceptible reaction; R= Resistant reaction; -=not tested
[b] Cultivar names (resistance genes)

There are two types of genetic resistance to blackleg in canola, Qualitative and quantitative. Qualitative resistance, also known as race-specific resistance is governed by single major genes and is expressed as hypersensitive reaction of plants to inoculation with a pathogen that carries the corresponding avirulence gene (Ansan-Melayah et al. 1997). This resistance can be expressed at all growth stages and in all parts of the plant, but because it can be identified at the seedling stage (Ansan-Melayah et al. 1998), it is also called seedling resistance. To date, 18 *L. maculans*-resistance genes have been identified among Brassica species (Zhang et al. 2015). Of these, genes *Rlm2* (Larkan et al. 2015) and *LepR3* (Larkan et al. 2013) which, interacts with a single avirulence gene, *AvrLm1*, and have been cloned in canola. Resistance genes *Rlm1*, *Rlm3*, *Rlm4*, *Rlm7* and *Rlm9* are located on chromosome A07 (Ferreira et al. 1995; Mayerhofer et al. 1997; Ansan-Melayah et al. 1998; Zhu and Rimmer 2003; Rimmer 2006; Delourme et al. 2006); resistance genes *Rlm2*, *LepR2* and *LepR3* are located on chromosome A10 (Yu et al. 2005; Larkan et al. 2013; Ansan-Melayah et al. 1998); and resistance gene *LepR1* is located on chromosome A02 (Yu et al. 2005). In contrast, quantitative resistance, or non-race specific is governed by multiple genes

and is more easily detected at the adult plant stage, quantitative resistance can be expressed in different ways, one of them being the production of smaller cankers on crown (Balesdent et al. 2001).

Qualitative resistance offers complete protection to plants and because of it, it creates high selection pressure on the pathogen to adapt. Once isolates with new virulence profiles are selected they can render qualitative genes useless in short periods of time (Delourme et al. 2006). Two approaches that can reduce the level of selection pressure on the pathogen and consequently extend the shelf-life of cultivars carrying major resistance genes are: the inclusion of strong adult-plant resistance traits into a plant genotype (Brun et al. 2010) and the establishment of variety rotations in production systems. To implement the latter, it is necessary to identify sources of major resistance genes on available germplasm as well as on breeding lines and commercial cultivars

The objective of this study was to infer the presence of R genes present in several commercial canola hybrids, elite *B. napus* plant introduction materials and elite canola breeding lines.

Materials and Methods

Two types of studies were conducted in greenhouse. The first was to infer the presence/absence of resistance genes based on the interaction of plant genotypes and *L. maculans* isolates with known avirulence genes. The second was to characterize the reaction of these genotypes to North Dakota isolates.

Brassica napus Commercial Hybrids, Breeding Lines and PIs

Twenty-four commercial canola hybrids grown in North Dakota *and* produced by 11 seed companies were used in this study (Table 5.2) along with nine elite plant introductions (PIs)

considered resistant to PG-4 of blackleg and five elite North Dakota State University (NDSU) canola breeding lines (Table 5.3).

Table 5.2

List of commercial canola hybrids used in this study

Seed producers	Varieties	Type [a]	Blackleg Rating [b]
Brett Young	6040RR	H, TR	R
Croplan	HyCLASS 940	H, TR	R
Croplan	HyCLASS 988	H, TR	R
Dekalb	DKL30-42	H, TR	R
Dekalb	DKL51-45	H, TR	R
Dekalb	DKL72-55	H, TR	MR
DL Seeds	30512-D8	H, TR	R
Bayer	InVigor 8440	H, LL, TR	R
Bayer	InVigor L130	H, LL, TR	R
Bayer	InVigor L150	H,LL, TR	R
Mycogen	CL166102H	HO	R
Mycogen	CL166103H	HO	R
Croplan Genetics	XCEED Oasis CL	OP, CL,TR	R
Pioneer	45S52	TR	MR
Croplan Genetics	HyCLASS 955	TR	R
Brett Young	BY11-860	TR	R
Integra Fortified Seed	7152R	TR	R
Monsanto	G99402	TR	R
Monsanto	G08039	TR	R
Monsanto	G99396	TR	R
Monsanto	G98739	TR	R
Monsanto	G88605	H, TR	R
Cargill	07H874	H, TR	R
Croplan Genetics	Exp021	H, TR	R

[a] LL : tolerance to Liberty (glufosinate ammonium) ; CL: Clearfield (imazamox) herbicides. H: Hybrid; TR: Traditional oil type; OP: Open pollinated

[b] The rating is provided by each seed producer. R: Resistance; MR: Moderately resistance

Table 5.3

List of B. napus Plant Introductions (PIs) and canola breeding lines used in this study

Genotypes	Type	Growth habit	Country of origin
77-71	Plant introduction	-[a]	United States
Aomori-1	Plant introduction	-	South Korea
Iwao natane	Plant introduction	-	South Korea
Nabo	Plant introduction	-	South Korea
Oleifera	Plant introduction	-	South Korea
Su Weon Cheg	Plant introduction	Semi-winter-type	South Korea
Bolko	Plant introduction	Winter-type	Poland
CR 165/76a	Plant introduction	-	Germany
CR 167/65a	Plant introduction	-	Germany
ND-662c	Breeding line	Spring-type	United States
NDSU-9071	Breeding line	Spring-type	United States
NDSU-9020	Breeding line	Spring-type	United States
NDSU-9067	Breeding line	Spring-type	United States
NDSU-h119	Breeding line	Spring-type	United States

[a] Not determined

Fungal Material

A set of four well-characterized *L. maculans* isolates, D4 (*AvrLm4,5,6,7,8,S,LepR1*), D5 (*AvrLm1,2,4,7,S,LepR1*), D10 (*AvrLm5,6,8,9,S,LepR1),* and D18 (*AvrLm3,5,6,(8),S,LepR1*), provided by Dr. Angela Van de Wouw (University of Melbourne, Australia) (Table 5.4) and called differentials from now on were used for the first study (Van de Wouw et al. 2017). Three *L. maculans* isolates BLK-428,295, and 85 from North Dakota were used for the second study.

Table 5.4

Presence/absence of different avirulence genes of different races of L. maculans used in this study

Avirulence genes	Isolates			
	D4	D5	D10	D18
AvrLm1	-[a]	+	-	-
AvrLm2	-	+	-	-
AvrLm3	-	-	-	+
AvrLm4	+[b]	+	-	-
AvrLm5	+	-	+	+
AvrLm6	+	-	+	+
AvrLm7	+	+	-	-
AvrLm8	+	_	+	nd [c]
AvrLm9	-	-	+	-
AvrLmS	+	+	+	+
AvrLmLepR1	+	+	-	+

[a] Absence of gene
[b] Presence of gene
[c] Not determined

Plant and Inoculum Preparation

Seeds from all plant genotypes were planted in batches containing 38 entries at a time and cv. Westar served as the susceptible control. Westar is no longer a commercial cultivar and has been used as standard susceptible control because it does not carry known blackleg resistance genes (Balesdent et al. 2005). Entries in each batch were evaluated using a randomized complete block design (RCBD) with three replications and conducted twice. Seeds were placed in individual plastic cells in trays (44 x 57 x 50 mm) filled with soilless potting mix (PRO-MIXR BX, Premier Tech Horticulture, Quakertown, PA) and kept in greenhouse room at 20 ± 2 C with 16 h light daily supplemented with 600-watt high pressure sodium lamps (P.L. Light Systems, Inc., Beamsville, Ontario, Canada). Single-spore cultures of each *L. maculans* isolate were produced in separate dishes containing V8 agar medium as described by Nepal et al. (2014). After harvest, spore

concentrations of each isolate were estimated with help of a hemocytometer and adjusted to 10^7 spores/ml.

Characterization of The Reaction of B. napus Materials to Inoculation

Twelve-days old seedlings were inoculated on the center of each cotyledon leaf by lightly pricking its surface once with sharp forceps and depositing a 10 µl droplet of the spore suspension on the wound. The inoculated seedlings were incubated in cool mist chambers at 20°C and 98% humidity in dark for 24 h and then returned to the greenhouse room. Each isolate was inoculated onto ten seedlings and the trial was repeated one more time. The cultivar Westar without any resistance genes was used as susceptible control for each trial. The reaction of cotyledon leaves was recorded ten days after inoculation based on the 0-9 scale of Williams and Delwiche (1979). The median response of each plant genotype to every isolate was calculated and the genotype was considered resistant if its median was ≤ 5 or susceptible if it was > 5 (Kutcher et al. 2007).

Results

Prevalence of Rlm Genes In Canola Hybrids/Accessions

The presence or absence of R genes were inferred based on reaction of the plant genotypes to inoculations with four *L. maculans* isolates. Of the 38 genotypes evaluated, 27 (71%) showed resistance to at least one differential race. Our results showed that *Rlm9* was the major R gene prevalent in commercial hybrids, while, *Rlm2* was dominant in *B. napus* PIs and only one breeding line was characterized which had *LepR1* and the rest remained unknown (Table 5.5). Around 55% of PIs, 83% of hybrids and all breeding lines showed seedling resistance to at least one of the differentials. Four PIs were susceptible to all the differentials isolates used and therefore no R genes were inferred for these accessions. Further, some of the PIs carried uncharacterized resistance genes that could not be inferred using the four differential isolates (Table 5.6). This type

of resistance was considered as unknown resistance in this study. Among the R genes detected, *Rlm9* was present in seven entries, both *Rlm2* and *Rlm3* were detected in six entries, while, *LepR1* in four and *Rlm4* and *Rlm5* in two genotypes. Four breeding lines had an unknown R gene. Three hybrids and four PIs were susceptible to all of isolates (Table 5.6). Three North Dakota races were evaluated based on their reaction to the hybrids, breeding lines and PIs and the reactions of entries were different from Australian races.

