University of Alberta

Screening of *Brassica* Germplasm for Resistance to *Plasmodiophora brassicae* Pathotypes Prevalent in Alberta, Canada

by

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A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Master of Science
in
Plant Science

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Edmonton, Alberta

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Spring, 2010
Edmonton, Alberta

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Abstract

Clubroot disease, caused by *Plasmodiophora brassicae*, poses a threat to the Canadian canola industry, and breeding of resistant cultivars is urgent. The objective of this study was to identify *Brassica* germplasm possessing resistance to Canadian *P. brassicae* pathotypes based on greenhouse screening. Germplasm resistant to local pathotype(s) is the prime requirement for breeding clubroot resistant cultivars. Race-specific to broad-spectrum resistance was identified in the diploid species *B. rapa* (AA) and *B. oleracea* (CC), and in the amphidiploid *B. napus* (AACC). The diploid *B. nigra* (BB) also showed race-specific to broad-spectrum resistance; however, the two amphidiploids with *B. nigra* as one of the parental species *viz.*, *B. juncea* (AABB) and *B. carinata* (BBCC) were completely susceptible. The occurrence of resistance in the diploid and amphidiploid *Brassica* species is discussed in the light of their evolution, and a differential set for identification of Canadian clubroot pathotypes is proposed.

Acknowledgements

I would like to express my heartily gratitude to Dr. Habibur Rahman for giving me the opportunity to work with Canola Breeding Program and also for the invaluable guidance, encouragement and advice throughout the project. His never tiring nature and supreme patience for introducing me to the world of research and thesis writing is gratefully acknowledged. I want to extend my sincere gratitude to Dr. Stephen Strelkov too, for providing valuable suggestions at several points of my research. Dr. Randolph S Currah is also gratefully acknowledged for his valuable suggestions as examination committee member.

I would like to acknowledge Agricultural Research Service of the United States Department of Agriculture (USDA), Department of Gene Bank of Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, Green Gene International, Hill Rising, Horse Castles Lane, Sherborn, United Kingdom, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh, Canola Breeding Program, University of Alberta, Bangladesh Agricultural University (BAU), Dr. Ronal J. Howard from Alberta Agriculture, Food and Rural Development, Alberta, Canada and Dr. Dean Spaner from Department of Agricultural, Food and Nutritional Science, University of Alberta for providing me all the *Brassica* germplasm for my research.

I would love to extend my gratitude to all the good people from Canola Breeding Program who have taught me so much and made my time so enjoyable-Dr. Mohan Thiagarajah, An Vo, Shakir Abdus Salam, Mirko, Zahidur Rahman, Salvador Lopez and many others. I want to thank Dr. Victor Manolii, Dr. Tiesen Cao from Plant Pathology Lab for helping me throughout my program with ideas and information.

I want to extend my heartily gratitude to Alberta Canola Producers Commissions (ACPC) and Alberta Canola Industry Development Fund (ACIDF) for funding this project.

All the cooperation I received from Mr. Bruce Alexander and Claudia throughout my research project is also sincerely acknowledged.

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Chapter 1

Literature Review

1.1 Introduction

Brassica, one of the 338 genera (Warwick *et al.*, 2006) in the tribe Brassicae of the family Brassicaceae (Rakow, 2004), is agriculturally the most important genus providing the greatest diversity of food products from its 39 species worldwide (Warwick *et al.*, 2006; Dixon, 2006b). For centuries, species in this genus have been a major source of food for humans' and fodder for domesticated animals. The seed is a valuable source of high quality edible oil and the seed meal is a high value protein for animal feed. In addition, some species of *Brassica* have been used as condiments in some Asian countries for centuries. In the western world, prior to World War II, *Brassica* oil was used as lamp oil as well as lubricant for steam engines (Downey, 1990). However, increased awareness of global warming has recently amplified an interest in these eco-friendly fuels, bio-diesel production from biodegradable components, which leads to the re-inauguration of use of this oil in the automotive industries. New avenues of use of *Brassica*, especially broccoli, cauliflower, brussels sprouts, collards, kohlrabi and kale are becoming apparent and they are now considered functional food with long-term roles in the fight against cancer (Lampe and Peterson, 2002; Fowke *et al.*, 2003; Brandi *et al.*, 2005) and coronary disease (Dixon, 2006b).

Brassica oil seeds, derived from *Brassica napus* and *B. rapa*, currently are together the second most widely cultivated crop in Canada. Acreage of this crop has increased steadily since its introduction in the 1940s; with a sharp increase in acreage since the introduction of low-erucic acid cultivars in the late 1960s. Since then, acreage has increased five-fold (Figure 1.2) and has recently reached 6.54 million hectares (Statistics Canada, 2008). Plant breeding and improved agronomic practices have played an important role in the increased productivity of this crop. Around an 11 fold increase per unit yield (Figure 1.3) has been achieved in last four decades, which is reflected in an 11 folds increase in total production

(Figure 1.2) accounting for 12.65 million tonnes in 2008 (Statistics Canada, 2008).

During the early stage of establishment of these oilseeds, *B. rapa* was the dominant species in western Canada (Downey *et al.*, 1974). However, its acreage has gradually diminished to less than 2.0% of the total acreage with the introduction of relatively early maturing *B. napus* cultivars. In 2008, cultivated species of *Brassica* were seeded in more than 6.55 million hectares of land of which 6.54 million hectares (99.98% of the total *Brassica* spp. growing area) was occupied by 'double low' or 'canola' quality cultivars (Statistics Canada, 2008). This 'canola' quality is characterized as any rapeseed having less than 1% erucic acid in the fatty acid profile of seed storage lipids and less than 18 micromoles aliphatic glucosinolates per gm seed at 8.5% moisture content (Canadian Food Inspection Agency, 1999).

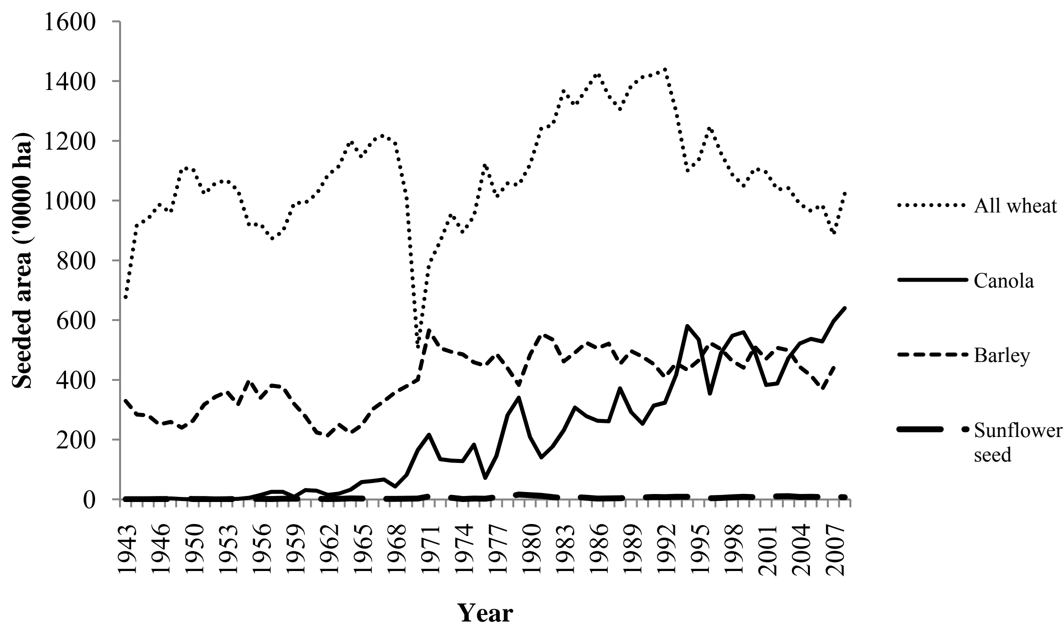

Figure 1.1: Agricultural land usage statistics of five most important agricultural crops in Canada from 1943 to 2008 (Statistics Canada, 2009; data retrieved on March 23 2009)

Improved quality oil from canola type cultivars, whose content is about two times higher than soybean [approximately 43% in canola (Casséus, 2009) versus

20% in soybean (Droff, 2007)] and its meal having good feed value makes this a highly profitable crop, which is believed to have played an important role in its increased acreage over the past years. Development of high yielding, open pollinated and hybrid canola cultivars possessing superior agronomic traits and tolerance to various insect pests and diseases on a regular basis by government research institutes and private seed companies has contributed to the steady increase in both average yield and national annual production of this crop.

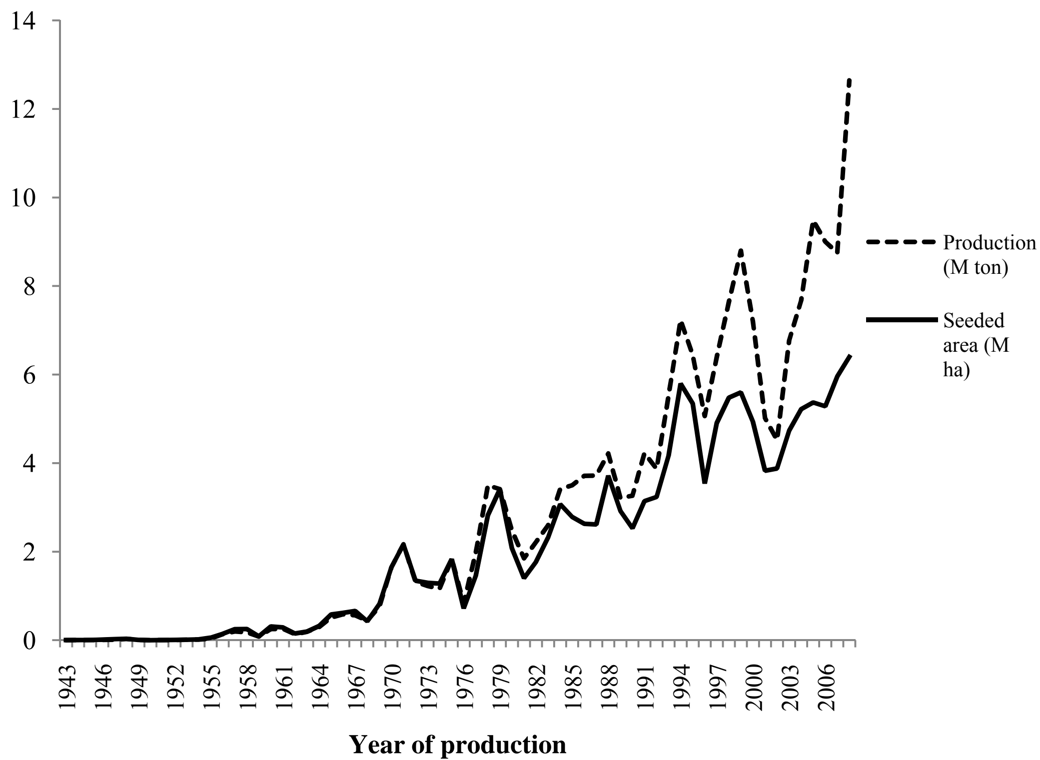

Figure 1.2: Production statistics of canola in Canada from 1943 to 2008 (Statistics Canada, 2009; data retrieved on March 23, 2009)

In the meantime, the demand for Canadian canola in the international market has increased significantly, for example during the period of 2000 to 2006 this demand increased four-fold (Mark Goodwin Consulting Ltd., 2008). As a result, the canola crop today has become a big industry in Canada and is being ranked as the number one cash crop (Saha and Trant, 2008) for its annual contribution of \$13.8 billion, accounting for 1.18% of the nation's GDP (Mark Godwin Consulting Ltd., 2008). Canola alone generated 17 percent of Canada's

$14.5 billion gross receipts from sale of crops in 2006 along with creation of around 216,000 jobs in eastern and western Canada (Mark Goodwin Consulting Ltd., 2008).

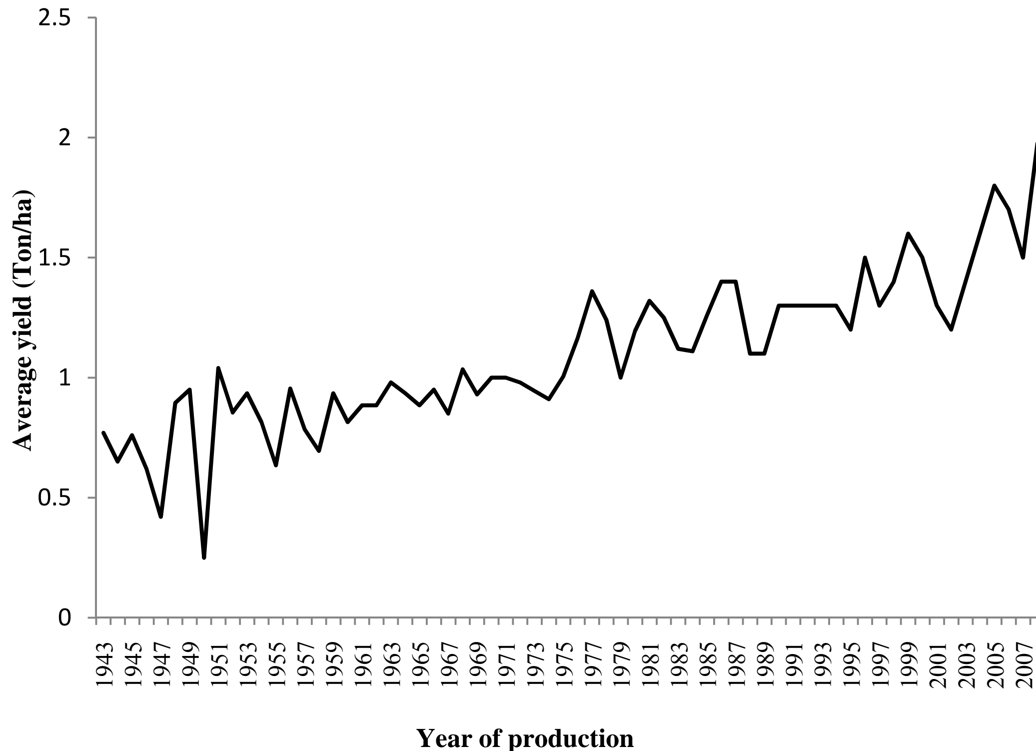

Figure 1.3: Per unit yield statistics of rapeseed in Canada from 1943 to 2008 (Statistics Canada, 2009; data retrieved on March 23, 2009)

Canada by far is the largest exporter of canola/rapeseed, accounting for 53% of global export trade in 2006 (FAOSTAT, 2009 - updated on December 9, 2008 and data retrieved July 21, 2009); although it produces only 18.4% of the world's canola/rapeseed (FAOSTAT, 2009 - updated on June 23, 2009 and data retrieve July 21, 2009). Moreover, canola is responsible for 18.2% of Canada's global trade in agricultural products (crops, livestock and their by-products), worth $15.6 billion in 2006 (Mark Goodwin Consulting Ltd., 2008). It is assumed that due to worldwide demand for biofuels and consumer preference for canola oil over soybean and palm oil for its health benefits, demand for this seed oil will be further boosted in the future. The price of canola seed and oil is also expected to increase in the future due to its increased use in bio-fuel production (G8 Hokkaido-Toyako Summit, 2008), ultimately which will boost up the contribution

of the canola industry to the national economy to an even a greater extent. Therefore, the Canola Council of Canada, the non-profit organization representing the entire Canadian canola industry (*viz.*, growers, crop development and protection companies, processors, and exporters) has set an ambitious goal to increase canola production to 15 million tonnes by 2015 known as "Growing Great 2015" (Canola Council of Canada, 2007). Increasing per hectare yield of seed and its oil content would be the most agriculturally sustainable practice to achieve this goal.

The requirements for optimal production of a crop include good cultivars with improved genetics, suitable environment for its cultivation as well as application of the best management practices. The package of improved genetics primarily includes different desirable morphological and physiological traits contributing to the increased yield of the crop and enhanced resistance or tolerance to different biotic and abiotic stresses. The abiotic stresses include water shortage and/or drought, water logged conditions, salinity, freezing temperature and/or heat shock, etc. On the other hand, the biotic stresses mainly include infection by different insect pest and pathogens. Generally, resistance and/or tolerance to biotic stresses in crop plants do not directly contribute to the yield potential; however, it is extremely important for secured production of the crop. Therefore, resistance breeding has received much attention from the plant breeders worldwide.

So far, different lepidopteran and coleopteran insects and some fungal diseases have been reported to be the major biotic stresses of canola in Canada. Among the diseases of canola, blackleg (*Leptosphaeria maculans*) and sclerotinia stem rot (*Sclerotinia sclerotiorum*) are considered as major diseases; while diseases like fusarium wilt (*Fusarium oxysporum*), clubroot (*Plasmodiophora brassicae*), downey mildew (*Peronospora parasitica*), alternaria black spot (*Alternaria brassicae, A. raphani, A. alternata*), white rust (*Albugo candida*), etc. have been listed as minor diseases (Agriculture and Agri-Food Canada, 2005).

However, in the recent years, clubroot disease has received a great deal of attention from the canola growers in Alberta because of its increasing occurrence in canola fields, nature of its devastation on yields and the ability of this pathogen to persist in soil by avoiding all kinds of management practices.

1.2 Clubroot disease of *Brassica* crops

Clubroot is a serious soil borne root disease of *Brassica* crops worldwide, caused by *Plasmodiophora brassicae* Woronin (Woronin, 1878 cited by Cook and Schwartz, 1930; Strelkov *et al.*, 2007). This disease has long been reported as a major limiting factor for successful production of vegetable and oil crop species of Brassicaceae in Australia (McAlpine, 1891 as cited by Donald *et al*., 2006), Japan (Ikegami *et al*., 1981 as cited by Hirai, 2006) and in most of the European countries (Wallenhammar, 1996; Voorrips, 1995; reviewed by Hirai, 2006). In North America, this disease was first reported in the United States of America in 1852 (Karling, 1968) while in Canada it was first reported in Quebec and New Brunswick in 1927 (Canadian Phytopathological Society, 1927-1928). Clubroot disease has been a problem for vegetable cole crop production in the east coast (Atlantic Region) of Canada and in British Columbia (Rimmer *et al.,* 2003). In Alberta, this disease was previously reported in home and market gardens (as mentioned in Strelkov *et al.*, 2006a); while in commercial canola (*Brassica napus*) fields, it was first reported in 2003 where more than 15% (12 out of 70 surveyed) fields showed presence of this disease (Strelkov *et al.*, 2005). Further surveys conducted in 2005 confirmed presence of the disease in more than three times number of fields than the previously reported in the Edmonton region of Alberta (Strelkov *et al*., 2006b). Occurrence of clubroot disease in canola has received much attention from the canola growers and researchers for its potential to cause significant reduction in seed yield and oil content (Pageau *et al*., 2006) as well as for its ability to disseminate at an epidemic rate (Clarkson and Brokenshire, 1984).

1.3 Disease cycle of *Plasmodiophora brassicae*

The clubroot pathogen *Plasmodiophora brassicae* was initially categorized into the phylum Plasmodiophorids, a group of obligate, inter-cellular plant parasites commonly known as slime moulds (Myxomycetes) (Karling, 1968); however, later it was grouped as Protista under the phylum Plasmodiophoromycota (Barr, 1992). The body of this pathogen is plasmodium in nature. These plasmodia produce numerous sub-spherical to spherical shaped haploid resting spores which are around 2.4 to 3.9 µm in diameter (Buczacki and Cadd, 1976). These resting spores upon germination produce spindle-shaped or pyriform zoospores (2.8 to 5.9 µm in diameter) having two flagella (a shorter flagellum which is blunt ended and a longer one which has a whiplash or tail piece) (Ayers, 1944). Germination of resting spores is stimulated by some kind of heat stable, fairly polar and low molecular weight chemical exudates, termed Germination-Stimulating Factor (GSF) (Suzuki *et al*., 1992). These stimulants are secreted from the roots of both susceptible and resistant crucifer host species (Macfarlane, 1970; Suzuki *et al*., 1992) as well as from non-host species such as lettuce (*Lactuca sativa*) (Suzuki *et al*., 1992), leek (*Allium porrum*), perennial ryegrass (*Lolium perenne*), winter rye (*Secale cereale*) (Friberg *et al*., 2005; Bochow, 1965; Kowalski and Bochow, 1996). Germination of resting spores to release zoospores occurs 26 to 30 hours after stimulation by GSF (Mehrotra and Aneja, 1990). The cell wall membrane of the resting spores consist of approximately 25.1% chitin, ≥2.5% other carbohydrates, 33.6% protein and ≥17.5% lipid (Moxham and Buczacki, 1983). The frequency of resting spore germination increases with the maturity of the spore and is influenced by various environmental factors like humidity, soil moisture, temperature, soil texture etc., as well as triggered by the presence of certain inorganic ions (e.g., Ca^{+}, B, N) in soil (Karling, 1968; Macfarlane, 1970; Yano *et al*., 1991; Takahashi, 1994a; Dixon, 2006a). Mature spores have also been found to germinate regardless of the presence of calcium ions, whereas the presence of calcium ions is essentially required for the germination of immature young spores (reviewed by Kageyama and Asano, 2009). On the other hand, the resting spores from old rotten galls have

been found to possess higher germination potential than those from young, firm galls (Macfarlane, 1970).

This pathogen is generally more prevalent in wet soil with moisture content of 70% of its maximum water holding capacity (Colhoun, 1953), which are acidic in nature (Karling, 1968; Strelkov *et al*., 2007). Palm (1963) reported that soil pH of 4.9 to be optimal for infestation by *P. brassicae* while Wallenhammar (1996) found highest infestation at soil pH of 5.2 to 6.6. Still, virulence of *P. brassicae* is not restricted only to acidic soils since clubroot disease was found in fields with soil pH of as high as 7.8 (Wellman, 1930 cited by Wallenhammar, 1996). However, higher germination frequency of resting spores in acidic condition than in alkaline soil (Bochow, 1960) and increased concentration of Ca^{+2} in root tip meristem at lower soil pH (Walter *et al.*, 2000) are believed to be the reasons for higher incidence of clubroot disease in acidic soil. In Alberta, clubroot disease was recorded in canola fields with soil pH as low as 4.8 to as high as 7.6 (Strelkov *et al*., 2007).

In addition to soil moisture and soil pH, soil texture (soil physical condition) has significant influence on infection by *P. brassicae* to its host plants. This pathogen was found to cause higher rate of infection in silty and clay type soils compared to sandy type soils which are low (<6%) in humus (Wallenhammar, 1996). Five times more *P. brassicae* spores are required for optimal infection in humus-rich soil than in clayey soil (Naumov, 1928).

Chupp (1917) observed *P. brassicae* resting spores to germinate at soil temperature between 16 and 21°C in presence of host plant. Ikegami *et al*. (1981) found 20 to 25^0C to be the most favourable spring soil temperature for germination of resting spores. On the other hand, Buczacki *et al.* (1978) reported mean daily temperature of not less than 19.5°C to be the most favourable for 100% infection by this pathogen. However, infection can occur at temperature as low as 12^0C (Porter, 1986). Furthermore, optimum temperature for germination of resting

spore and successful infection is also influenced by soil pH. According to Sherf and MacNab (1986) the ranges of minimum, maximum and optimum temperature for germination of resting spores and successful infection of host plants in acidic soil are, respectively 10 to14°C, 30 to 35°C and 20 to 25°C; while in case of alkaline soil resting spores are able to germinate at a lower temperature. A swarm of primary zoospores are released from resting spores after germination, which are able to survive for relatively short periods of time in absence of the host (Karling, 1968).