Table 5.5

Summary of R genes in B. napus hybrids, Plant introductions and breeding lines

	Frequencies			
Putative R gene	Hybrids	PIs	Breeding lines	Total
Rlm9	6	1	0	7
Rlm3	5	1	0	6
LepR1	2	1	1	4
Rlm2	4	2	0	6
Rlm4	2	0	0	2
Rlm5	2	0	0	2
None [a]	3	4	0	7
Unknown [b]	0	0	4	4

[a] None means no resistance gene(s) could not be found.
[b] Unknown means the resistance gene(s) could not be determined

Table 5.6

Reaction of B. napus hybrids, Plant Introductions (PI) and breeding lines (B-line) to the different races [a] of Leptosphaeria maculans

Entries	Entry type	D4 (*AvrLm4, 5,6,7,8,S, LepR1*)	D5 (*AvrLm1,2,4, 7,S,LepR1*)	D10 (*AvrLm5,6,8,9,S ,LepR1)*	D18 (*AvrLm3,5,6,(8),S, LepR1*)	BLK-428 *(AvrLm 6, 11)*	BLK-295 *(AvrLm 6, 11)*	BLK-85 *(AvrLm 6, 11)*	R gene
EXP021	Hybrid	7[b]	2	7	3	7	7	7	*Rlm2*
InVigor L150	Hybrid	9	7	1	7	7	7	7	*Rlm9*
InVigor L130	Hybrid	7	1	9	5	3	7	9	*Rlm2*
InVigor 8440	Hybrid	1	7	1	3	7	8	9	*Rlm5*
BY11-860	Hybrid	7	7	7	7	9	9	7	None
6040RR	Hybrid	7	1	7	9	3	9	6	*Rlm2*
07H874	Hybrid	1	1	7	7	3	1	2	*Rlm4*
HyClass 988	Hybrid	7	5	1	7	5	7	7	*Rlm9*
HyClass 955	Hybrid	9	7	1	5	7	5	9	*Rlm9*
HyClass 940	Hybrid	7	1	7	7	-[b]	7	9	*Rlm2*
XCEED Oasis CL	Hybrid	0	0	0	0	0	0	0	*LepR1*
DKL72-55	Hybrid	5	7	7	1	1	5	-	*Rlm3*
DKL30-42	Hybrid	7	7	9	2	7	7	7	*Rlm3*
DKL51-45	Hybrid	7	7	1	7	7	5	7	*Rlm9*
30512-D8	Hybrid	7	5	9	7	5	8	7	None
7152R	Hybrid	7	9	7	1	7	9	7	*Rlm3*
G99396	Hybrid	1	1	9	7	5	2	3	*Rlm4*
G98739	Hybrid	7	7	7	2	7	8	9	*Rlm3*
G88605	Hybrid	7	7	7	9	9	9	7	None
G08039	Hybrid	1	7	1	1	7	9	7	*Rlm5*
G99402	Hybrid	7	9	7	3	9	5	7	*Rlm3*
CL166103H	Hybrid	9	7	1	7	7	3	9	*Rlm9*
CL166102H	Hybrid	7	7	1	7	9	7	7	*Rlm9*
45S52	Hybrid	1	1	1	3	-	1	3	*LepR1*
469821	PI	7	9	7	7	-	-	-	None

Table 5.6

Reaction of B. napus hybrids, Plant Introductions (PI) and breeding lines (B-line) to the different races [a] of Leptosphaeria maculans (continued)

Entries	Entry type	D4 (*AvrLm4, 5,6,7,8,S, LepR1*)	D5 (*AvrLm1,2,4, 7,S,LepR1*)	D10 (*AvrLm5,6,8,9,S ,LepR1)*	D18 (*AvrLm3,5,6,(8),S, LepR1*)	BLK-428 *(AvrLm 6, 11)*	BLK-295 *(AvrLm 6, 11)*	BLK-85 *(AvrLm 6, 11)*	R gene
458980	PI	2	1	2	3	-	-	-	*LepR1*
633120	PI	7	1	7	9	-	-	-	*Rlm2*
470000	PI	7	7	9	3	-	-	-	*Rlm3*
469944	PI	5	7	3	7	-	-	-	*Rlm9*
469726	PI	7	1	7	9	-	2	3	*Rlm2*
470031	PI	7	7	9	9	7	7	9	None
633134	PI	9	7	7	7	7	8	7	None
633135	PI	9	7	7	7	-	-	-	None
ND-662c	B-line	2	1	3	1	3	2	9	*LepR1*
NDSU-9071	B-line	3	0	7	2	9	1	3	Unknown
NDSU-9020	B-line	1	1	1	9	1	1	4	Unknown
NDSU-9067	B-line	1	1	2	7	7	7	7	Unknown
NDSU-h119	B-line	7	1	7	3	7	5	9	Unknown

[a] Race is a subgroup or biotype within a species or variety, distinguished from other races by virulence, symptom expression, or host range, but not by morphology.

[b] 0-5 consider resistant; 6-9 consider susceptible; each number is average median severity of 120 readings of two trials.

[nd] accession was not tested.

Discussion

The qualitative resistance in background of quantitative is one of the best ways to have a durable and stable resistance. Qualitative resistance is controlled by single major genes. While qualitative resistance is more effective in protecting the plants, it also exerts high selection pressure on the pathogen to evolve. This change will most likely occur through mutations in their avirulence genes that allow it to escape from recognition by corresponding *Rlm* gene products (effector) in plant. When this happens, resistance breaks. On the other hand, quantitative resistance is controlled by multiple genes. Since each gene contributes low levels of protection, the selection pressure exerted on the pathogen is lower and thus quantitative resistance lasts longer than qualitative resistance. In many instances, the expression of quantitative resistance genes is affected by environmental conditions and thus is difficult to measure (McDonald & Linde 2002b). At the same time, the pathogen can evolve to overcome quantitative resistance. This process is called "erosion". Marcroft et al. (2012) showed that the protection provided by major gene *Rlm6*, to a group of isogenic lines without quantitative resistance was overcome in three years, but cultivars carrying *Rlm6* in a quantitative resistance background still was effective after three years.

Of the 24 hybrids evaluated, 58 to 75% of them were susceptible to the three North Dakota isolates used. This is significant because it means not enough progress has been made to incorporate major resistance genes that are effective against blackleg isolates prevalent in the region. A study conducted by Marino (2011) on seedlings in greenhouse conditions, indicated that all commercial hybrids were susceptible to PG-4 of blackleg. Marino (2011) examined reaction of 75 hybrids to PG-4 and found all were susceptible. While all evaluations were made at seedling stage, there is a significant correlation between reaction of seedling and adult plants as qualitative resistance is expressed at all stages of plant development. To make a more accurate assessment of

the progress made by the seed industry, however, additional work is required to determine whether quantitative resistance is present in these hybrids.

Pyramiding multiple resistance genes and planting mixture of cultivars with different resistance genes are strategies intended to reduce selection pressure on the pathogens and to slow down development of epidemics. These cause direct selection towards races with all corresponding virulence alleles. Gene pyramiding will be ineffective, however, when the pathogen population has recombination events (McDonald & Linde 2002a). Since the number of recombination events in *L. maculans* is high, gene pyramiding may not be an effective management strategy to control blackleg. For example, *L. maculans* overcame *Rlm1* and *RlmS* in sylvestris-derived cultivars within three years from their release (Rouxel et al. 2003). A management alternative would be to rotate cultivars that contain different resistance genes. The information generated by this research provides a start point for this.

Resistance genes *Rlm2* and/or *Rlm3* were widely used at the beginning of the 1990s to provide protection against strains of PG2, which were the most prevalent group during that time (Nepal et al. 2014). In this study, their presence was inferred on several hybrids and is likely a consequence of using lines derived from parental materials developed back then (Zhang et al. 2016).

Presence of resistance genes could not be inferred in 11 genotypes evaluated in this study. It is possible that the avirulence genes load on these differentials has not been thoroughly characterized. It is also possible that gene interactions may be masking the effect of some avirulence genes (Larkan et al. 2015; Plissonneau et al. 2016).

Our study showed that most of hybrids carry *Rlm9*, *Rlm2* and *Rlm3*. The former could be a useful source of resistance but the latter two have already been defeated by strains of PG-4

common in the region (del Río Mendoza et al. 2012). In addition, a recent study that characterized the prevalence of avirulence genes of *L. maculans* in North Dakota (Chittem et al. 2015) revealed a high frequency of prevalence of *AvrLm4-7*, *AvrLm6* and *AvrLm11*. Consequently, the incorporation of resistance genes *Rlm6*, *Rlm4-7*, *Rlm9*, and *Rlm11* in cultivars to be used in North Dakota should provide effective protection.

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CHAPTER 6. GENERAL CONCLUSION

Leptosphaeria maculans, causal agent of blackleg, represents a serious threat to the canola (*Brassica napus*) industry in North Dakota as its capability to inflict severe yield reductions is complemented by the widespread prevalence of isolates for which effective genetic resistance is still not commercially available. The most prevalent *L. maculans* pathogenicity group (PG) in North Dakota is PG-4, which can overcome resistance genes *Rlm1*, *Rlm2* and *Rlm3*; the second most prevalent group is PG-3, which also overcomes resistance genes *Rlm2* and *Rlm3*. The objectives of this study were to identify sources of resistance to these groups among a worldwide collection of *B. napus* germplasm; to identify molecular markers associated with disease resistance; and to characterize the reaction of elite germplasm, commercial and otherwise, to several blackleg races

For the first objective, replicated greenhouse screenings were conducted in the greenhouse, using mixtures of five races belonging to each PG. Plants were inoculated at the seedling stage and their reaction was recorded 12 after inoculation. A total of 60 seedlings from each accession were evaluated in replicated trials conducted twice. A group of 29 plant introduction materials was considered highly resistant to PG-4 at seedling stage. However, none of these materials was resistant to both groups. Nineteen PG-4 resistant accessions were evaluated at the adult plant stage in replicated field trials conducted for three years. Accessions, CR 165/76a, Aomori-1 and Sumner showed significantly greater levels of resistance to blackleg than the commercial hybrids used as controls in all trials. These three lines should be considered as strong sources of resistance to be transferred into modern canola breeding lines.

For the second objective, molecular markers associated with resistance to PG-3 and PG-4 were identified using genome-wide association study (GWAS). 32,527 and 33,266 single

nucleotide polymorphisms were obtained from seedlings of the 78 and 213 accessions screened in the greenhouse for resistance to PG-3 and PG-4, respectively. A total of 36 significant markers were identified; this includes 26 markers for PG-3, each of them explaining on average 14 to 26% of the phenotypic variability and 10 markers for PG-4 each of them explaining on average 8 to 10% of the phenotypic variation observed. The markers were in 14 of the 19 chromosomes of *B. napus* genome. Three of these markers were successfully validated under field conditions. Future work will be conducted to validate the remaining markers. Mining of the Arabidopsis data bank using the sequences of markers identified in this study led to the identification of eight candidate genes whose functions have been associated with plant defense response system. Additional studies are needed to characterize the role these genes play during the infection process.

For the third objective, 24 hybrids, 5 breeding lines and 9 elite accessions were inoculated with four differential races of *L. maculans* (*AvrLm4,5,6,7,8,S,LepR1*, *AvrLm1,2,4,7,S,LepR1*, *AvrLm5,6,8,9,S,LepR1*, and *AvrLm3,5,6,(8),S,LepR1*) at the seedling stage in greenhouse conditions. At the end, it was revealed that *Rlm9* was present in 18% of the plant genotypes evaluated, while, *Rlm2* and *Rlm3* were each present in 16% of them, *LepR1* was present in 11% of them, and *Rlm4* and *Rlm5* were each present in 5% of them. We were not able to infer the presence of R gene(s) for 29% of the plant genotypes evaluated. Two thirds of the commercial hybrids evaluated were susceptible to blackleg races from North Dakota but almost 40% of the NDSU breeding lines evaluated were resistant to them.

In summary, sources of effective resistance against the two most prevalent pathogenicity groups of *L. maculans* in North Dakota and molecular markers associated with it have been identified. These accessions are a valuable resource for canola breeding programs that are creating blackleg-resistant canola materials for the region.

APPENDIX A. MEDIAN, MEAN RANK, AND RELATIVE TREATMENT EFFECTS ALONG WITH 95% CONFIDENCE INTERVALS FOR BLACKLEG SEVERITY RATINGS IN RELATION TO *BRASSICA NAPUS* ACCESSIONS AND PG-4 ISOLATES OF *LEPTOSPHAERIA MACULANS*

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
Ames 2793	Ceskia Tabor	Czechoslovakia	9	443.42	0.89	0.81	0.94
Ames 6099	Gorczanski		9	373.75	0.76	0.53	0.90
Ames 6102	Tandem	France	9	387.00	0.78	0.57	0.91
Ames 15650	Arco C10-2	Netherlands	3	103.33	0.20	0.15	0.28
Ames 15651	BO-63	Canada	9	419.08	0.85	0.66	0.94
Ames 15652	BO-72	United States, California	8	283.33	0.56	0.29	0.81
Ames 15653	CrGC-5	United States, California	9	451.19	0.90	0.81	0.95
Ames 15654	Bienvenu	United States, California	9	351.00	0.70	0.42	0.89
Ames 18935	Hobson	United Kingdom, England	3	85.58	0.16	0.08	0.31
Ames 26626	Siberian	United States, California	9	317.08	0.63	0.34	0.85
Ames 26627	Siberian	United States, California	8	286.75	0.57	0.29	0.82
Ames 26635	Polo Canola	United States, Wisconsin	7	305.17	0.60	0.35	0.81
Ames 26636	Hobson		2	64.08	0.12	0.06	0.22
Ames 26645	Red Russian	United States, Maine	7	359.58	0.73	0.58	0.84
169075		Turkey	3	114.42	0.22	0.10	0.40
169080		Turkey, Samsun	2	87.08	0.16	0.06	0.39
169083		Turkey, Tekirdag	9	326.75	0.65	0.34	0.87
184452		Germany	9	244.42	0.48	0.19	0.78
184453		Germany	5	254.50	0.51	0.36	0.66
221971		Japan	9	397.67	0.80	0.58	0.92
232895		Hungary	9	369.67	0.75	0.53	0.89
250135	NU 52585	Pakistan	4	186.58	0.38	0.26	0.52
251236		Pakistan, Punjab	2.5	127.33	0.26	0.14	0.42
251614		Serbia	5	159.00	0.30	0.16	0.51
269449		Pakistan	5.5	265.53	0.53	0.13	0.89
271452	1187	India, Gujarat	9	319.42	0.65	0.43	0.82
282571	NU 52589	Japan	9	337.83	0.61	0.35	0.81
284859	A 19890	Poland	9	324.83	0.65	0.34	0.87
286418		Nepal	7	295.92	0.60	0.39	0.78

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
305278	NU 51623	Sweden		261.66	0.54	0.53	0.55
305279	NU 52072	Sweden	5	255.83	0.52	0.40	0.63
305280	NU 52073	Sweden	7	266.93	0.58	0.25	0.85
305281	NU 52074	Sweden	7	332.09	0.68	0.38	0.88
305282	NU 51627	Sweden		162.45	0.34	0.17	0.55
311727	Bronowski	Poland	7	327.08	0.66	0.54	0.76
311728	Czyzowskich	Poland	7	298.01	0.62	0.29	0.87
311729	Gorczanski	Poland	7	328.51	0.68	0.37	0.89
311730	Mazowiecki	Poland	8	291.67	0.61	0.30	0.85
311731	Mlochowski	Poland	5	251.16	0.50	0.41	0.58
311732	Skrzeszowicki	Poland	8	314.09	0.63	0.12	0.95
311733	Warszawski	Poland	9	355.16	0.65	0.42	0.82
357374	Esenska Mesana	Former Serbia and Montenegro	9	391.74	0.81	0.47	0.95
365644	Turret	Canada, Manitoba	9	367.27	0.68	0.38	0.87
383422	Tantal	France	7	419.88	0.83	0.62	0.94
384536	NORDE	Sweden		214.78	0.44	0.26	0.64
391552	Chun-Nung 1	China, Shaanxi	9	310.17	0.61	0.31	0.84
391553	Shang-You	China, Shaanxi	7.5	295.32	0.56	0.32	0.78
399418	Trebicska	Czech Republic, North Moravia	9	314.58	0.63	0.34	0.85
409022	Erra	Germany	7	308.75	0.62	0.39	0.81
409023	Lesira	Germany	9	326.75	0.65	0.34	0.87
409024	Rapora	Germany	2.5	196.92	0.40	0.17	0.69
431571	Midas	Canada, Saskatchewan	7	323.00	0.65	0.46	0.80
431572	Regent	Canada, Saskatchewan	7	385.17	0.78	0.64	0.87
431574	Tower	Canada, Saskatchewan	9	294.50	0.63	0.27	0.89
432391	BAU-M/49	Bangladesh	7.5	274.32	0.50	0.23	0.77
432392	BAU-M/50	Bangladesh	7	381.42	0.77	0.67	0.85
432393	BAU-M/53	Bangladesh	7	312.76	0.67	0.40	0.86
432394	BAU-M/58	Bangladesh		173.12	0.34	0.17	0.55
432395	BAU-M/71	Bangladesh	5	264.58	0.52	0.35	0.69
436554	Gan You No. 1	China	5	275.38	0.54	0.53	0.55
436555	Gan You No. 2	China	5	241.67	0.49	0.39	0.59
436556	Gan You No. 3	China	7	309.50	0.63	0.43	0.79
436557	Gan You No. 4	China	5	267.83	0.54	0.53	0.55
436558	Gan You No. 5	China	6	214.67	0.42	0.20	0.67
443015	Gry	Norway	7	411.05	0.82	0.81	0.83
458605	Calder Swede	New Zealand	3	145.75	0.28	0.18	0.42
458606	Crimson King Swede	New Zealand	5	317.05	0.68	0.51	0.81

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
458607	Doon Major Swede	New Zealand	5	387.07	0.76	0.54	0.90
458608	Doon Spartan Swede	New Zealand	7	322.75	0.65	0.49	0.79
458609	Kiri Swede	New Zealand	8	320.98	0.65	0.14	0.95
458610	Wilhelmsburger	New Zealand	5	313.42	0.63	0.48	0.76
458919	Brio	France	7	325.58	0.66	0.54	0.76
458920	Cresor	France	7	325.03	0.61	0.47	0.74
458921	Cresus	France	7	267.93	0.56	0.30	0.79
458922	Crop	France	7	236.08	0.46	0.17	0.78
458923	Kentan	France	3	113.88	0.24	0.23	0.25
458924	Parapluie	France	5	265.17	0.54	0.39	0.68
458925	Primor	France	8	309.07	0.55	0.30	0.78
458930	Oro	Canada, Saskatchewan	7	314.83	0.63	0.43	0.79
458935	Brink	Sweden	4.5	231.25	0.46	0.30	0.64
458936	Gulle	Sweden	7	277.67	0.56	0.36	0.75
458937	Gulliver	Sweden	7	231.75	0.46	0.23	0.70
458939	Asahi natane	Japan	5	207.42	0.40	0.29	0.53
458940	Chisaya natane	Japan	7	271.75	0.54	0.33	0.73
458941	Norin 16	Japan	3	183.33	0.37	0.23	0.53
458944	Arwin	Germany	5	228.50	0.46	0.27	0.66
458945	Eragi	Germany	5	192.92	0.38	0.20	0.60
458946	Gido	Germany	3	67.33	0.14	0.07	0.25
458947	Girita	Germany	4	193.41	0.38	0.26	0.52
458948	Gisora	Germany	3	167.33	0.35	0.20	0.54
458949	Gora	Germany	5	181.92	0.36	0.25	0.49
458950	Kara	Germany	4	150.33	0.31	0.20	0.44
458951	Kosa	Germany	5	207.33	0.41	0.30	0.53
458952	Laura	Germany	3	110.58	0.22	0.18	0.26
458953	Lisora	Germany	3	174.92	0.35	0.20	0.54
458954	Luna	Germany	3	138.17	0.29	0.20	0.40
458955	Prota	Germany	5	214.32	0.51	0.25	0.76
458956	Rico	Germany	3	93.33	0.18	0.13	0.26
458957	Sera	Germany	3	193.73	0.35	0.22	0.50
458958	Vanda	Germany	3.25	164.54	0.34	0.19	0.54
458959	Wira	Germany	2	32.16	0.07	0.05	0.10
458964	77-60	New Zealand	6	296.82	0.58	0.50	0.65
458965	77-58	New Zealand	5.5	189.02	0.34	0.13	0.66
458967	Jet Neuf	France	2	79.75	0.15	0.09	0.24
458968	Orpal	France	5	291.75	0.59	0.49	0.67
458969	Primor	France	2.5	97.22	0.21	0.15	0.27