A zoospore infects the host plant by injecting its cellular contents into the root hair cell (Aist and Williams, 1971). This infection process involves attachment of the zoospore to the wall of the root hair by evaginating its long tube like structure called 'Rohr' into a bulbous structure called 'adhesorium', puncturing the wall with a bullet shaped, rod like structure, called 'Stylet' located within the Rohr and finally transfer of the cellular contents into the cell of the root hair (Mehrotra and Aneja, 1990). After penetration, a papilla of callose develops beneath the adhesorium around the point of penetration to heal the wound. The zoospore takes about three hours to penetrate the wall of the root hair, whereas injection of genetic material occurs in one second. In the infested soil and under favourable environmental conditions, this primary infection on host root hairs takes place within two weeks of seeding (Williams *et al.*, 1971; Ingram and Tommerup, 1972). Naiki *et al.* (1978) observed higher incidence of root hair infection at spore density of 10^6 to 10^7 spore/g dry soil, whereas no incidence of root hair infection was found at a spore density of less than 10^3 spore/g dry soil. After a few days of primary infection, repeated mitotic division of the pathogen nucleus take place, which leads to the development of a multinucleate plasmodium. This multinucleate plasmodium later develops into tens or hundreds of zoosporangia, each containing four to eight haploid zoospores. These zoospores are then either released from zoosporangia directly into the rhizosphere by disintegration of the host cell wall (Mehrotra and Aneja, 1990) or infect neighbouring cortical cells directly from inside the root hair (Aist, 1977 as cited

by Diederichsen *et al*., 2009). These newly formed haploid zoospores undergo plasmogamy prior to penetration and infection, where the cytoplasm of two haploid zoospores fuse (but nuclei remain separate) to form a dikaryotic secondary zoospore (Ingram and Tommerup, 1972). This kind of root hair infection by primary zoospores and formation of zoosporangia and secondary zoospores has also been observed in non crucifers such as *Raphanus sativus, Lolium perenne, Reseda odorata*, and *Tropaeolum majus* (Webb, 1949; Macfarlane, 1952). These secondary zoospores released in the rhizosphere have been found to re-infect root hairs, resulting in a rapid, asexual propagation of the pathogen (Naiki *et al.,* 1984).

The second phase in the pathogenesis of *P. brassicae* to the host species is known as the cortical infection stage, as identified by Cook and Swartz (1930). It is induced only by the secondary zoospores which are 1.9 to 2.3 µm in diameter and possess two equal size flagella (Ayers, 1944). They infect either young root tissue through direct penetration or the older, thickened roots and even underground stems by penetration through wounds to produce new plasmodia. These binucleate plasmodia then undergo synchronous mitotic division and become multinucleate. Resistance and/or susceptibility of the host species towards *P. brassicae* pathotypes become apparent at the secondary phase of infection (Kobelt *et al.*, 2000; Tanaka *et al*., 2006; Donald *et al*., 2008).

These multinucleate plasmodia then spread to cortical cells and reach the cambium through direct penetration from where they spread in all directions in the cambium, outward into the cortex and inward toward the xylem (Figure 1.4). As the plasmodia pass through the cells, they become established in some of the cells; and this process is stimulated by elevated concentration of cytokinins (Dekhuijzen and Overeem, 1971; Dekhuijzen, 1980) and auxins (reviewed by Ludwig-Müller, 1999). The invaded cells enlarge and divide abnormally resulting in mass of cells with root size up to five or more times larger compared to normal

root and thus lead to the formation of characteristic galls, known as clubbed root (Agrios, 2005).

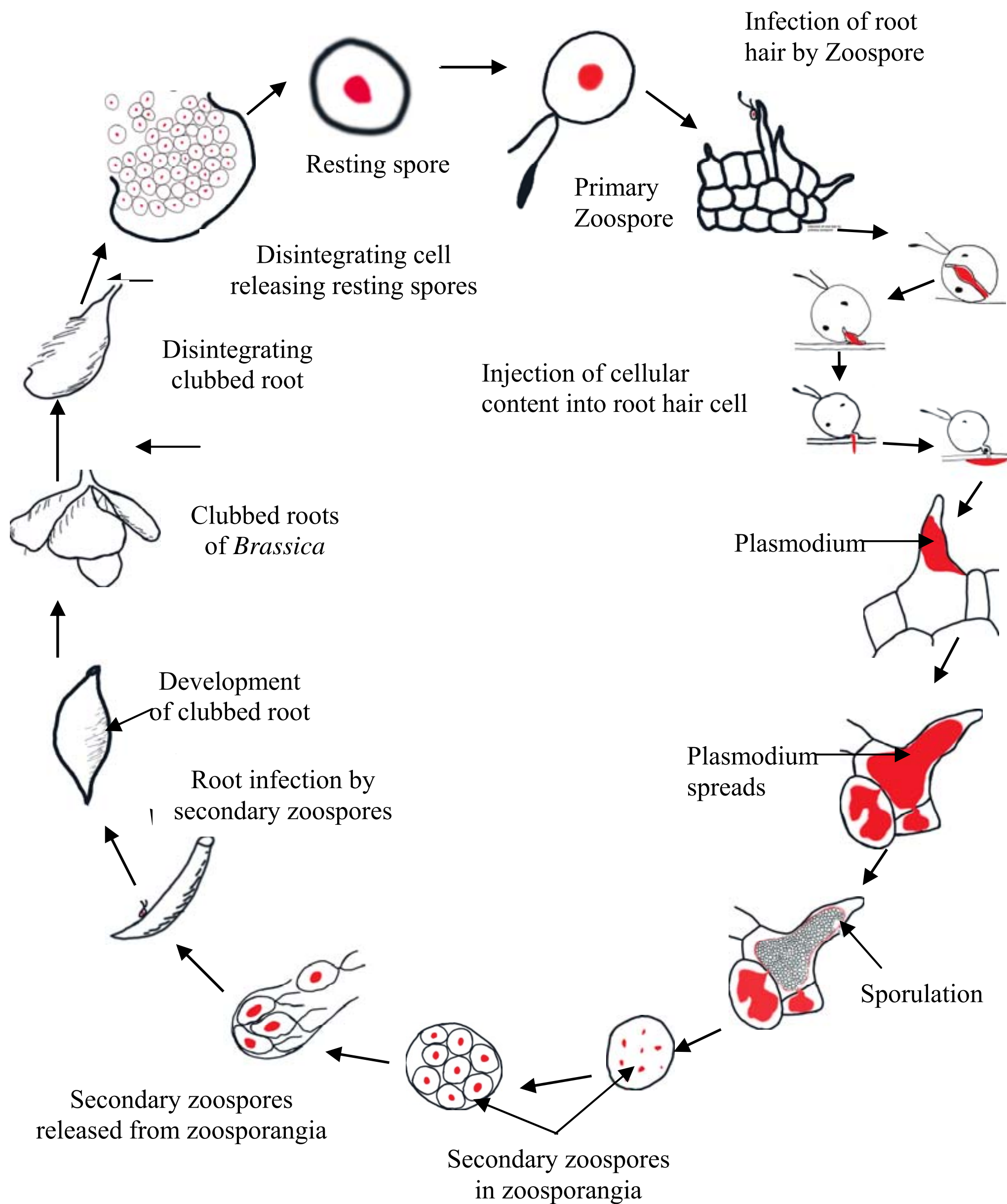

Figure 1.4: Disease cycle of clubroot of crucifers caused by *Plasmodiophora brassicae*

Raa (1971) found presence of 50-100 times higher concentration of IAA (indole-3-acetic acid, auxin) in the gall tissue from infected plants compared to those from uninfected plants. Clubbed roots not only use much of the nutrients normally available for growth and development of the plant but also interfere with

absorption and translocation of mineral nutrients and water through the root system, resulting in gradual stunting and wilting of the above ground parts of the plant, which consequently result in severe reduction in the crop yield (Hirai *et al.,* 2004; Agrios, 2005).

Later in the development of secondary multinucleate plasmodia, two haploid nuclei in plasmodia fuse (process known as karyogamy) and become diploid. Karyogamy is closely followed by meiosis, resulting in the formation of haploid type plasmodia (Buczacki, 1983). These plasmodia finally develop into resting spores (Ikegayami *et al*., 1982). Upon decay of the clubs, as many as 10^{11} resting spores per plant are released into soil (Voorrips, 1995) where they can survive up to 20 years even in absence of any host plant (Wallenhammar, 1996). On the other hand, zoospores unable to invade host plant are reported to survive for only short periods (Karling, 1968; Suzuki *et al.*, 1992; Takahashi, 1994b).

Majority of the cultivable land in western Canada is either alkaline (pH >7.5) or neutral (pH = 6.5 to 7.5) in pH, but over three million hectares of land in this region has been reported to be medium (pH 5.6 to 6.0) to strongly acidic (pH 5.1 to 5.5) in nature (Canola Council of Canada, 2003). Average temperature in this region during the canola growing period (April 15 to September 15) ranges from 15 to 23° C (Howard *et al.*, 2008). All these factors may have significant contribution to the increased incidence of clubroot disease in Alberta. Occurrence of this disease at the field entrance and their patchy distribution throughout the infested field suggests farm equipments contaminated with soil and pathogen as important mode of its dissemination from one field to the other (Strelkov *et al*., 2007). Earthworms, moles, root nematodes and insects can transport this pathogen over shorter distances, while rain and flood water can disperse them especially on sloping land (reviewed by Dixon, 2009b); however, spores attached to light, dry, dusty soil particles can be disseminated even over greater distances by wind. Both farm animals and their food supplies are believed to have played major roles in the worldwide dissemination of this pathogen since spores can withstand highly

acidic gut environment. Before *P. brassicae* was declared as an Agricultural Pest in Alberta in 2007, there was no restriction on farm equipment movement among the fields. In Alberta, clubroot disease was first reported in 12 canola fields in 2003 (Strelkov *et al*. 2005); however by 2008 the number of infested fields has jumped to more than 400 (Cao *et al*., 2009). Therefore, this disease is thought to be more prevalent among *Brassica* crops in Alberta.

1.4 Pathogenic variability of *Plasmodiophora brassicae*

Honig (1931) was the first to report pathogenic variability among the field populations of *P. brassicae*. Since then, more studies have been conducted by researchers around the world to define this pathogenic variability (*i.e.* physiologic specialization) of this pathogen from populations collected from different fields using sets of differential hosts. A large number of *P. brassicae* pathotypes with various degrees of virulence have been reported to occur in Europe (Macfarlane, 1955; Tajllingii, 1965; Buczacki *et al*., 1975; Nowicki, 1978; Crute *et al*., 1980; Voorrips, 1995; Somê *et al*., 1996), Asia (Yoshikawa, 1993; Kuginuki *et al*., 1999; Hatakeyama *et al.*, 2004), Australia (Donald *et. al*., 2006) and in North America (Ayers, 1957; Williams, 1966; Strelkov *et. al.*, 2007). Multiple pathotypes of this pathogen have also been reported to coexist in the same field (Ayers, 1972; Jones *et al*., 1982; Xue *et al.*, 2008).