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
458970	Rafal	France	5	169.67	0.34	0.23	0.47
458971	Romeo	France	3	177.38	0.31	0.15	0.52
458979	77-70	United States, Oregon	3	155.64	0.30	0.14	0.53
458980	77-71	United States, Oregon	2.5	73.58	0.15	0.08	0.25
469724	Aomori	Korea, South	2	128.70	0.25	0.08	0.55
469726	Aomori-1	Korea, South	2	59.83	0.12	0.06	0.22
469728	Armander	Korea, South	2	71.24	0.16	0.06	0.37
469729	Austria-3	Korea, South	9	249.17	0.49	0.21	0.77
469730	Azuma	Korea, South	0	126.12	0.36	0.16	0.63
469731	Azuma 22	Korea, South	9	279.78	0.44	0.15	0.78
469732	Azuma 156	Korea, South	4.5	204.92	0.40	0.23	0.60
469733	Azumasho	Korea, South	7	309.03	0.59	0.38	0.78
469734	Azumasho	Korea, South	6	322.75	0.65	0.38	0.84
469735	Bansai	Korea, South		127.95	0.24	0.13	0.41
469736	Barplina	Korea, South	9	291.33	0.46	0.15	0.80
469737	Borowski	Korea, South	9	282.33	0.55	0.27	0.81
469738	Buk Wuk 3	Korea, South	7	287.75	0.59	0.37	0.77
469739	Buk Wuk 4	Korea, South	7.5	258.48	0.59	0.12	0.94
469740	Buk Wuk 7	Korea, South	9	307.11	0.63	0.25	0.89
469741	Buk Wuk 12	Korea, South		344.78	0.70	0.21	0.95
469742	Buk Wuk 13	Korea, South	1	151.84	0.41	0.18	0.69
469743	Buk Wuk 14	Korea, South		331.12	0.69	0.51	0.82
469744	Buk Wuk 15	Korea, South	9	368.54	0.71	0.48	0.87
469745	Buk Wuk 16	Korea, South	9	328.92	0.57	0.26	0.83
469746	Buk Wuk 17	Korea, South	7	273.35	0.48	0.20	0.77
469747	Buk Wuk 20	Korea, South	4.5	279.41	0.57	0.23	0.86
469748	Buk Wuk 21	Korea, South	9	371.00	0.67	0.13	0.97
469749	Buk Wuk 23	Korea, South	9	393.72	0.78	0.46	0.93
469750	Buk Wuk 24	Korea, South		173.12	0.34	0.17	0.55
469751	Buk Wuk 26	Korea, South		282.45	0.59	0.49	0.69
469753	C73/1262	Korea, South	9	319.74	0.60	0.28	0.85
469754	Cescaljarni repka	Korea, South	9	487.22	0.97	0.97	0.97
469755	Chon nam	Korea, South	9	487.67	0.97	0.97	0.97
469758	Dae cho sen	Korea, South	1	75.92	0.16	0.08	0.31
469759	Dong Buk	Korea, South	2.5	150.42	0.33	0.12	0.63
469760	Dong Hae	Korea, South	1.5	101.08	0.24	0.10	0.48
469761	Dong Hae 1	Korea, South	5	291.25	0.59	0.49	0.67
469762	Dong Hae 2	Korea, South	5	181.92	0.36	0.25	0.49
469763	Dong Hae 3	Korea, South	5	182.17	0.37	0.23	0.53
469764	Dong Hae 4	Korea, South	3	201.00	0.41	0.24	0.60

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
469765	Dong Hae 6	Korea, South	3	201.85	0.44	0.25	0.64
469766	Dong Hae 9	Korea, South	3	194.67	0.39	0.27	0.53
469767	Dong Hae 10	Korea, South	2	130.33	0.28	0.14	0.48
469768	Dong Hae 11	Korea, South	3	100.29	0.23	0.08	0.51
469769	Dong Hae 12	Korea, South	7	230.01	0.41	0.16	0.72
469770	Dong Hae 14	Korea, South	4	203.32	0.37	0.17	0.62
469771	Dong Hae 15	Korea, South	3	145.33	0.29	0.16	0.47
469772	Dong Hae 16	Korea, South	2	54.25	0.11	0.07	0.17
469773	Dong Hae 18	Korea, South	7	312.25	0.63	0.51	0.74
469774	Dong Hae 19	Korea, South	3	131.50	0.26	0.12	0.48
469775	Dong Hae 20	Korea, South	2	160.50	0.30	0.14	0.54
469776	Dong Hae 21	Korea, South	5	293.58	0.60	0.40	0.77
469777	Dong Hae 22	Korea, South	4	168.58	0.33	0.24	0.44
469778	Dong Hae 23	Korea, South	5	155.42	0.31	0.17	0.48
469779	Dong Hae 24	Korea, South	5	240.52	0.46	0.32	0.61
469780	Dong Hae 25	Korea, South	3	113.88	0.24	0.23	0.25
469781	Drawft	Korea, South	3	112.97	0.24	0.14	0.37
469782	Drawft	Korea, South	4	155.22	0.31	0.10	0.65
469783	Dwarf Essex	Korea, South	2.5	153.29	0.27	0.10	0.54
469784	Eckendorfer Mali	Korea, South	3	127.25	0.27	0.12	0.48
469786	Enshu	Korea, South	3	170.72	0.34	0.17	0.55
469787	Expander	Korea, South	3	117.17	0.24	0.15	0.35
469788	Fertodi	Korea, South	2.5	110.00	0.23	0.14	0.35
469789	Fonto	Korea, South	3	142.00	0.28	0.14	0.48
469790	Fonto	Korea, South	2	101.25	0.21	0.10	0.39
469791	France 1	France	4	209.67	0.43	0.19	0.71
469792	France 2	France	4	194.58	0.40	0.22	0.61
469793	France 3	France	3.5	147.17	0.30	0.25	0.36
469794	France 5	France	5	191.19	0.43	0.18	0.72
469795	France 6	France	5	264.88	0.53	0.38	0.67
469796	France 8	France	2	94.25	0.20	0.06	0.49
469797	France 9	France	5	294.42	0.59	0.53	0.66
469798	France 10	France	5	289.08	0.57	0.40	0.73
469799	France 11	France	7	289.75	0.57	0.28	0.82
469800	France 12	France	4	184.81	0.37	0.36	0.38
469801	Fuji	Korea, South	4	162.50	0.33	0.27	0.38
469802	Gebr Dippes	Korea, South	1	55.83	0.11	0.06	0.22
469804	Germany	Germany	4	158.67	0.31	0.16	0.52
469805	Giant rape	Korea, South	3	119.89	0.24	0.23	0.25
469806	Giant rape	Korea, South	1.5	7.72	0.04	0.02	0.10

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
469807	Gogane	Korea, South	4	159.17	0.33	0.27	0.38
469808	Gokstad	Korea, South	3	115.17	0.24	0.13	0.39
469809	Gokstad	Korea, South	1.5	83.09	0.15	0.04	0.44
469810	Gorozanski	Korea, South	4	282.47	0.57	0.28	0.81
469811	Gorozanski	Korea, South	3	165.94	0.33	0.21	0.48
469812	Gylle	Korea, South	6	244.00	0.51	0.29	0.72
469813	Gylle	Korea, South	1.5	147.83	0.30	0.15	0.51
469814	Hamburg	Korea, South	2.5	85.83	0.18	0.08	0.35
469815	Hamburg 1	Korea, South	5	220.17	0.44	0.32	0.57
469816	Hok Kaidoshu	Korea, South	1	138.61	0.27	0.10	0.54
469818	Hwa 318	Korea, South	5	259.00	0.51	0.21	0.80
469819	Isek urodane	Korea, South	1	117.08	0.25	0.11	0.48
469821	Iwao natane	Korea, South	1	16.25	0.03	0.02	0.06
469822	Iwashiro-natane	Korea, South	2.5	149.50	0.30	0.11	0.61
469823	Iwawoochi	Korea, South	3	128.58	0.27	0.12	0.48
469824	Iwawoochi	Korea, South	3	174.83	0.35	0.21	0.53
469825	Janetzki	Korea, South	3	178.98	0.36	0.22	0.53
469826	Janetzkis	Korea, South	5	261.00	0.53	0.33	0.72
469828	Kani	Korea, South	6	357.92	0.72	0.56	0.84
469829	Karafuto	Korea, South	3	120.83	0.24	0.17	0.34
469830	Kasuya	Korea, South	3	165.00	0.34	0.23	0.47
469831	Kasuyashu	Korea, South	7	358.58	0.73	0.60	0.83
469832	Kinki 18	Korea, South	7	328.42	0.67	0.47	0.82
469833	Kinki 20	Korea, South	4	190.00	0.38	0.28	0.50
469834	Kinki 21	Korea, South	2	54.64	0.10	0.07	0.13
469837	Kinki 29	Korea, South	6.5	272.17	0.57	0.21	0.87
469838	Kinki 30	Korea, South	5	294.42	0.59	0.53	0.66
469839	Kinki wase	Korea, South	4	158.88	0.31	0.20	0.44
469840	Klinki	Korea, South	4.5	232.14	0.47	0.24	0.71
469841	Koubun	Korea, South	4.5	242.45	0.48	0.37	0.59
469842	Kraphhauser	Korea, South	4	188.60	0.38	0.23	0.55
469843	Kritmar rape	Korea, South	6	366.17	0.74	0.68	0.79
469844	Kritmar rape	Korea, South	5	289.00	0.58	0.41	0.73
469845	Kuju	Korea, South	7	412.58	0.84	0.80	0.87
469846	Kuju 4	Korea, South	7	319.75	0.65	0.46	0.80
469847	Kuju 7	Korea, South	6	317.69	0.63	0.56	0.71
469848	Kuju 8	Korea, South	5	313.07	0.62	0.44	0.77
469849	Kuju 9	Korea, South	5	275.97	0.54	0.53	0.55
469850	Kuju 11	Korea, South	5.5	298.89	0.62	0.53	0.70
469851	Kuju 13	Korea, South	7	400.59	0.82	0.65	0.91