Based on the differential hosts of *B. rapa*, *B. nigra*, several genotypes of *B. oleracea* and *Sisymbrium altissimum*, Ayers (1957) reported the occurrence of six pathotypes of *P. brassicae* in Canada. Later, Ayers (1972) adopted the Williams (1966) system, which is based on the reaction of infection to four crucifer cultivars: *B. oleracea* var. *capitata* ‘Jersey Queen’ and ‘Badger Shipper’ and *B. napus* subsp. *napobrassica* ‘Laurentian’ and ‘Wilhelmsburger’, to determine the pathotypes of *P. brassicae* and identified seven races [race 1, race 2, race 3, race 4, race6, race 6a (later identified as race-5) and race 7] of this pathogen in Canada. Pathotype 6 was initially identified by Williams (1966) in a *P. brassicae* population from Quebec along with pathotype 2 from British

Columbia. However, pathotype 6 was later found to be the dominant one in cole crops in Ontario (Reyes *et al.,* 1974). Strelkov *et al*. (2006a) collected seven *P. brassicae* populations from Alberta where five were identified as pathotype 3 and the remaining two populations were identified as pathotype 5. Based on single-spore isolates obtained from Canadian *P. brassicae* populations, Xue *et al*. (2008) identified five pathotypes of which pathotypes 2, 3, 6 and 8 occur in the canola fields of Alberta, pathotypes 3, 5 and 8 in Ontario and pathotype 6 occurs in British Columbia. Among the *P. brassicae* pathotypes identified in Alberta, pathotype 3 was found to be the most prevalent one and virulent on all Canadian canola (*B. napus*) cultivars (Strelkov *et al*. 2006a and 2007; Xue *et al.*, 2008). Pathotype 3 was also reported to be a dominant pathotype in Nova Scotia (Hildebrand and Delbridge, 1995). However, all these *P. brassicae* populations in Canada are considered to be less diverse than those of Europe (Donald *et al.*, 2006).

1.5. Impact of clubroot disease on yield

Clubroot disease causes significant yield loss in *Brassica* oil seed crops. Wallenhammar *et al.* (1999) observed up to 50% yield loss in oilseed *B. napus* due to 91% infestation by this pathogen. In Quebec, Canada, up to 91% yield loss with 4.7 to 6.1% reduction in oil content was reported in the infected canola plants (Pageau *et al*., 2006); while in Alberta, almost 100% yield loss was observed in the most severely affected field (Strelkov *et al*., 2007). Therefore, clubroot was added as a declared pest to *Alberta's Agricultural Pests Act* (APA) in April 2007 (http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex8593).

1.6 Management of clubroot disease

A disease of an organism is an abnormal condition caused by a pathogen and expressed as specific signs and/or symptoms that impairs that organism's normal physiological functions. The visible symptoms of a disease are the result of interaction of three components: virulent pathogen, suitable environment and susceptible host; and in the absence of any one of these three, a disease cannot be

established. Therefore, manipulation and/or control of any one or all of these components, depending on their suitability, feasibility and effectiveness are obligatory for effective management of diseases.

Traditional disease management strategies primarily focus on eliminating or suppressing the pathogen after its destructive effects become visible (Sullivan, 2004). In case of soil borne diseases, this strategy is usually performed by using either chemical (*e.g.*, soil drenching fungicides, fumigants etc.) and/or biological (*e.g.,* Trichoderma, *Phoma glomerata* etc.) agents. Various fungicides, for example benomyl, captafol (Tate, 1977; Naiki and Dixon, 1987), quintozene, trichlamide, calcium cyanamide, EF70513 (Naiki and Dixon, 1987), chlorothalonil, fostyl-Al, thiabendazole, thiophanate-methyl (Humpherson-Jones, 1993) fluazinam, flusulfamide (Humpherson-Jones, 1993; Cheah *et al*., 1998), mancozeb (Cheah *et al*., 1998), etc. were tested to control clubroot disease of *Brassica*s. However, efficacy of these fungicides for controlling *P. brassicae* was found to be inconsistent (Naiki and Dixon, 1987; Cheah *et al*., 1998). Moreover, use of different chemical pesticides is generally discouraged for their potential hazardous effect on the ecosystem (Kesavachandran *et al*., 2009).

A number of surfactant products have been evaluated for their ability to control clubroot (Humpherson-Jones, 1989 and 1993) of which alkyl phenol ethylene oxide (Agral^{TM}) was found effective in controlling the disease in glasshouse experiments with consistent increases in yield and non-phytotoxicity to young plants (Humpherson-Jones, 1993). However, in field trials when applied in the absence of disease pressure, all the treatments of Agral were found to be phytotoxic (Hildebrand and McRae, 1998).

Plant nutrients (mostly calcium, magnesium, potassium and boron) management has been considered an important strategy for decades to control clubroot disease of *Brassica*. A number of studies indicated that the changes in available calcium, the ratio of calcium to potassium, pH and the ratio of calcium

to boron can affect infection and establishment of the pathogen and subsequent gall development (reviewed by Donald and Porter, 2009). Higher concentration of calcium ions at high soil pH reduces root hair infection effectively (Webster, 1986 as cited by Dixon, 2009a), as calcium ion in the form of calcium pectate increases the tolerance of the cell wall to the action of cell wall-degrading enzymes (Punja *et al*., 1986) by strengthening the middle lamella of epidermal cell walls and by impairing flagellar motion and chemotaxic movement of zoospores towards the host root (Marschner, 1995). This ion also inhibits the production of differentiated and dehisced sporangia of *P. brassicae* within infected root hairs (Webster and Dixon, 1991a). However, this ability of calcium ion to inhibit disease development is hampered in a boron deficient environment; and abundant supplies of potassium following infection encourage an expansion of gall tissue (Palm, 1963). On the other hand, calcium in the form of calcium nitrate directly affects the viability of *P. brassicae* resting spores and reduces the extent of root galling even when applied after infection (Page, 2001 as cited by Dixon, 2009a).

In addition to many other forms of liming, application of calcium in the form of calcium cyanamide ($CaCN_2$) has been widely tested worldwide as control measure of clubroot disease (Naiki and Dixon, 1987; Klasse, 1996; Tremblay *et al*., 2005). Upon hydrolysis, $CaCN_2$ increases soil pH and releases an intermediate anion (CN^{-2}) which is fungitoxic in nature (Conforth, 1971). Phytotoxic effect of this intermediate anion (CN^{-2}) has also been reported in cabbage species both in field (Naiki and Dixon, 1987) and glasshouse experiments (Williamson and Dyce, 1989). Therefore, time break is usually advised between product applications and seeding or transplantation to ensure proper decomposition of calcium cyanamide. The rate of decomposition of calcium cyanamide is influenced by soil type, temperature, application rate, humidity, crop type and cultivation techniques (Klasse, 1996; Tremblay *et al*., 2005). The efficacy of this chemical is also dependent on its particle size (Murakami *et al*., 2002; Donald *et al*., 2004). No report on the efficacy of $CaCN_2$ is yet available in canola cropping system under the conditions of Canadian prairie.

In contrast to calcium and soil pH, boron inhibits intra-cellular dissemination of infection both in primary and secondary stages (Webster, 1986; Webster and Dixon, 1991b) by disrupting the movement of *P. brassicae* within the host plant. This results from its (boron) role in the regulation of endogenous auxin (Bohnsack and Albert, 1977), which is required for the synthesis of cell wall precursors (Marschner, 1995) and thus increases the integrity of the cell wall. However, this interrelationship between mineral nutrients in the soil system is very complex and is governed by a number of factors like soil type, soil pH, soil temperature, presence of other mineral elements and also applicable form of the mineral elements and their method of application. More research is necessary to standardize a suitable nutrient system by considering all the factors to manage clubroot disease of canola successfully.

Possibility to control clubroot disease by using soil borne microorganisms has also been explored. Several soil borne fungal isolates *e.g.*, *Phoma glomerata* (Arie *et al.*, 1998), *Heteroconium chaetospira* (Narisawa *et al.*, 1998), *Trichoderma* (Cheah *et al.*, 2000) and also bacterial isolates *e.g*., *Streptomyces* spp. (Cheah *et al.*, 2000; Cheah *et al*., 2001) have been reported to reduce the severity of clubroot disease in vegetable *Brassica* crops. Narisawa *et al.* (1998) isolated a root endophytic fungus *Heteroconium chaetospira*, which was effective against *P. brassicae* in Chinese cabbage (*Brassica rapa* var. *chinensis*) at low to moderate soil moisture and at pathogen resting-spore densities up to 10^5 spores per gram of soil (Narisawa *et al*., 2005). Teruyoshi *et al*. (2001) also identified two isolates of the root endophytic fungus *H. chaetospira,* which were successful in reducing clubroot in Chinese cabbage significantly. On the other hand, Arie *et al.* (1998) observed complete control of clubroot in Chinese cabbage, turnip (*B. rapa* var. *rapifera*), cabbage (*B. oleracea* var. *oleracea*) and broccoli (*B. oleracea* var. *italica*) hosts in greenhouse tests following application of culture broth of a fungal isolate of *Phoma glomerata*. However, there is no report of any bio agents capable of suppressing the clubroot disease development, either completely or partially in *Brassica* oil crop species.

So far there is no curative method for successful eradication of *P. brassicae* pathogen from the field; but several cultural practices (*e.g.*, crop rotation, farm equipment sanitation etc.) have been recommended to suppress the development of the disease as well as to prevent further dissemination of the pathogen to the non-infested fields. Most important of them is crop rotation with non-host species. However, all kinds of cultural practices have been found unsuccessful to manage this disease due to the extreme longevity of *P. brassicae* resting spores in soil. Once this pathogen is established in a field, it is almost impossible to get rid of it.

Host resistance to clubroot has always been desired since, complete or at least significant resistance in the host could provide growers relatively cheap, reliable and environmentally friendly way of controlling the disease. However, in reality, commercial lines with resistance become susceptible to the disease very quickly as a result of emergence of new virulent pathotypes of *P. brassicae*. Therefore, integrated approaches for disease management have been recommended where cultivars with durable resistance plays the central role not only to ensure maximum production of the crop, but also to reduce inoculum count and prevent the occurrence of new virulent pathotypes in the already infested fields.

1.7 *Brassica* germplasm for disease resistance

A good plant breeding strategy to introduce durable disease resistance necessitates better understanding of the sources of resistance and their inheritance (Grandclement *et al.*, 1996; Polak and Bartos, 2002) as well as the nature and diversity in virulence of the pathogen (Hayes, 1930; Manzanares-Dauleux *et al.*, 2001). Resistant cultivars, landraces and wild forms, collectively known as the primary gene pool, are usually breeder's first choice as source of natural resistance for any breeding programs. However, presence of greater diversity among the sheer number (total number) of *Brassica* germplasm available in gene bank has made it difficult to identify relevant germplasm. Therefore, a well characterized core collection of *Brassica* species representing maximum possible

diversity can be a valuable resource for utilization as novel germplasm in a breeding program. A large number of cruciferous crop species have been evaluated and characterized through several national and international research efforts for morphological diversity, seed quality traits, genetic resistance to insect pests and diseases and agronomic traits. Those well characterized genotypes were then rationalized to generate core collections representing the highest possible diversity useful for identification of traits of agronomical interest and their introgression into oilseed *Brassica* species and finally documented as primary, secondary and tertiary gene pool (Table 1.1) (Snowdon *et al*., 2007). Germplasm in primary gene pool of *Brassica* oilseed includes both wild and cultivated races of the crop species. Gene transfer from primary gene pool through crossing is easy, hybrids are generally fertile with good chromosome pairing and gene segregation is approximately normal. Transfer of desirable gene from secondary gene pool is possible with some limitations. Hybrids tend to be sterile, chromosomes pair either poorly or not at all; some hybrids may be weak and difficult to bring to maturity; recovery of desired types in advanced generations is difficult. On the other hand, germplasm from tertiary gene pool can also be used to make cross, but the hybrids tend to be anomalous, lethal or completely sterile. Gene transfer is either not possible with known techniques or rather extreme or radical measures *e.g.* embryo culture to obtain hybrids, doubling chromosome number or using bridging species, are required for introgression of the traits (Harlan and deWet, 1971). A large number of *Brassica* genotypes listed in the primary and secondary gene pools of *Brassica* oilseed crops were evaluated for resistance to clubroot disease; and genotypes with race-specific resistance (Toxopeus and Janssen, 1975; Voorrips and Visser, 1993; Hirai *et al*., 2004) as well as resistance to a broad-spectrum of *P. brassicae* pathotypes (Johnston, 1970; Diederichsen and Sacristan, 1996; Some *et al.*, 1996) have been identified.

Resistance to clubroot disease caused by Canadian *P. brassicae* pathotypes was not reported in Canadian canola quality oilseed cultivars (Strelkov *et al*., 2006a) until recently.

Table 1.1 Selected members of the primary, secondary and tertiary gene pools for potential transfer of traits of interest to *Brassica* oilseed crops (Snowdon *et al*., 2007)

Chromosome number (n)		Primary gene pool
17	:	*Brassica carinata* A. Braun (Ethiopian mustard)
18	:	*Brassica juncea* Czern (Indian mustard, brown mustard)
19	:	*Brassica napus* subsp. *napus* (Oilseed rape, kale)
19	:	*Brassica napus* subsp. *napobrassica* (Swede)
19	:	*Brassica napus* subsp. *napus* var. *pabularia* (leaf rape, kale)
		Secondary Gene pool
8	:	*Brassica nigra*
9	:	*Brassica oleracea*
		(includes crop varieties, *B. alboglabra, B. bourgeaui, B. cretica*, *B. hilarionis*, *B. incana*, *B. insularis*, *B. macrocarpa*, *B. montana*, *B. rupestris*, *B. villosa*)
10	:	*Brassica rapa* (includes wild and cultivated varieties)
		Tertiary Gene Pool
8	:	*Brassica fruticulosa*
10	:	*Brassica gravinae*
8	:	*Brassica maurorum*
9	:	*Brassica oxyrrhina*
10	:	*Brassica repanda* (includes *B. desnottesii*, *B. nudicaulis*, *B. saxatilis*)
11	:	*Brassica souliei* (syn. *B. amplexicaulis*)
10	:	*Brassica tournefortii*
12	:	*Coincya* spp. (includes all species in the genus)
45	:	*Crambe abyssinica*
11	:	*Diplotaxis acris*
9	:	*Diplotaxis assurgens*
9	:	*Diplotaxis berthautii*
9	:	*Diplotaxis catholica*
7	:	*Diplotaxis cossoniana*
13	:	*Diplotaxis harra* (includes *D. crassifolia*, *D. gracilis*, *D. hirtum*, *D. lagascana*)
21	:	*Diplotaxis muralis* (*D. tenuifolia* × *D. viminea*)
8	:	*Diplotaxis siettiana* (includes *D. ibicensis*)
10	:	*Diplotaxis siifolia*
11	:	*Diplotaxis tenuifolia*

Table 1.1 (Continued)

Chromosome number (n)		Tertiary Gene Pool
9	:	*Diplotaxis tenuisiliqua*
10	:	*Diplotaxis viminea*
9	:	*Diplotaxis virgata*
10	:	*Enarthrocarpus* ssp. (includes *E. lyratus*, *E. pterocarpus*, *E. strangulatus*)
11	:	*Eruca* spp. (includes *E. vesicaria*, *E. sativa*, *E. pinnatifida*
8	:	*Erucastrum abyssinicum*
9	:	*Erucastrum canariense* (includes *E. cardaminoides*)
15	:	*Erucastrum elatum* (*E. littoreum* × *E. Virgatum*)
15	:	*Erucastrum gallicum* [*E. leucanthum* × *Diplotaxis erucoides*/*D. cossoniana*]
8	:	*Erucastrum nasturtiifolium* (includes *E. leucanthum*)
8	:	*Erucastrum strigosum*
7	:	*Erucastrum varium*
7	:	*Erucastrum virgatum*
12	:	*Onchophragmus violaceus*
9	:	*Raphanus* ssp. (includes *R. raphanistrum*, *R. sativus*, *R. caudatus*, *R. maritimus*, *R. landra*)
10	:	*Sinapidendron* spp. (includes *S. angustifolium*, *S. frutescens*, *S. rupestre)*
12	:	*Sinapis alba* (includes *S. dissecta*)
9	:	*Sinapis arvensis* (includes *S. allioni*, *S. turgida*)
7	:	*Sinapis aucheri* (syn. *Raphanus aucheri*)
12	:	*Sinapis flexuosa*
9	:	*Sinapis pubescens* (includes *S. aristidis*, *S. boivinii*, *S. indurata*)

Note: Secondary and tertiary gene pool species listed together are members of the same cytodeme, *i.e*., they share a single diploid chromosome number and are generally fully inter-fertile, while square brackets indicate amphidiploid taxa.

In 2009, Pioneer Hi-Bred released a clubroot resistant hybrid canola cultivar '45H29' for the Canadian prairies (http://www.pioneer.com/canada). On the other hand, there is no report of occurrence of this resistance in Canadian *B. napus* land races. However, several Swede or rutabaga (*B. napus* subsp. *napobrassica*) and forage rape (*B. napus* subsp. *napus* var. *pabularia*) genotypes were reported to possess race-specific resistance against *P. brassicae* pathotypes (Colhoun, 1958; Karling, 1968; Johnston, 1970; Ayers and Lelacheur, 1972; Buczacki *et al*., 1975; Crute *et al.*, 1983; Gustafsson and Fält, 1986). Of the two parental species of

B. napus, the European storage root type *B. rapa* genotypes, commonly known as Dutch or Belgian stubble turnip (*B. rapa* var. *rapifera*) (Karling, 1968; Buczacki *et al*., 1975; Yoshikawa, 1993) and white cabbage or kale type *B. oleracea* (Crisp *et al.*, 1989; Manzanares-Dauleux *et al*., 2000; reviewed by Hirai, 2006) have frequently been reported to carry resistance to clubroot disease (Hirai *et al*., 2004; Nomura *et al*., 2005; Wallenhammar *et al*., 2000). Some of these European turnips *e.g*., 'Siloga' (Kuginuki *et al*., 1997; Suwabe *et al*., 2003 and 2006), 'Gelria' (Piao *et al*., 2004), 'Milan White' (Hirai *et al*., 2004) and 'Debra' have been used for breeding different clubroot resistant Chinese cabbage cultivars (for review see Hirai, 2006) as well as clubroot resistant *B. napus* germplasm (Diederichsen and Sacristan, 1996). Some of these have also been used as differential hosts *e.g*., ECD-1 (from 'Debra') and ECD-2 (from 'Gelria') for physiological classification of *P. brassicae* pathotypes (Crute *et al*., 1983). These European stubble turnips are regarded as the most effective resistance source, showing broad-spectrum resistance to *P. brassicae* pathotypes (Toxopeus *et al*., 1986).

On the other hand, clubroot disease resistance in *B. oleracea* hosts has been reported less frequently compared to *B. rapa* and *B. napus* hosts. Often, *P. brassicae* pathotypes which were found to be non-virulent to *B. rapa* and *B. napus* hosts were found highly virulent to *B. oleracea* hosts (Crute *et al*., 1983). Again, resistance of *B. oleracea* alone was found barely effective in *B. napus* unless it is combined with clubroot resistance from *B. rapa* (Diederichsen and Sacristan, 1996). However, to our knowledge, there is no report on clubroot disease resistance in wide genotypes of *B. carinata B. juncea* and *B. nigra*.

1.8 Genetic basis of clubroot disease resistance

Inheritance of clubroot disease resistance in *Brassica* has been reported to be dependent on the nature of germplasm as well as virulence of *P. brassicae* pathotype. Wit (1965) first reported three independent dominant genes controlling clubroot resistance in turnip (*B. rapa* var. *rapifera*). However, recently published

articles indicated the presence of at least eight clubroot resistance (CR) genes in turnip, seven as major genes and one as a minor gene (Kuginuki *et al*., 1997; Suwabe *et al*., 2003 and 2006; Hirai *et al*., 2004; Piao *et al*., 2004; Sakamoto *et al*., 2008). Two independent major genes named as *Crr1 and Crr2* and one QTL (Quantitative Trait Loci) for clubroot resistance have been identified in European fodder turnip 'Siloga' (Kuginuki *et al*., 1997; Suwabe *et al*., 2003 and 2006) of which clubroot resistance (CR) gene *Crr1* alone shows resistance to a mild *P. brassicae* population Ano-01, which is identified as race 2 according to Williams (1966) system by Yoshikawa (1981) and as race 16 using ECD-1 to ECD-5 (Buczacki *et al.*, 1975). On the other hand, the CR gene *Crr1* alone was completely susceptible to a more virulent *P. brassicae* isolate Wakayama-01, which is classified as race 4 by Kuginuki *et al*. (1999) system; however, *Crr1* and *Crr2* genes together in homozygous condition were found to confer complete resistance to Wakayama-01 (Suwabe *et al*., 2003). Hirai *et al*. (2004) identified another dominant gene named as *Crr3* in another European fodder turnip 'Milan White' which also shows resistance to *P. brassicae* population Ano-01. Strandberg and Williams (1967) first reported monogenic inheritance of clubroot resistance in one Chinese cabbage (*B. rapa* var. *pekinensis*) cultivar towards multiple *P. brassicae* pathotypes. Piao *et al*. (2004) mapped one dominant gene named *CRb* from European fodder turnip 'Gelria R', which confer resistance in Chinese cabbage against *P. brassicae* races 2, 4 and 8. Monogenic inheritance of clubroot resistance to one single spore isolate of *P. brassicae* has also been reported to be present in Chinese cabbage (Cho *et al*., 2008).

The polygenic nature of clubroot resistance among the *B. oleracea* germplasm tested has been reported from both classical genetic studies (Walker and Larson, 1951; Vriesenga and Honma, 1971; Laurens and Thomas, 1993; Yoshikawa, 1993) and modern genetic mapping studies using molecular markers (Landry *et al*., 1992; Figdore *et al.*, 1993; Voorrips *et al*., 1997; Rocherieux *et al*., 2004; Nomura *et al*., 2005). Walker and Larson (1951) first observed polygenic recessive inheritance of clubroot resistance in cabbage (*B. oleracea* var. *capitata*).

Chiang and Crete (1970) found two recessive genes in another cabbage cultivar conferring resistance to Canadian *P. brassicae* race 6A (later identified as race 5). Multiple dominant alleles with additive genetic effect have also been reported to be involved in clubroot resistance in green curly kale (*B. oleracea* var. *acephala*) (Laurens and Thomas, 1993).

Piao *et al*. (2009) reviewed the occurrence of at least 22 QTLs in *Brassica oleracea* genotypes to date, where most of the QTLs confer race-specific resistance to clubroot disease. Landry *et al.* (1992) for the first time mapped two dominant QTLs by using restriction fragment length polymorphism (RFLP) markers in one cabbage cultivar which confer resistance to race 2 of *P. brassicae*. This resistant cabbage cultivar was developed from crossing of a clubroot susceptible tetraploid (2n = 4x = 36) cabbage (*B. oleracea* var. *capitata* 'Châteauguay') with a rutabaga line (*B. napus* subsp. *napobrassica* 'Wilhelmsburger') resistant to race 2 of *P. brassicae* (Chiang *et al*., 1980). Voorrips *et al*. (1997) mapped two QTLs, designated *pb-3* and *pb-4* in *B. oleracea* for clubroot resistance of which *pb*-3 alone was responsible for 54% of the phenotypic variation. On the other hand, Rocherieux *et al*. (2004) identified nine putative QTLs on seven linkage groups in *B. oleracea* involved in the control of isolate-specific to broad-spectrum resistance against five *P. brassicae* isolates belong to the pathotype 1, 2, 4 and 7 as per the classification proposed by Somé *et al*. (1996); among them, QTL *Pb-Bol* alone responsible for 20.7 to 80.7% of the phenotypic variation. Moriguchi *et al*. (1999) identified single QTL in *B. oleracea* showing resistance against a mixture of *P. brassicae* populations containing race 1 and 3, classified as per Williams (1966) system of classification. Similarly, Yoshikawa (1983) reported one recessive gene control resistance in *B. oleracea*. On the other hand, Grandclement *et al.* (1996) reported both additive and dominant type of genetic effect involved in the control of clubroot resistance.

Among the *B. napus* germplasm rutabagas (*B. napus* subsp. *napobrassica*) are well reported to carry isolate-specific resistance against clubroot pathogen.