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
469852	Kuju 15	Korea, South	5.5	316.99	0.62	0.46	0.76
469853	Kuju 16	Korea, South	7	358.58	0.73	0.60	0.83
469854	Kuju 17	Korea, South	5	291.25	0.59	0.53	0.66
469856	Kuju 19	Korea, South	5.5	293.34	0.59	0.49	0.69
469857	Kuju 22	Korea, South	4	243.42	0.49	0.27	0.72
469858	Kuju 24	Korea, South	4	269.63	0.54	0.25	0.81
469859	Kuju 25	Korea, South	5	315.58	0.63	0.51	0.74
469860	Kuju 26	Korea, South	6	337.74	0.68	0.38	0.88
469861	Kuju 27	Korea, South	7	384.00	0.78	0.59	0.89
469862	Kuju 29	Korea, South	7	385.75	0.78	0.72	0.83
469863	Kuju 32	Korea, South	7	362.33	0.73	0.60	0.83
469864	Kuju 33	Korea, South	5	263.76	0.54	0.53	0.55
469865	Kuju 35	Korea, South	5	267.83	0.54	0.53	0.55
469866	Kuju 36	Korea, South	4.5	223.89	0.46	0.36	0.55
469867	Kuju 37	Korea, South	5	271.66	0.54	0.53	0.55
469868	Kuju 39	Korea, South	4.5	222.83	0.46	0.36	0.55
469869	Kuju 40	Korea, South	2.5	73.59	0.13	0.12	0.14
469870	Kuju 41	Korea, South	5	241.25	0.48	0.41	0.55
469871	Kuju 45	Korea, South	5	315.58	0.63	0.51	0.74
469872	Kuju 47	Korea, South	9	489.47	0.97	0.97	0.97
469874	Kuju 49	Korea, South	4.5	228.24	0.46	0.30	0.62
469875	Kuju 51	Korea, South	7	395.83	0.80	0.64	0.90
469877	Kuju 54	Korea, South	7	405.63	0.82	0.81	0.83
469878	Kuju 55	Korea, South	7	442.19	0.91	0.81	0.96
469879	Kuju 56	Korea, South	7	414.50	0.83	0.67	0.93
469880	Kuju 57	Korea, South	8	441.08	0.89	0.66	0.97
469881	Kuju 58	Korea, South	7	353.33	0.71	0.56	0.82
469882	Kutkowski	Korea, South	7	419.17	0.85	0.74	0.92
469883	Lembkes	Korea, South	6	349.42	0.70	0.52	0.84
469884	Lembkes	Korea, South	5	375.45	0.78	0.46	0.93
469885	Lembkes malchower	Korea, South	2.5	247.58	0.50	0.31	0.68
469886	Lenora	Korea, South	6	282.33	0.58	0.25	0.85
469887	Lieikoposki	Korea, South	4	336.28	0.68	0.55	0.79
469888	Lifura	Korea, South	3	319.00	0.65	0.41	0.84
469889	Linus	Korea, South	3	185.67	0.38	0.25	0.55
469890	Maintainer for GHR MS	Korea, South	5	283.29	0.45	0.17	0.77
469891	Major	Korea, South	9	189.67	0.38	0.28	0.50
469892	Mulchower	Korea, South	8	291.50	0.58	0.25	0.85
469893	Mulchower	Korea, South	9	413.00	0.83	0.67	0.93

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
469894	Mali	Korea, South	5	305.92	0.62	0.29	0.86
469896	Mang woon	Korea, South	5	228.08	0.46	0.32	0.61
469897	Marcus	Korea, South	7	234.17	0.47	0.32	0.61
469898	Marcus	Korea, South	5	285.75	0.56	0.29	0.79
469899	Matador	Korea, South	7	251.08	0.50	0.31	0.70
469900	Matador	Korea, South	4	340.36	0.72	0.48	0.88
469901	Miekuro Dane	Korea, South	9	340.25	0.69	0.43	0.86
469902	Miochowski	Korea, South	6	419.92	0.84	0.56	0.95
469903	Mihonatane	Korea, South	7	281.52	0.61	0.41	0.77
469904	Mijagi Bansai	Korea, South	5	417.05	0.83	0.62	0.94
469905	Mokpo # 2	Korea, South	1.5	275.38	0.54	0.53	0.55
469906	Mokpo 2	Korea, South	1	11.07	0.02	0.01	0.05
469907	Mokpo # 3	Korea, South	2	55.25	0.11	0.05	0.22
469908	Mokpo	Korea, South	3	74.39	0.15	0.08	0.24
469909	Mokpo # 4	Korea, South	5	114.17	0.24	0.11	0.43
469910	Mokpo 4	Korea, South	4	216.51	0.44	0.30	0.60
469911	Mokpo 5	Korea, South	3	151.23	0.28	0.15	0.47
469912	Mokpo 6	Korea, South	5	100.38	0.21	0.07	0.47
469913	Mokpo 7	Korea, South		183.17	0.39	0.23	0.59
469915	Mokpo 9	Korea, South	4	209.00	0.38	0.27	0.51
469916	Mokpo 10	Korea, South	2.5	134.00	0.25	0.09	0.55
469917	Mokpo 13	Korea, South	7	327.08	0.66	0.54	0.76
469918	Mokpo 14	Korea, South	3.5	245.81	0.40	0.16	0.70
469919	Mokpo 15	Korea, South	4	225.33	0.44	0.26	0.64
469920	Mokpo 16	Korea, South	3	268.22	0.46	0.22	0.72
469921	Mokpo 17	Korea, South	3	143.00	0.28	0.18	0.42
469922	Mokpo 18	Korea, South	3	164.88	0.34	0.17	0.55
469923	Mokpo 19	Korea, South	5	253.75	0.51	0.46	0.57
469925	Mokpo 22	Korea, South	6	288.38	0.58	0.25	0.85
469926	Mokpo 23	Korea, South	4	189.88	0.38	0.23	0.56
469927	Mokpo 24	Korea, South	5	215.57	0.45	0.24	0.69
469928	Mokpo 25	Korea, South	7	246.20	0.57	0.22	0.86
469929	Mokpo 26	Korea, South	3	175.08	0.36	0.25	0.49
469930	Mokpo 27	Korea, South	5	241.00	0.49	0.39	0.59
469931	Mokpo 28	Korea, South	5	194.08	0.38	0.21	0.59
469932	Mokpo # 29	Korea, South	2.5	182.28	0.32	0.13	0.60
469933	Mokpo 30	Korea, South	3	145.33	0.29	0.19	0.42
469934	Mokpo # 32	Korea, South	3	200.08	0.41	0.26	0.58
469935	Mokpo # 33	Korea, South	5	275.38	0.54	0.53	0.55
469936	Mokpo # 38	Korea, South	5	261.92	0.53	0.33	0.72

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
469937	Mokpo # 40	Korea, South	3	223.08	0.46	0.22	0.72
469939	Mu.che!	Korea, South	6	373.25	0.75	0.65	0.83
469940	Murame nadame	Korea, South	3	210.57	0.39	0.24	0.57
469941	Mura yamasho	Korea, South	4.5	292.68	0.62	0.44	0.77
469942	Mutsumi	Korea, South	3	271.97	0.51	0.25	0.76
469943	N001-28-246-5-4	Korea, South	3	93.00	0.18	0.13	0.26
469944	Nabo	Korea, South	3	115.17	0.24	0.13	0.39
469945	Niedera-rubacher	Korea, South	3	94.38	0.20	0.14	0.28
469946	Nilla glossy	Korea, South	6	373.55	0.74	0.65	0.81
469947	Nilla: 1022	Korea, South	5	322.22	0.63	0.44	0.79
469948	Noda 1	Korea, South	5	192.92	0.38	0.20	0.60
469949	Norin # 1	Japan		240.78	0.49	0.25	0.74
469950	Norin # 1	Japan	6	276.85	0.50	0.32	0.68
469951	Norin # 2	Japan	4	235.58	0.47	0.32	0.64
469952	Norin 2	Japan	4	225.17	0.45	0.30	0.61
469953	Norin # 3	Japan	5	291.90	0.61	0.47	0.74
469954	Norin 3	Japan	3	257.08	0.53	0.30	0.76
469955	Norin # 4	Japan	6	287.41	0.62	0.43	0.77
469956	Norin 4	Japan	5.5	309.01	0.63	0.51	0.73
469957	Norin # 5	Japan	6	244.58	0.49	0.33	0.65
469958	Norin 5	Japan	2.5	144.90	0.36	0.19	0.58
469959	Norin # 6	Japan	5	276.75	0.56	0.40	0.71
469960	Norin 6	Japan	3	194.58	0.41	0.22	0.63
469961	Norin 8	Japan	4	273.05	0.58	0.38	0.76
469962	Norin 9	Japan	3.5	158.49	0.33	0.08	0.76
469963	Norin 10	Japan		308.45	0.64	0.54	0.74
469964	Norin 11	Japan	5	281.08	0.57	0.51	0.62
469965	Norin 12	Japan	4	152.17	0.35	0.21	0.53
469966	Norin 13	Japan	5	205.42	0.41	0.30	0.53
469967	Norin 14	Japan	5	310.67	0.63	0.43	0.79
469968	Norin 15	Japan	5	311.08	0.64	0.38	0.83
469969	Norin # 15	Japan	3	85.55	0.18	0.10	0.31
469970	Norin # 17	Japan	3	285.17	0.58	0.35	0.78
469971	Norin 17	Japan	4	285.08	0.58	0.35	0.78
469972	Norin 18	Japan	5	241.00	0.49	0.39	0.59
469973	Norin 19	Japan	3	200.00	0.41	0.26	0.58
469974	Norin 20 (michinoku natane)	Japan	4	193.55	0.38	0.23	0.56
469975	Norin 21	Japan	3	185.75	0.38	0.25	0.55
469976	Norin # 21	Japan	5	318.55	0.63	0.44	0.79

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
469977	Norin # 22	Japan	4	190.88	0.38	0.23	0.56
469978	Norin 22	Japan	5	275.38	0.54	0.53	0.55
469979	Norin # 25	Japan	3	268.17	0.55	0.32	0.76
469980	Norin # 26	Japan	3	282.25	0.58	0.35	0.78
469981	Norin 26	Japan	4	249.42	0.51	0.36	0.66
469982	Norin 27	Japan	5	184.83	0.36	0.22	0.53
469983	Norin 28	Japan	5	194.00	0.39	0.27	0.53
469984	Norin 29	Japan	3	201.50	0.41	0.22	0.63
469985	Norin 30	Japan	2.5	213.46	0.55	0.27	0.80
469986	Norin 31	Japan	3	165.08	0.35	0.17	0.59
469987	Norin 31 (Gogane)	Japan	5	236.19	0.46	0.22	0.72
469988	Norin 32 (Tokiwa)	Japan	5	300.50	0.61	0.39	0.80
469989	Norin 33	Japan	2	130.42	0.28	0.14	0.49
469990	Norin # 33	Japan	5	267.83	0.54	0.53	0.55
469991	Norin 34	Japan	3	218.25	0.46	0.23	0.70
469992	Norin 35	Japan	2	154.83	0.33	0.15	0.59
469993	Norin 36	Japan	3	138.17	0.29	0.20	0.40
469994	Norin 37	Japan	3	113.88	0.24	0.23	0.25
469995	Norin 39	Japan	5	365.10	0.79	0.56	0.91
469997	Norin 41	Japan	2.5	180.33	0.38	0.15	0.67
469998	Norin 42	Japan	3.5	261.28	0.56	0.30	0.79
469999	Nugget	Korea, South	4	256.92	0.57	0.38	0.73
470000	Oleifera	Korea, South	3	113.92	0.23	0.12	0.40
470001	Olguell	Korea, South	3	254.80	0.60	0.33	0.83
470002	Panter	Korea, South		322.62	0.67	0.39	0.86
470003	Petanova-lihonova	Korea, South	3	244.58	0.50	0.26	0.73
470004	Poland 1	Poland	3	249.00	0.51	0.26	0.77
470005	Poland 3	Poland	5	253.17	0.51	0.41	0.61
470006	Poland 4	Poland	3.5	193.50	0.40	0.22	0.61
470007	Poland 5	Poland	5	232.58	0.47	0.34	0.61
470008	Polnoslaski	Korea, South	5	321.92	0.65	0.43	0.82
470009	Primer	Korea, South	7	342.58	0.70	0.42	0.88
470010	R. Creeus	Korea, South	3	260.00	0.53	0.29	0.76
470011	R. janus	Korea, South	3	142.38	0.29	0.10	0.59
470012	Ramses	Korea, South	3	138.92	0.28	0.14	0.49
470013	Rang	Korea, South	5	240.58	0.49	0.32	0.66
470014	Rang	Korea, South	1	171.34	0.44	0.12	0.82
470015	Rapifera	Korea, South	5.5	352.66	0.73	0.52	0.87
470016	Rapol	Korea, South	8	345.05	0.70	0.21	0.95
470017	Rapol	Korea, South	5	302.25	0.61	0.48	0.73

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
470018	RD-6	Korea, South	6	325.00	0.66	0.54	0.76
470019	Regal	Korea, South	5	275.38	0.54	0.53	0.55
470020	Rumania 1	Romania	4	252.17	0.51	0.37	0.65
470021	Russia 5	Soviet Union, Former		447.95	0.92	0.75	0.98
470022	Russia 6	Soviet Union, Former	5	318.55	0.63	0.44	0.79
470023	Salamander	Korea, South	6	375.22	0.74	0.65	0.81
470024	Sapporo	Korea, South	5	289.25	0.59	0.49	0.67
470025	Scherwitz	Korea, South	5	325.08	0.66	0.50	0.79
470026	Sei yoshu	Korea, South	3	246.58	0.51	0.26	0.76
470027	Sznes zowicki	Korea, South	7	381.92	0.77	0.67	0.85
470028	Skrzeszowicki	Korea, South	3	256.75	0.53	0.29	0.76
470029	Spote zollerngold	Korea, South	7	346.75	0.71	0.59	0.80
470030	SR-37 IGHR MS	Korea, South	5	221.50	0.44	0.32	0.57
470031	Su weon cheg	Korea, South	2	53.50	0.11	0.06	0.22
470032	S. V. Gulle	Korea, South	3	236.92	0.49	0.32	0.66
470033	Svalof gullen	Korea, South	3	279.17	0.57	0.29	0.82
470034	Svalof Victoria	Korea, South	3	239.42	0.49	0.32	0.66
470035	Synra	Korea, South	2	151.58	0.33	0.11	0.67
470036	Taichang	Korea, South	5	386.58	0.78	0.57	0.91
470037	Taichang	Taiwan	1	219.50	0.46	0.18	0.77
470038	Taiwan	Taiwan	2	198.92	0.42	0.17	0.73
470039	Taiwan	Taiwan	3	258.33	0.53	0.33	0.72
470040	Taiwan # 2	Taiwan	3	237.58	0.49	0.23	0.76
470041	Taiwan 2	Korea, South	4	193.55	0.38	0.23	0.56
470043	Takegis MS	Korea, South	5	349.00	0.70	0.54	0.83
470044	Tanka	Korea, South	5	247.00	0.49	0.39	0.59
470045	Target	Korea, South	3	117.33	0.24	0.23	0.25
470046	Titus	Korea, South	7	333.58	0.68	0.46	0.83
470047	Titus	Korea, South	2	201.83	0.43	0.16	0.74
470048	Todane	Korea, South	3	201.67	0.41	0.26	0.58
470049	Tokiwa	Korea, South	5	275.38	0.54	0.53	0.55
470050	Tonus	Korea, South	5	319.25	0.65	0.43	0.82
470051	Tosharshu	Korea, South	3	167.55	0.34	0.17	0.55
470052	Trebicska (ozima repka)	Korea, South	3	281.25	0.58	0.35	0.78
470053	Tsukushishu	Korea, South	2	212.83	0.45	0.19	0.73
470054	Wasefuji	Korea, South	5	221.72	0.44	0.26	0.64
470055	Weal Dong Cho	Korea, South	5	339.70	0.64	0.36	0.85
470056	Weibulls margo	Korea, South	2	188.17	0.39	0.15	0.70
470057	Wielkoposki	Korea, South	2	250.58	0.52	0.24	0.78
470058	Willa	Korea, South	3	227.17	0.47	0.32	0.61

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470059	Willa	Korea, South	5	313.58	0.63	0.51	0.74
470060	Yong dang	Korea, South	2	187.25	0.39	0.19	0.64
470061	Yonkkaichi Kwo	Korea, South	2	188.75	0.40	0.18	0.67
470062	Yonkokuban	Korea, South	3	164.88	0.34	0.17	0.55
470063	Yonkoku ban	Korea, South	5	273.69	0.54	0.36	0.70
470065	Yudal	Korea, South	2	229.84	0.33	0.07	0.77
470066	Yudal	Korea, South	5	333.12	0.61	0.47	0.74
470067	4x8-1-1	Korea, South	3	314.08	0.64	0.32	0.86
470068	65/685	Korea, South	5	295.08	0.58	0.39	0.75
470069	72/438/5	Korea, South	2	186.80	0.35	0.13	0.65
470070	72/891	Korea, South	5	312.00	0.63	0.43	0.79
470071	651-685	Korea, South	3	247.42	0.51	0.28	0.73
470073	7003-2B-23-1	Korea, South	3	283.58	0.58	0.35	0.78
470074	7003-2B-36	Korea, South	3	209.00	0.39	0.24	0.57
470075	7003-2B-38	Korea, South	3	280.75	0.58	0.31	0.80
470076	7006-2B-13-2	Korea, South	3	282.25	0.58	0.35	0.78
470077	7008-2B-36	Korea, South	5	224.38	0.44	0.26	0.64
470078	7008-2B-36-1	Korea, South	4	296.58	0.60	0.39	0.78
470079	7008-2B-226-5-3	Korea, South	5	334.08	0.68	0.46	0.83
470080	7115-2B-28	Korea, South	3	247.33	0.51	0.23	0.78
470081	7115-2B-70	Korea, South	3	271.65	0.46	0.22	0.72
470083	79-71	Korea, South	7	340.25	0.68	0.46	0.83
470084	79-353	Korea, South	3	141.75	0.30	0.14	0.54
470085	79-355	Korea, South	3	269.75	0.55	0.32	0.76
470086	79-389	Korea, South	3	244.92	0.50	0.27	0.73
470087	79-390	Korea, South	4	211.33	0.43	0.22	0.68
478339	O 54	China	2	197.92	0.42	0.16	0.72
478340	O 84	China	5	216.67	0.44	0.32	0.57
502303	AR-115	Russian Federation	5	250.90	0.50	0.27	0.73
502304	AR-256	Russian Federation	5	268.87	0.54	0.53	0.55
531273	Attila	Hungary	2	154.66	0.30	0.14	0.53
531274	Barkant	Netherlands	2	146.79	0.28	0.09	0.61
531275	BNW. 1 61/83	Germany	3	221.70	0.42	0.21	0.66
531276	BNW. 1 62/83	Germany	7	325.50	0.66	0.54	0.76
531277	Darmar	France	7	278.08	0.57	0.32	0.79
531278	Elena	Germany	5	250.42	0.51	0.36	0.66
531279	GK Savaria	Hungary	2	149.33	0.32	0.14	0.59
531280	IR-2	Hungary	7	365.38	0.73	0.51	0.87
531281	Librador	Germany	2	112.13	0.22	0.09	0.44
531282	Lindore	Germany	5	199.18	0.42	0.28	0.57

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
531283	Linglandor	Germany	5	231.59	0.48	0.36	0.60
531284	Liratrop	Germany	3	162.10	0.33	0.18	0.52
531285	Liropa	Germany	2	70.17	0.15	0.09	0.25
531287	Santana	Germany		333.41	0.68	0.38	0.88
531288	Ujfertodi	Hungary	5	228.67	0.48	0.21	0.75
537090	Seoul	Korea, South	5	283.83	0.56	0.42	0.70
542983	Tri-Cascade	United States, Idaho	3	218.14	0.41	0.22	0.65
594321	KS3579	United States, Kansas	3	211.42	0.44	0.27	0.62
597828	112-3690-75	Algeria	3	211.00	0.43	0.26	0.63
604608	KS1701	United States, Kansas	2	137.43	0.26	0.08	0.60
610258	AR91004	United States, Arkansas	3	226.82	0.36	0.13	0.68
612846	Wichita	United States, Kansas	2	47.67	0.10	0.05	0.17
619618	AR91017	United States, Arkansas	3	81.45	0.20	0.13	0.29
632400	Abilene	United States, Kansas	2	80.17	0.18	0.04	0.55
633118	Legend	United States, New York	3	111.00	0.24	0.13	0.39
633119	Colt	United States, New York	3	96.08	0.19	0.14	0.26
633120	Bolko	Poland, Warszawa	3	83.08	0.17	0.12	0.24
633121	Mar	Poland, Warszawa	2.5	53.16	0.13	0.07	0.22
633122	BRA 1168/85	Italy	3	222.71	0.45	0.26	0.65
633123	E94197	Mongolia	7	256.32	0.62	0.29	0.86
633124	NU 51084	Sweden, Malmohus	5	267.64	0.53	0.37	0.68
634754	Sumner	United States, Kansas	3	139.75	0.28	0.14	0.48
649126	Ames 1670	Canada, Saskatchewan	7	314.22	0.62	0.25	0.89
649127	Ames 6073	Canada, Ontario	2	162.12	0.38	0.05	0.88
649128	Ames 6069	Sweden	6	337.74	0.68	0.38	0.88
649130	Ames 15939	Sweden	3	179.00	0.37	0.19	0.59
649131	Ames 19144	Germany	5	300.05	0.59	0.49	0.69
649133	Ames 22550	Poland, Warszawa	4	174.05	0.35	0.16	0.60
649134	Ames 24221	Canada, Ontario	5	221.72	0.44	0.26	0.64
649135	Ames 24222	Turkey	5	144.78	0.35	0.17	0.59
649136	Ames 26628	Germany	5	130.88	0.32	0.13	0.60
649137	Ames 26631	Germany	3	85.55	0.18	0.10	0.31
649138	Ames 26632	Germany	3	170.72	0.34	0.17	0.55
649140	Ames 26634	Germany	4	114.73	0.28	0.12	0.53
649142	Ames 26638		5	230.17	0.47	0.29	0.66
649143	Ames 26639		3	164.08	0.33	0.15	0.59
649144	Ames 26640		4	128.23	0.31	0.15	0.52
649145	Ames 26641		3	107.39	0.23	0.13	0.38