The rutabaga cultivar ‘York’ was found to carry one dominant gene for resistance to each of race 2 and 3; while another rutabaga genotype ‘Wilhelmsburger’ carry one gene for resistance to race 3 and two genes for resistance to race 2 of *P. brassicae* (Ayers and Lelacheur, 1966). Manzanares-Dauleux *et al*. (2000) identified one major gene (*Pb-Bn1*) on the linkage group DY4 of *B. napus* ‘Darmor-*bzh*’ showing high level resistance to *P. brassicae* isolate Pb137-522 and two additive QTLs on the linkage group DY4 and DY15 showing partial additive resistance to isolate K92-16. These two QTLs explained 62% to 81% phenotypic variation depending on *P. brassicae* isolates. Gustafsson and Fält (1986) reported four dominant genes in the *B. napus* hosts of ECD series confer resistance against three Scandinavian *P. brassicae* isolates.

So far, more than 55 CR loci have been detected in *Brassica* species of which 16 are distributed on the chromosome of ‘A-genome’ and 39 on ‘C-genome’ (as reviewed by Piao *et al*., 2009). Several race-specific clubroot resistant cultivars have also been developed for each of the *Brassica* species with varying degrees of success. However, durability of this resistance has always been tested by the expansion of physiological races of *P. brassicae*. Therefore, broadening the genetic basis of clubroot resistance by pyramiding different CR genes into a single line will be an indispensible mean to increase durability of resistance against a wide range of physiological races as well as to stop outbreak of new physiological races.

1.9 Broadening genetic diversity for clubroot resistance

Genetic diversity among the existing cultivated species is of utmost importance for their increased adaptive fitness against insect pest and disease epidemics or unpredicted climatic effects. It is also a valuable means for introduction of improved heterotic potential in the parents of hybrid cultivars. However, the needs and priorities for any base-broadening program vary from crop to crop, and also from production area to production area. ‘Introgression’ and ‘Incorporation’ are the two approaches generally practiced for broadening genetic

diversity of crops for the traits of interest (as reviewed by Simmonds, 1993). Introgression is defined as the movement of mostly monogenic traits (sometimes oligogenic traits) from one species into the gene pool of another by backcrossing. On the other hand, 'incorporation' includes systematic exploitation of a wide range of germplasms, representing a large array of variability, consistent with the specific objective of the program to generate a mass of newly adapted germplasm stocks usable as parents in breeding programmes.

Resynthesis of *B. napus* from *B. rapa* and *B. oleracea* has proven successful to expand the genetic base of oilseed rape (*B. napus* subsp. *napus*) (Becker *et al*., 1995; Seyis *et al*., 2003b). These resynthesized oilseed rape lines were found highly genetically diverse compared to modern spring type rapeseed and fodder type *B. napus* (Becker *et al*., 1995; Seyis *et al*., 2003a) and are considered as a valuable source for widening the genetic base of cultivated oilseed rape cultivars.

Diederichsen and Sacristan (1996) resynthesized several *B. napus* lines by embryo rescue assisted interspecific hybridization between clubroot resistant *B. rapa* var. *rapifera* (ECD-04) and *B. oleracea* cultivar (ECD-15 or 'Böhmerwaldkohl'). These resynthesized *B. napus* lines showed broad spectrum resistance to clubroot disease both in greenhouse and field trials. These materials were used in the breeding program to introgress resistance into elite winter type rapeseed materials; and the winter oilseed rape cultivar 'Mendel' was released in the early 2000s to combat this disease in the affected areas of Britain and Germany (Frauen, 1999). However, resistance of this rapeseed cultivar has been broken down due to the evolution of new *P. brassicae* pathotypes. Therefore, the objective of resistance breeding research has shifted to the introduction of durable resistance to wide range of pathotypes by pyramiding more than one resistance gene into a cultivar.

Other *Brassica* species and sometimes related species can be used as potential sources to broaden genetic diversity of oilseed rape for disease

resistance; and introduction of blackleg disease resistance in rapeseed from *Brassica* spp. carrying B-genome through interspecific hybridization is the prime example of its kind. *Brassica* species containing B genome *viz.*, *B. nigra*, *B. juncea* and *B. carinata*, are well documented for absolute and stable mono- or oligogenic resistance to most of the aggressive isolates of *Leptosphaeria maculans* at the seedling and later stages (Rimmer and van den Berg, 1992; Dixelius, 1999) and has been extensively used as a genetic pool to develop resistant oilseed rape germplasm (Roy, 1978; Sjodin and Glimelius, 1989; Chevre *et al*., 1996; Struss *et al*., 1996; Dixelius, 1999).

Intergeneric hybridization technique is another interesting alternative to introgress durable, broad spectrum resistance into oilseed rape from wild and less closely related crucifers. This technique has been applied to introgress resistance to beet cyst nematodes (*Heterodera schachtii*) and alternaria black spot (*Alternaria brassicae*) disease into *B. napus* from *Raphanus sativus (*Thierfelder and Friedt, 1995; Voss *et al*., 2000; Peterka *et al.*, 2004) and *Brassica elongata*, *Sinapis alba*, *Diplotaxis tenuifolia*, and *D. erucoides* (Klewer *et al*., 2003) respectively. Therefore, the entire Brassicaceae family is theoretically considered as a source of potential germplasm for the identification of novel genes/alleles for use in *Brassica* oilseed crop breeding (Snowdon *et al.*, 2007).

1.10 Research Objectives

Based on the literature reviewed, it is clearly apparent that primary zoospores of *P. brassicae* can cause infection both in susceptible and resistant *Brassica* hosts and also in some non-host species. However, infection by secondary zoospores causes economic destruction to the crop plants. Resistance and/or susceptibility of the host species towards *P. brassicae* pathotypes become apparent at the secondary phase of infection. So far, several pathotype-specific clubroot resistant vegetable *Brassica* cultivars were released to combat this pathogen. In case of oilseed *Brassica*, very limited work has been done which is primarily on winter type of *B. napus*. Meanwhile, the resistance of these cultivars

have been compromised due to the evolution of new virulent pathotypes. Therefore, breeding of new cultivars by pyramiding resistance genes from various sources is recommended for durable resistance against a wide range of pathotypes.

Before initiating any breeding program, it is important to identify germplasm resistant to specific pathotypes prevalent in the region and understand the genetic nature of resistance. The long term goal of this project is to develop canola germplasm resistant to multiple clubroot pathotypes. Therefore, it is important to identify different resistance sources in *Brassica* for characterization and introgression into canola-quality *B. napus*. Thus, the objective of this research is to collect germplasm of the diploid and amphidiploid *Brassica* species belonging to the primary and secondary gene pools of rapeseed from gene banks and other sources and to evaluate for resistance to Canadian clubroot pathotypes. The results from this study will lay the foundation for further research for pyramiding the resistance genes into Canadian canola cultivars for durable resistance to this disease.

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Chapter 2

Screening of *Brassica* Germplasm for Resistance to Clubroot Disease Caused by *Plasmodiophora brassicae*

2.1 Introduction

Clubroot disease of *Brassica* spp., caused by *Plasmodiophora brassicae* (Woronin, 1878 cited by Cook and Schwartz, 1930; Strelkov *et al.*, 2007) has been reported as a major disease of canola because of its potential to cause almost total destruction to the crop yield (Wallenhammar *et al.*, 1999; Strelkov *et al.*, 2006b) along with significant reduction in oil content of the seed (Pageau *et al.*, 2006). Though the disease was recorded in Western Europe in the 13th century (Mehrotra and Aneja, 1990; for detailed review see Dixon, 2009) and caused epidemic destruction to cabbage crops in St. Petersburg, Russia in the late 19th century, effective control measures are yet to be discovered. Moreover, the ability of the pathogen to survive in the soil as resting spores for long periods of time even in the absence of host plants (Wallenhammar, 1996) and its transmission to non-infested fields through unrestricted movement of farm equipment, humans and animals has made the disease virtually impossible to control and its spread unstoppable. Cultivation of susceptible hosts in the infested field facilitates the build-up of the pathogen population through reproduction as well as evolution of new pathogenic races with increased virulence. Therefore, cultivation of resistant cultivars in infested fields is the most reliable strategy to prevent yield loss, build-up of the pathogenic populations and the evolution of new virulent populations.

Pre-requisite for resistance breeding is to identify the sources of resistance based on host-pathogen interactions. The objective of this study was to identify the natural sources of resistance to clubroot disease in germplasm of different *Brassica* species. Two important factors *viz.*, characteristics of germplasms and physiological ranking of the pathogen based on their virulence were considered carefully before initiating this screening program for clubroot disease resistance.

2.2 Materials and Methods

2.2.1 Selection of *Brassica* germplasm for screening

Terminology:

The terminology ‘sub-species’ was used to refer to a group of plants within a species which differ morphologically or sometimes only genetically from other groups of plants from the same species. The group of plants which differ slightly from the plants of a species were defined as ‘variety’ (abbreviated ‘var.’). However, domesticated form of plant species that arose through deliberate hybridisation was defined as ‘cultivar’ (Sivarajan and Robson, 1991). Cultivars and lines/genotypes of *Brassica* spp. used in this research are written within single inverted commas (‘’).

2.2.1.1 Selection of diploid *Brassica* germplasm

***Brassica rapa* (2n=20, AA):** A total of five turnip type genotypes were collected along with 13 vegetable and 18 rapeseed and/or canola quality winter and spring type oilseed *B. rapa* from United States Department of Agriculture (USDA), Green Gene International, United Kingdom, Canola Breeding Program, University of Alberta and Bangladesh Agricultural Research Institute (BARI) to evaluate for resistance to five Canadian *P. brassicae* pathotypes (Table A-1). Six *B. rapa* genotypes were also kindly provided by Dr. Ronald J. Howard, Alberta Agriculture, Food and Rural Development.

***Brassica oleracea* (2n=18, CC):** A total of 48 genotypes from its three distinct groups were used in this research project (Table A-2) of which 38 were kindly provided by Dr. Ronald J. Howard.

***Brassica nigra* (2n=16, BB):** A total of 77 *B. nigra* genotypes, originating from different geographic regions, were collected from the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany (IPK, Germany) (Table A-3).

2.2.1.2 Selection of amphidiploid *Brassica* germplasm

***Brassica napus* (2n=38, AACC):** This species evolved into two sub-species *viz.*, '*napobrassica*' (includes fodder, vegetable and rutabaga types) and '*napus*' (includes oilseed form). These two forms are known to be genetically diverse from each other (Hasan *et al.*, 2006). A total of 44 rutabagas, fodder rape and spring type oilseed *B. napus* genotypes were collected from USDA, Green Gene International, BINA and IPK, Germany to evaluate for resistance to Canadian *P. brassicae* pathotypes (Table A-4). Two Canadian rutabaga genotypes *viz.*, 'Brookfield-9005' and 'Polycross-9006' were kindly provided by Dr. Dean Spaner, Professor, Department of Agricultural, Food and Nutritional Science, University of Alberta.

***Brassica juncea* (2n=36, AABB) and *B. carinata* (2n=34, BBCC):** A total of 48 *B. juncea* (Table A-5) and 24 *B. carinata* (Table A-6) genotypes, obtained from Bangladesh Agricultural University (BAU), Bangladesh Agricultural Research Institute (BARI) and IPK, Germany were evaluated to extend the search for resistance in these two amphidiploid species, although these two species are generally reported to be lacking resistance to clubroot disease (as reviewed by Diederichsen *et al.*, 2009).

Thus, a total of 277 *Brassica* genotypes, 36 of *B. rapa*, 48 of *B. oleracea*, 77 of *B. nigra*, 44 of *B. napus*, 48 of *B. juncea* and 24 of *B. carinata* were evaluated for clubroot resistance in this research project.