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
649146	Ames 26642		5	193.23	0.42	0.19	0.69
649147	Ames 26644		4	120.75	0.23	0.11	0.42
649148	Ames 26646	Albania	5	176.29	0.43	0.23	0.65
Ames 26657	Per	Canada, Ontario	6	207.42	0.50	0.24	0.76
535847	Czyzowski	poland	7	239.68	0.58	0.27	0.83
535848	Mlochowski	poland	6.5	245.20	0.49	0.21	0.78
535849	Skrzeszowicki	poland	6	192.92	0.37	0.17	0.63
535850	Valdor	France	7	168.92	0.33	0.12	0.66
535851	Beryl	Poland	7	184.90	0.41	0.15	0.73
535852	Doral	Germany	3.5	119.20	0.24	0.16	0.35
535853	Gundula	Germany	2.5	243.68	0.55	0.25	0.82
535854	Herkules	Sweden	6	379.92	0.77	0.65	0.86
535855	Janpol	Poland	7	227.25	0.44	0.18	0.74
535856	Korina	Germany	7	207.75	0.42	0.19	0.70
535857	Lester	Germany	5	212.08	0.43	0.20	0.70
535858	Lirabou	Germany	5	134.83	0.30	0.14	0.53
535859	Lirakotta	Germany	5	201.33	0.41	0.20	0.67
535860	Lirama	Germany	7	306.25	0.61	0.34	0.82
535861	Marinus	Germany	7	286.08	0.58	0.36	0.78
535862	Mirander	Germany	6	188.83	0.36	0.16	0.64
535864	Quinta	Germany	5	163.58	0.32	0.20	0.47
535865	Ridana	Germany	4.5	178.20	0.35	0.15	0.62
535866	Silesia	Czechoslovakia	7	262.24	0.58	0.25	0.85
535867	SL-29	Czechoslovakia	5	221.50	0.43	0.26	0.63
535868	Status	Sweden	5	181.00	0.36	0.22	0.53
535869	Svaloefs	Sweden	7	411.05	0.82	0.81	0.83
535870	Tamara	Germany	5	265.38	0.53	0.23	0.81
535871	Wipol	Poland	7	317.05	0.71	0.45	0.87
535872	Darmor	Poland	8	355.70	0.72	0.48	0.88
535873	Jantar	Poland	5	241.15	0.48	0.29	0.68
535874	Licantara	Germany	5	299.74	0.53	0.31	0.75
535875	Liglandor	Germany	9	465.42	0.94	0.87	0.97
535876	Liropa	Germany	7	317.05	0.71	0.45	0.87
535877	Rubin	Germany	7	263.43	0.60	0.33	0.83
535878	Start	Poland	7	284.92	0.57	0.30	0.80
633125	Mar'janovskij	Ukraine	7	419.83	0.85	0.74	0.92
633126	Vostochno-sibirskii	Russian Federation	7	416.33	0.84	0.75	0.91
633127	Vinnickij 15/59	Ukraine	4	307.00	0.63	0.38	0.82
633128	Nemercanskij 2268	Ukraine	5	310.25	0.63	0.43	0.79

PI Number	Plant Name	Country originated/obtained from	Median	Mean Rank	RE	Lower	Upper
633129	Krasnodarskii	Russian Federation	6	345.75	0.64	0.36	0.85
633130	Kubanskii 1	Russian Federation	7	439.92	0.89	0.81	0.94
633131	Evvin	Russian Federation	7	425.25	0.86	0.80	0.90
633132	Kovalevskij	Ukraine	5	339.50	0.68	0.55	0.79
633133	CR 157/87a	Germany	4	213.42	0.44	0.30	0.59
633134	CR 165/76a	Germany	3	191.00	0.38	0.20	0.60
633135	CR 167/65a	Germany	7	382.50	0.77	0.67	0.85
633136	CR 813/81	Germany	4	268.58	0.55	0.31	0.76
633137	CR 764/93a	Germany	6	344.00	0.70	0.49	0.85
633138	CR879/83	Germany	5	276.16	0.54	0.36	0.70
633139	CR 1029/86	Germany	5	338.92	0.68	0.55	0.79
633140	CR 1034/80a	Germany	5	295.42	0.60	0.46	0.74
633141	CR 1051/83	Germany	3	198.75	0.41	0.22	0.63
649149	Ames 22974	Germany	3	173.00	0.36	0.25	0.49
649150	Ames 26653	United States, Idaho	7	363.08	0.72	0.50	0.87
649151	Ames 26654	United States, Idaho	7	321.33	0.65	0.49	0.79
649152	Ames 26655	United States, Idaho	7	361.08	0.73	0.60	0.83
649153	Ames 26656	Canada, Ontario	7	420.88	0.84	0.74	0.91
Westar		Canada	7	406.49			

APPENDIX B. LIST OF ACCESSIONS USED FOR IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO PG-4

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
Ames 15651	BO-63	Canada	Spring	7
Ames 15654	Bienvenu	United States	Winter	5
Ames 26626	Siberian	United States	Winter	5
Ames 26635	Polo Canola	United States	Spring	6
Ames 26636	Hobson		Winter	2
Ames 26645	Red Russian	United States	Winter	7
Ames 2793	Ceskia Tabor	Czechoslovakia	Spring	7
311728	Czyzowskich	Poland	Spring	7
311732	Skrzeszowicki	Poland	Winter	7
311733	Warszawski	Poland	Winter	5
357374	Esenska Mesana	Serbia	Winter	9
365644	Turret	Canada	Spring	5
391552	Chun-Nung 1	China	winter	7
391553	Shang-You	China	Semi-winter	5
409022	Erra	Germany	Winter	5
409023	Lesira	Germany	Winter	9
409024	Rapora	Germany	Winter	5
431571	Midas	Canada	Spring	7
431572	Regent	Canada	Spring	7
431574	Tower	Canada	Spring	8
458607	Doon Major Swede	New Zealand	Winter	6
458609	Kiri Swede	New Zealand	Winter	7
458610	Wilhelmsburger	New Zealand	Rutabaga	7
458919	Brio	France	Spring	5
458920	Cresor	France	Spring	5
458922	Crop	France	Spring	5
458923	Kentan	France	winter	3
458924	Parapluie	France	Winter	5
458930	Oro	Canada	Spring	5
458935	Brink	Sweden	Winter	5
458936	Gulle	Sweden	Spring	5
458937	Gulliver	Sweden	Spring	5
458945	Eragi	Germany	Winter	3
458946	Gido	Germany	Spring	3
458947	Girita	Germany	Semi-winter	5
458948	Gisora	Germany	Spring	3
458949	Gora	Germany	Spring	3

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
458951	Kosa	Germany	Spring	5
458952	Laura	Germany	Spring	3
458953	Lisora	Germany	Semi-winter	3
458954	Luna	Germany	Winter	3
458955	Prota	Germany	Spring	5
458956	Rico	Germany	Spring	3
458957	Sera	Germany	Semi-winter	3
458958	Vanda	Germany	Winter	3
458959	Wira	Germany	Winter	2
458967	Jet Neuf	France	Winter	2
458968	Orpal	France	Spring	5
458970	Rafal	France	Winter	3
458971	Romeo	France	Spring	3
469724	Aomori	South Korea	Winter	3
469727	Aoyagi	South Korea	Winter	3
469730	Azuma	South Korea	Semi-winter	5
469734	Azumasho	South Korea	Semi-winter	7
469736	Barplina	South Korea	Winter	3
469738	Buk Wuk 3	South Korea	Spring	5
469754	Cescaljarni repka	South Korea	Semi-winter	9
469755	Chon nam	South Korea	Semi-winter	9
469757	Colza 18 Miroc	South Korea	Semi-winter	9
469758	Dae cho sen	South Korea	Semi-winter	3
469759	Dong Buk	South Korea	Winter	2.5
469782	Drawft	South Korea	Winter	3
469783	Dwarf Essex	South Korea	Winter	3
469784	Eckendorfer Mali	South Korea	Semi-winter	3
469787	Expander	South Korea	Winter	3
469788	Fertodi	South Korea	Winter	3
469789	Fonto	South Korea	Spring	3
469791	France 1	South Korea	Spring	5
469801	Fuji	South Korea	Spring	3
469802	Gebr Dippes	South Korea	Winter	1
469806	Giant rape	South Korea	Winter	1.5
469807	Gogane	South Korea	Winter	4
469811	Gorozanski	South Korea	Winter	3
469812	Gylle	South Korea	Semi-winter	4
469814	Hamburg	South Korea	Winter	2.5
469818	Hwa 318	South Korea	Winter	7
469822	Iwashiro-natane	South Korea	Winter	2.5
469823	Iwawoochi	South Korea	Winter	3