2.2.2 *Plasmodiophora brassicae* population

In this study, single-spore derived isolates of five *P. brassicae* pathotypes (Table 2.1), collected from Canadian canola fields (Xue *et al.*, 2008) was used to evaluate the *Brassica* genotypes for resistance to clubroot disease. 'Single-spore isolate' refers to a population derived from a club inoculated with a single resting spore and maintained in isolation. This resting spore population possesses the genetic uniformity necessary to apply the terminology 'race' (Parlevliet, 1985).

However, the terminology ‘pathotype’ was used to address these single-spore isolate derived populations of *P. brassicae* (as per Xue *et al*., 2008) since they were distinguished from each other based on their pathogenicity on specific hosts rather than genetic dissimilarities. According to Voorrips (1995), precisely ranked *P. brassicae* isolates based on their virulence is imperative to identify race-independent resistance in *Brassica* genotypes. Though these pathotypes were not precisely ranked based on their virulence and genetic variability, pathotype 3 was reported to be the most virulent on all canola cultivars tested from Canada, followed by pathotype 5 and pathotype 6 (Strelkov *et al*., 2006a). On the other hand, pathotype 2 and 8 were reported to be the least virulent on the canola cultivars. Single-spore isolates of these five pathotypes were kindly provided by Dr. Stephen Strelkov, Associate Professor of Plant Pathology at the University of Alberta, Edmonton, Alberta, Canada.

Table 2.1 Single-spore derived isolates of *Plasmodiophora brassicae* from Canada used to evaluate the *Brassica* genotypes for clubroot resistance

ID of single spore isolate	Origin	Pathotype	Original host
SACAN-ss-3	St. Albert, Alberta, Canada	2	Canola
SACAN-ss-1	St. Albert, Alberta, Canada	3	Canola
ORCA-ss-4	Orton, Ontario, Canada	5	Cabbage
AbotJE-ss-1	Abbottsford, BC, Canada	6	Soil
CDCN-ss-1	CDC, North, Alberta, Canada	8	Canola

Note: As classified on differentials of Williams (1966) by Xue *et al*. (2008)

2.2.3 Experimental Design

Resistance to Canadian *P. brassicae* pathotypes was evaluated in two phases. In the first phase, all 277 *Brassica* genotypes were evaluated for resistance to pathotypes 2, 3, 5, 6 and 8. For this, nine plants per genotypes were tested against each pathotype in nine celled trays without replication. After this initial screening, a total of 59 genotypes were selected, based on their resistance to multiple pathotypes, for characterization in second phase of the experiment.

In the second phase, the selected 59 genotypes were evaluated for resistance to the most virulent Canadian *P. brassicae* pathotype 3 (Strelkov *et al*., 2006a) and pathotype 5. In this case, for testing against each pathotype, 18 plants of each genotype were grown in 18-celled trays. Each treatment was repeated three times and was considered as three replications. Single-spore derived isolate suspension of the pathotypes was used to inoculate the plants.

These two phases of the research are hereafter referred to as Experiment 1 and Experiment 2, respectively.

2.2.4. Preparation of plant material/seedlings for inoculation

Seeds were germinated on moistened Whatman filter paper No. 1 in 90 × 15 mm^2 size Petri plates. Plates were kept wet with tap water to provide adequate moisture for germination of the seeds and their initial development. *Brassica* seeds usually start to germinate 2-3 days after plating. Seedlings at the age of seven days after plating were inoculated with single-spore isolates of *P. brassicae* pathotypes.

2.2.5 Preparation of inoculum

Single-spore isolate derived resting spores of Canadian *P. brassicae* were received in the form of clubbed root, preserved at -20°C temperature. Resting spore suspensions were prepared on the day of inoculation from the preserved gall following modified version of Williams (1966) protocol as described by Strelkov *et al*. (2007). Approximately 2.0–2.5 g of preserved clubbed roots was ground in a mortar with a pestle by adding 50 ml of sterile deionized water (sdH_2O). The homogenate was filtered through eight layers of cheesecloth (American Fiber and Finishing Inc., Albemarle, NC, USA) to separate spore suspension from plant debris. The resting spores in suspension was quantified with a haemocytometer (VWR, Mississauga, ON Canada) and adjusted to minimum suspension concentration of 1.0×10^7 resting spores ⁄ ml, which is reported to be the optimum concentration for successful inoculation (Voorrips and Visser, 1993).

2.2.6 Inoculation of seedlings

Seedlings were inoculated by dipping the entire root in resting spore suspension for 10 seconds (Nieuwhof and Wiering, 1961). The inoculated seedlings were immediately planted in 9- or 18-celled trays filled with Sunshine Professional Growing Mix (Sunshine Horticulture, Bellevue, USA). The seedlings were grown in greenhouse at 21 ± 2°C temperature with 16 hour photoperiod. To ensure optimum environment for resting spore germination and root hair infection by zoospores, the trays with inoculated seedlings were kept saturated with water for the first week. From the second week, watering was done once a day. Quick release mixed fertilizer *viz.*, 15-30-15 (N-P-K) was applied once a week to provide nutrients for seedlings as well as to ensure acidic conditions in the soil. To prevent dissemination of *P. brassicae*, percolated water was collected and discarded after autoclaving for 60 minutes at 121°C and 1.757 ksc.

2.2.7 Screening for resistance, data collection and statistical analysis

Seedlings at 42 to 45 days after inoculation were evaluated for clubroot resistance. Roots were dug out, washed in tap water and examined for gall formation. The severity of gall development was rated on a 0 to 3 scale as described by Kuginuki *et al.* (1999), where 0 = no galling, 1 = one or few small galls on the lateral roots, 2 = moderate galling on the lateral roots and 3 = severe galling on the lateral roots or on the main root (Figure 2.1).

Figure 2.1: Disease severity classes for clubroot disease in *Brassica* based on gall development (Score 0= No visible gall; Score 1= One or few small galls on lateral roots; Score 2= Moderate galling on lateral roots; Score 3= Sever galling on the lateral roots or in the main root)

In case of Experiment 1, percent resistance for each genotype against each pathotype was calculated using the following formula:

$$\text{Percent resistance} = \frac{\text{No. of resistant plants}}{\text{No. of plants inoculated}} \text{X } 100$$

Plant with no visible gall at 42-45 days after inoculation was considered as resistant (Figure 2.2)

Figure 2.2: *Plasmodiophora brassicae* infected *Brassica rapa* plant with no visible gall

In case of the Experiment 2, disease score was calculated for each genotype using the following formula (Strelkov *et al*., 2007):

$$\text{Score} = \sum (n\ x\ 0 + n\ x\ 1 + n\ x\ 2 + n\ x\ 3$$

Where *n* is the number of plants in each class and 0, 1, 2 and 3 are the symptom severity classes as described above. An Index of Disease (ID) was also calculated for each genotype, using the method of Horiuchi and Hori (1980) as modified by Strelkov *et al.* (2006a):

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

Where *n* is the number of plants in each class, *N* was the total number of plants, and 0, 1, 2 and 3 were the symptom severity classes. The Index of Disease (ID) value was calculated for each genotype against each treatment from each replication. Experiment was arranged in a split-plot design where *Brassica* genotypes were main plot and *P. brassicae* pathotypes were sub-plot. The data were analysed according to generalized linear mixed model of split-plot design by using SAS software version 9.2 (SAS Institute Inc., 2008), where *P. brassicae* pathotypes and *Brassica* genotypes were considered as fixed effect and replication as random effect. Significance of resistance of the *Brassica* genotypes to *P. brassicae* pathotypes 3 and 5 were calculated, where ID value of 13% and 12.38% respectively were considered as the cut-off between a resistant and susceptible reaction. *P. brassicae* pathotypes 3 and 5 were compared for virulence on the tested germplasm. Pair wise comparisons of *Brassica* genotypes were performed at 5% level of significance.

2.3 Results

2.3.1 Experiment 1

The objective of this experiment was to identify *Brassica* germplasm having resistance to five Canadian *P. brassicae* pathotypes and thus to select experimental materials for Experiment 2.

2.3.1.1 *Brassica rapa*

In Experiment 1, 36 *B. rapa* genotypes were evaluated. Among them, American turnip cultivar ‘Royal Gam’ and four European turnip genotypes *viz.*, ‘Debra’, ‘Gelria’, ‘ECD-3’ and ‘ECD-4’ were found to possess resistance to all five Canadian *P. brassicae* pathotypes (Table 2.2). Resistance to all these pathotype was also observed in one hybrid Chinese cabbage (*B. rapa* var. *chinensis*) cultivar ‘Bilko’ (Table 2.3). Hybrid cultivar ‘Mirako’ also showed some resistance to only pathotypes 3 and 5. However, six other leafy vegetable type *B. rapa* var. *chinensis* cultivars evaluated did not show resistance to any of the *P. brassicae* pathotype.

Table 2.2: Resistance response of *Brassica rapa* var. *rapifera* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	*Plasmodiophora brassicae* pathotypes				
	2	3	5	6	8
Debra	100	100	100	100	100
Gelria	100	100	100	100	100
ECD-3	100	77.8	100	100	100
ECD-4	100	100	100	100	100
Royal Gam	66.7	100	55.6	55.6	55.6

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

Resistance to *P. brassicae* pathotypes was also absent in five *B. rapa* var. *pekinensis* cultivars tested in the first phase of the experiment (Table 2.3).

Table 2.3: Resistance response of *Brassica rapa* var. *chinensis* and var. *pekinensis* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm[1]	*Plasmodiophora brassicae* pathotypes				
	2	3	5	6	8
Granaat	00	00	00	00	00
Qui Lu 75	00	00	00	00	00
Mirako (F_1)	00	11.1	44.4	00	00
Bilko (F_1)	100	100	88.9	100	100
Feng Quig Choi	00	00	00	00	00
Blues	00	00	00	00	00
Kasumi	00	00	00	00	00
Nikko (F_1)	00	00	00	00	00
Nai Bai Cai	00	00	00	00	00
Feng Rang- 70	00	00	00	00	00
Qingmayc	00	00	00	00	00
BARI Chinashak -1	00	00	00	00	00
BARI Shak-1	00	00	00	00	00

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance

[1]The last five genotypes, Nai Bai Cai, Feng Rang-70, Qingmayc, BARI Chinashak-1 and BARI Shak-1 belongs to *Brassica rapa* var. *pekinensis* and all other genotypes belong to *Brassica rapa* var. *chinensis*

On the other hand, the winter type *B. rapa* genotypes showed variability in resistance to Canadian *P. brassicae* pathotype. Some of the *B. rapa* genotypes *e.g.*, 'Largo', 'JSv01-11403', 'JSv01-11449', 'JSv00-15588' etc. showed resistance to all five pathotypes. However, *B. rapa* genotypes 'Prisma', 'JSv01-

13102' and 'JSv01-13084' were resistant to pathotypes 3, 5, 6 and pathotype 8 (Table 2.4). On the other hand, the spring type *B. rapa* cultivar 'Parkland', 'Horizon', 'Tobin', showed resistance to only pathotypes 6 and 8 (Table 2.5).

Table 2.4: Resistance response of winter type *Brassica rapa* var. *oleifera* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
Largo	33. 3	33.3	33.3	100	100
Prisma	00	55.6	33.3	100	100
JSv01-11403	22.2	22.2	11.1	88.9	100
JSv01-13102	00	33.3	11.11	100	100
JSv01-11449	33.3	44.4	11.1	88.9	100
JSv01-13051	44.4	44.4	11.1	100	100
JSv01-13084	00	11.1	11.1	55.6	88.9
JSv00-13426	11.1	11.1	11.1	88.9	100
JSv00-15588	33.3	33.3	22.2	22.2	100

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance

Of the 36 *B. rapa* genotypes evaluated in Experiment 1, five turnip genotypes *viz.*, 'Debra', 'Gelria', 'ECD-3', 'ECD-4' and 'Royal Gam', three Chinese cabbage genotypes *viz.*, 'Bilko', 'Mirako' and 'BARI Shak-1' and nine winter type rapeseed genotypes *viz.*, 'Largo', 'Prisma', 'JSv01-11403', 'JSv01-13102', 'JSv01-11449', 'JSv01-13051', 'JSv01-13084', 'JSv00-13426' and 'JSv00-15588' were selected for further evaluation against pathotype 3 and 5 in Experiment 2.