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
469826	Janetzkis	South Korea	Spring	5
469828	Kani	South Korea	Winter	7
469829	Karafuto	South Korea	Winter	3
469830	Kasuya	South Korea	Winter	3
469831	Kasuyashu	South Korea	Winter	7
469840	Klinki	South Korea	Spring	4
469841	Koubun	South Korea	Spring	5
469842	Kraphhauser	South Korea	Spring	5
469843	Kritmar rape	South Korea	Spring	7
469845	Kuju	South Korea	Winter	7
469883	Lembkes	South Korea	Winter	5
469890	Maintainer for GHR MS	South Korea	Winter	5
469944	Nabo	South Korea	Semi-winter	3
469948	Noda 1	South Korea	Winter	3
469999	Nugget	South Korea	Semi-winter	5
470000	Oleifera	South Korea	Semi-winter	3
470002	Panter	South Korea	Winter	5
470003	Petanova-lihonova	South Korea	Semi-winter	5
470008	Polnoslaski	South Korea	Winter	5
470010	R. Creeus	South Korea	Winter	7
470012	Ramses	South Korea	Winter	3
470013	Rang	South Korea	Semi-winter	5
470015	Rapifera	South Korea	Winter	7
470016	Rapol	South Korea	Winter	7
470019	Regal	South Korea	Winter	5
470020	Rumania 1	Romania	Winter	5
470021	Russia 5	Russia	Spring	9
470023	Salamander	South Korea	Winter	7
470024	Sapporo	South Korea	Winter	5
470025	Scherwitz	South Korea	Winter	5
470026	Sei yoshu	South Korea	Semi-winter	5
470027	Sznes zowicki	South Korea	Semi-winter	7
470029	Spote zollerngold	South Korea	Winter	7
470030	SR-37 IGHR MS	South Korea	Winter	5
470031	Su weon cheg	South Korea	Semi-winter	2
470032	S. V. Gulle	South Korea	Spring	3
470033	Svalof gullen	South Korea	Spring	5
470034	Svalof Victoria	South Korea	Winter	5
470036	Taichang	South Korea	Semi-winter	7
470038	Taiwan	Taiwan	Spring	3
470043	Takegis MS	South Korea	Semi-winter	7

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
470044	Tanka	South Korea	Semi-winter	5
470045	Target	South Korea	Spring	3
470046	Titus	South Korea	Winter	7
470048	Todane	South Korea	Semi-winter	5
470049	Tokiwa	South Korea	Semi-winter	5
470050	Tonus	South Korea	Spring	7
470051	Tosharshu	South Korea	Winter	3
470052	Trebicska (ozima repka)	South Korea	Winter	5
470054	Wasefuji	South Korea	Spring	5
470055	Weal Dong Cho	South Korea	Semi-winter	5
470056	Weibulls margo	South Korea	Semi-winter	5
470057	Wielkoposki	South Korea	Winter	7
470058	Willa	South Korea	Spring	5
470060	Yong dang	South Korea	Semi-winter	5
470061	Yonkkaichi Kwo	South Korea	Semi-winter	6
470062	Yonkokuban	South Korea	Winter	3
470065	Yudal	South Korea	Spring	3
478340	O 84	China	Spring	5
502304	AR-256	Russia	Winter	5
531273	Attila	Hungary	Winter	3
531274	Barkant	Netherlands	Winter	3
531275	BNW. 1 61/83	Germany	Winter	5
531277	Darmar	France	Winter	5
531278	Elena	Germany	Winter	5
531279	GK Savaria	Hungary	Winter	5
531280	IR-2	Hungary	Spring	7
531281	Librador	Germany	Winter	3
531282	Lindore	Germany	Winter	5
531283	Linglandor	Germany	Winter	5
531284	Liratrop	Germany	Winter	5
531285	Liropa	Germany	Winter	2
531287	Santana	Germany	Winter	7
531288	Ujfertodi	Hungary	Winter	3
535848	Mlochowski	Poland	Semi-winter	5
535850	Valdor	France	Winter	3
535851	Beryl	Poland	Winter	3
535853	Gundula	Germany	Winter	7
535854	Herkules	Sweden	Winter	7
535855	Janpol	Poland	Winter	3
535856	Korina	Germany	Winter	3
535857	Lester	Germany	Winter	3

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
535858	Lirabou	Germany	Winter	3
535859	Lirakotta	Germany	Winter	5
535860	Lirama	Germany	Winter	7
535861	Marinus	Germany	Winter	7
535862	Mirander	Germany	Winter	3
535864	Quinta	Germany	Winter	3
535865	Ridana	Germany	Winter	3
535866	Silesia	Czechoslovakia	Winter	7
535868	Status	Sweden	Winter	5
535869	Svaloefs Karab	Sweden	Winter	7
535870	Tamara	Germany	Winter	5
535871	Wipol	Poland	Semi-winter	7
535872	Darmor	Poland	Winter	7
535873	Jantar	Poland	Winter	5
535874	Licantara	Germany	Winter	5
535877	Rubin	Germany	Winter	7
535878	Start	Poland	Winter	7
542983	Tri-Cascade	United States	Winter	5
542984	Tri-Bridger	United States	Winter	4
594321	KS3579	United States	Winter	3
604608	KS1701	United States	Winter	2
610258	AR91004	United States	Winter	3
612846	Wichita	United States	Winter	2
619618	AR91017	United States	Winter	3
633118	Legend	United States	Spring	3
633119	Colt	United States	Spring	3
633120	Bolko	France	Winter	3
633121	Mar	Poland	Winter	3
633123	E94197	Mongolia	Spring	7
633124	NU 51084	Sweden	Spring	5
633125	Mar'janovskij	Ukraine	Spring	7
633126	Vostochno-sibirskii	Russia	Spring	7
633127	Vinnickij 15/59	Ukraine	Winter	5
633128	Nemercanskij 2268	Ukraine	Winter	5
633129	Krasnodarskii	Russia	Winter	5
633131	Evvin	Russia	Spring	7
633132	Kovalevskij	Ukraine	Spring	7
634754	Sumner	United States	Semi-winter	3
649126	Ames 1670	Canada	Spring	7
649128	Ames 6069	Sweden	Winter	5
649132	Ames 22548	Poland	Spring	5

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
649135	Ames 24222	Turkey	Spring	5
649136	Ames 26628	Germany	Semi-winter	3
649137	Ames 26631	Germany	Winter	3
649138	Ames 26632	Germany	Winter	3
649140	Ames 26634	Germany	Winter	3
649142	Ames 26638		Rutabaga	5
649143	Ames 26639		Rutabaga	3
649144	Ames 26640		Winter	3
649146	Ames 26642		Rutabaga	5
649147	Ames 26644		Rutabaga	3
649148	Ames 26646	Albania	Winter	5
649152	Ames 26655	United States	Spring	7

APPENDIX C. LIST OF ACCESSIONS USED FOR IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH RESISTANCE TO PG-3

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
311728	Czyzowskich	Poland	Spring	7
311732	Skrzeszowicki	Poland	Winter	7
469758	Dae cho sen	South Korea	Semi-winter	7
469759	Dong Buk	South Korea	Winter	7
469782	Drawft	South Korea	Winter	7
469783	Dwarf Essex	South Korea	Winter	7
469784	Eckendorfer Mali	South Korea	Semi-winter	7
469787	Expander	South Korea	Winter	3
469788	Fertodi	South Korea	Winter	2
469789	Fonto	South Korea	Spring	7
469791	France 1	South Korea	Spring	7
469801	Fuji	South Korea	Spring	5
469802	Gebr Dippes	South Korea	Winter	7
469806	Giant rape	South Korea	Winter	7
469807	Gogane	South Korea	Winter	5
469811	Gorozanski	South Korea	Winter	6
469812	Gylle	South Korea	Semi-winter	5
469814	Hamburg	South Korea	Winter	5
469818	Hwa 318	South Korea	Winter	2
469822	Iwashiro-natane	South Korea	Winter	7
469823	Iwawoochi	South Korea	Winter	3
469826	Janetzkis	South Korea	Spring	3
469828	Kani	South Korea	Winter	7
469829	Karafuto	South Korea	Winter	2
469830	Kasuya	South Korea	Winter	2
469831	Kasuyashu	South Korea	Winter	2
469840	Klinki	South Korea	Spring	3
469841	Koubun	South Korea	Spring	5
469842	Kraphhauser	South Korea	Spring	2
469843	Kritmar rape	South Korea	Spring	6
469845	Kuju	South Korea	Winter	2
469883	Lembkes	South Korea	Winter	7
469890	Maintainer for GHR MS	South Korea	Winter	5
469999	Nugget	South Korea	Semi-winter	7
470000	Oleifera	South Korea	Semi-winter	7
470002	Panter	South Korea	Winter	3
470003	Petanova-lihonova	South Korea	Semi-winter	5

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
470008	Polnoslaski	South Korea	Winter	9
470010	R. Creeus	South Korea	Winter	7
470012	Ramses	South Korea	Winter	7
470013	Rang	South Korea	Semi-winter	5
470015	Rapifera	South Korea	Winter	5
470016	Rapol	South Korea	Winter	7
470019	Regal	South Korea	Winter	5
470020	Rumania 1	Romania	Winter	5
470021	Russia 5	Russia	Spring	7
470055	Weal Dong Cho	South Korea	Semi-winter	5
470056	Weibulls margo	South Korea	Semi-winter	2.5
470057	Wielkoposki	South Korea	Winter	4
470058	Willa	South Korea	Spring	7
470060	Yong dang	South Korea	Semi-winter	2
470061	Yonkkaichi Kwo	South Korea	Semi-winter	7
470062	Yonkokuban	South Korea	Winter	7
470065	Yudal	South Korea	Spring	5
535848	Mlochowski	Poland	Semi-winter	7
535850	Valdor	France	Winter	7
535851	Beryl	Poland	Winter	5
535853	Gundula	Germany	Winter	5
535854	Herkules	Sweden	Winter	5
535855	Janpol	Poland	Winter	5
535856	Korina	Germany	Winter	5
535857	Lester	Germany	Winter	5
535858	Lirabou	Germany	Winter	5
535859	Lirakotta	Germany	Winter	5
535860	Lirama	Germany	Winter	5
535861	Marinus	Germany	Winter	1
535862	Mirander	Germany	Winter	3
535864	Quinta	Germany	Winter	3
535865	Ridana	Germany	Winter	3
535866	Silesia	Czechoslovakia	Winter	4
535868	Status	Sweden	Winter	7
535869	Svaloefs Karab	Sweden	Winter	6
535870	Tamara	Germany	Winter	5
535871	Wipol	Poland	Semi-winter	5
535872	Darmor	Poland	Winter	1
535873	Jantar	Poland	Winter	6
535874	Licantara	Germany	Winter	5

PI number	Plant Name	Country originated/obtained from	Growth habit	Median Severity
649128	Ames 6069	Sweden	Winter	5