Table 2.5: Resistance response of spring type *Brassica rapa* var. *oleifera* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
CR 25-7	00	00	00	00	00
YS 49	00	00	00	00	00
TR 7	00	00	00	00	00
TR 4-3-3-1	00	00	00	00	00
3.0026.027	00	00	00	00	00
Tori-7	00	00	00	00	00
Parkland	00	00	00	33.3	22.2
Horizon	00	00	00	88.9	55.6
Tobin	00	00	00	100	88.9

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

2.3.1.2 *Brassica oleracea*

Among the 48 *B. oleracea* genotypes evaluated, the cabbage (*B. oleracea* var. *capitata*) cultivar 'Badger Shipper' showed resistance to pathotypes 3, 5, 6 and 8 and 'Bindsachsenn' to pathotypes 2, 3, 5 and 8. The cabbage cultivar 'SCB 7322' and 'Silver Dynasty' showed some resistance to pathotypes 3 and 8, respectively. Also, a single plant of 'Jersey Queen' was found to be resistant to pathotype 3 (Table 2.6). A single plant of the white cabbage cultivar 'Blue Thunder' showed resistance to pathotype 3 and another single plant showed resistance to pathotype 5; whereas 44% of the total plants of 'Head Start' showed resistance to pathotype 3 only (Table 2.6).

Table 2.6: Resistance response of *Brassica oleracea* var. *capitata* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
Badger Shipper	00	100	33.3	100	100
Bindsachsenn	22.2	66.7	33.3	0	11.1
Jersey Queen	00	11.1	00	00	00
Septa	00	00	00	00	00
Supreme Vantage	00	00	00	00	00
SCB 7322	00	11.1	00	00	00
Silver Dynasty	00	00	00	00	22.2
Optiko	00	00	00	00	00
Manoko	00	00	00	00	00
Blue Thunder	00	11.1	11.1	00	00
Joi Choi	00	00	00	00	00
Loughton (F_1)	00	00	00	00	00
Lennox (F_1)	00	00	00	00	00
Balaton (F_1)	NT	00	00	00	NT
Farao (F_1)	00	00	00	00	00
Head start	00	44.4	00	00	00

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.
[1]The first six genotypes, Badger Shipper, Bindsachsenn, Jersey Queen, Septa, Supreme Vantage and SCB 7322 are cabbage (*Brassica oleracea* var. *capitata*) and all other genotypes are white cabbage.
NT- Not Tested

None of the 14 cauliflower (*B. oleracea* var. *botrytis*) cultivars tested showed resistance to any of the Canadian *P. brassicae* pathotypes (Table 2.7).

Table 2.7: Resistance response of *Brassica oleracea* var. *botrytis* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	*Plasmodiophora brassicae* pathotypes				
	2	**3**	**5**	**6**	**8**
Dok Elgon	00	00	00	00	00
Nimba Meda	00	00	00	00	00
BARI Cauliflower-1	00	00	00	00	00
BARI Cauliflower-2	00	00	00	00	00
Symphony	00	00	00	00	00
Amazing	00	00	00	00	00
Fremont	00	00	00	00	00
Cortes	00	00	00	00	00
Shasta	00	00	00	00	00
Concept	00	00	00	00	00
Concert (F_1)	00	00	00	00	00
Arctic	00	00	00	00	00
Tower (F_1)	00	00	00	00	00
Hateman	00	00	00	00	00

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

A total of 16 broccoli (*B. oleracea* var. *italica*) genotypes were tested in Experiment 1. A single plant of the hybrid broccoli cultivar 'Premium Crop' showed resistance to pathotype 3; while 33% of the tested plants of cultivar 'Iron' showed resistance to pathotype 8 only (Table 2.8).

Of the three brussels sprout (*B. oleracea* var. *gemmifera*) genotypes, 'Diablo' and 'Hybrid Spouket' were tested against *P. brassicae* pathotypes 3 and 5 only due to seed shortages. The cultivar 'Diablo' showed good resistance to both pathotypes; while a single plant of 'Hybrid Spouket' was resistant to only pathotype 3 (Table 2.9). On the other hand, the cultivar 'Oliver' was susceptible to all five pathotypes. Also, the single Chinese kale (*B. oleracea* var. *alboglabra*) genotype tested was found to be susceptible to all five pathotypes. *Brassica oleracea* var. *villosa* genotype 'BRA 1896' was found to show good resistance to pathotypes 2 and 8; however, single plant of this genotype showed resistance to pathotype 5 (Table 2.9)

Table 2.8: Resistance response of *Brassica oleracea* var. *italica* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
Iron	00	00	00	00	33.3
Premium Crop (F_1)	00	11.1	00	00	00
Major (F_1)	00	00	00	00	00
Surveyor (F_1)	00	00	00	00	00
Captain	00	00	00	00	00
Coroado	00	00	00	00	00
Acadia	00	00	00	00	00
Windsor	00	00	00	00	00
SBC 93 11	00	00	00	00	00
Sessantina Grossa	00	00	00	00	00
Lucky	00	00	00	00	00
Belstar (F_1)	00	00	00	00	00
Alborado (F_1)	00	00	00	00	00

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

Of the 48 *B. oleracea* genotypes evaluated in Experiment 1, six cabbage genotypes *viz.*, 'Badger Shipper', 'Bindsachsenn', 'Jersey Queen', 'Emblem', 'Augusta' and 'Balbro', two white cabbage cultivar *viz.*, 'Head Start' and 'Blue Thunder', two brussels sprout genotypes *viz.*, 'Hybrid Spouket' and 'Diablo', and two broccoli genotypes *viz.*, 'Monterey' and 'Premium Crop', were selected for further evaluation against pathotypes 3 and 5 in Experiment 2. The single *B. oleracea* var. *villosa* genotype, 'BRA 1896' was also selected for further evaluation against pathotypes 3 and 5 in Experiment 2.

Table 2.9: Resistance response of *Brassica oleracea* var. *gemmifera*, var. *alboglabra* and var. *villosa* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
Hybrid Spouket	NT	11.1	00	NT	NT
Oliver	00	00	00	00	00
Diablo (F_1)	NT	77.8	100	NT	NT
CD-2	00	00	00	00	00
BRA 1896	66.7	00	11.1	00	88.9

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.
[1] Genotypes CD-2 is *B. oleracea* var. *alboglabra* (Chinese kale), BRA 1896 is *B. oleracea* var. *villosa* and all other are *B. oleracea* var. *gemmifera*

2.3.1.3 *Brassica nigra*:

Among the three diploid *Brassica* species, the B-genome species showed a wide range of resistance to Canadian *P. brassicae* pathotypes. A total of 38 genotypes of *B. nigra* subsp. *nigra* var. *nigra* were tested in Experiment 1 of which 29 showed resistance to all five pathotypes. Of the 29 genotypes, 100% plants of 'Hneda Z Danska Hneda', 'Primus', 'Lu De no. 2', 'Alsaska', 'CR 2143', 'CR 2144', 'CR 2716' and 'Balkan' showed resistance to all pathotypes (Table 2.10). The Italian genotype 'CR 2115' was the most susceptible showing resistance to only pathotype 8 followed by 'Black Mustard' and Ethiopian genotype 'CR 2746'. The genotype 'Black Mustard' showed resistance to pathotypes 2, 6 and 8 whereas 'CR 2746' showed resistance to pathotypes 3, 5 and 8. However, 'CR 2704', 'Gibera', 'CR 2709' and 'CR 2755' showed resistance to pathotypes 2, 3, 6 and 8 but not to pathotype 5. The Greek genotype 'CR 2093' did not show any resistance to pathotype 6; while the Dutch genotype 'CR 2734' was resistant to all five pathotypes except pathotype 2 (Table 2.10). Two *B. nigra* subsp. *nigra* var. *pseudocampestris* genotypes were tested of which 'CR 2141' did not show any resistance to pathotypes 2, 4 and 6, whereas 'CR 1210' showed resistance to all five pathotypes (Table 2.10).

Six genotypes of *Brassica nigra* subsp. *hispida* were tested in Experiment 1. All five genotypes of subsp. *hispida* var. *orientales* showed varying degrees of resistance to all five pathotypes. All plants of the genotypes 'CR 2120', 'Sv76-39011' and 'CR 2762' showed resistance to all pathotypes. However, the *B. nigra* subsp. *hispida* var. *orientales* genotype 'CR 2128' did not show any resistance to pathotype 3 and 6 (Table 2.11).

In addition to the above mentioned *B. nigra* genotypes from subsp. *nigra* and subsp. *hispida*, 31 additional genotypes without known taxonomic classification at the sub-species level were tested (Table 2.12).

Table 2.10: Resistance response of *Brassica nigra* subsp. *nigra* var. *nigra* and var. *pseudocampestris* Sinskaya genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm[1]	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
Alsaska	100	88.9	44.4	100	100
CR 1199	100	100	66.7	100	100
Hneda Z Danska Hneda	100	100	100	100	100
Primus	100	100	100	100	100
Sizaja	100	100	88.9	100	100
CR 1214	77.8	25	88.9	100	100
Lu De no. 2	100	100	100	100	100
CR 2093	22.2	44.4	44.4	00	100
Alsaska	100	100	100	100	100
CR 2115	00	00	00	00	100
CR 2127	22.2	100	77.8	77.8	100
CR 2130	100	88.9	100	66.7	100
CR 2142	100	77.8	77.8	88.9	100
CR 2143	100	100	100	100	100
CR 2144	100	100	100	100	100
Black Mustard	33.3	00	00	11.1	88.9
CR 2697	100	88.9	77.8	88.9	100
CR 2704	100	100	00	100	100
CR 2706	55.6	100	55.6	100	100
CR 340	100	100	100	66.7	100
Gibera	44.4	22.2	00	44.4	55.6
Alaska	77.8	100	66.7	100	100
CR 2709	33.3	22.2	00	33.3	100
Junius	100	100	77.8	100	100
CR 2714	55.6	55.6	66.7	55.6	100
CR 2715	100	100	66.7	66.7	100
CR 2716	100	100	100	100	100
CR 2717	100	100	55.6	100	100
CR 2718	100	100	66.7	100	100
CR 2719	88.9	88.9	77.8	88.9	100
Balkan	100	100	100	100	100
CR 2721	100	66.7	77.8	100	100
CR 2724	55.6	88.9	100	100	100
CR 2727	100	55.6	77. 8	66.7	100
CR 2734	00	88.9	100	100	100
CR 2735	44.4	33.3	100	100	100
CR 2746	00	11.1	11.1	00	88.9
CR 2755	11.1	11.1	00	33.3	66.7
CR 2141	00	00	22.2	00	33.3
CR 1210	100	100	88.9	22.2	77.8

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.
[1]The genotype, CR 2141 and CR 1210 belong to *Brassica nigra* subsp. *nigra* var. *pseudocampestris* and all other genotypes belong to *B. nigra* subsp. *nigra* var. *nigra*.

Twenty four of them *viz*., 'CR 1216', 'CR 2094', 'CR 2095', 'CR 2096', 'CR 2097', 'CR 2098', 'CR 2101', 'CR2102', 'CR 2104', 'CR 2105', 'CR 2107', 'CR 2109', 'CR 2121', 'CR 2122', 'CR 2123', 'CR 2124', 'CR 2126', 'CR 2136', 'CR 2137', 'CR 2703', 'Gibera', 'CR 2723', 'CR 2725' and 'CR 2731' showed resistance to all five Canadian pathotypes. The genotypes 'CR 2106' and 'CR 2110' showed resistance to pathotypes 3, 5, 6 and 8; while 'CR 2112' was resistant to pathotypes 3, 5, and 8. The genotype 'CR 2135' showed resistance to pathotypes 2 and 8 only, whereas 'Pavlikenski' showed resistance to only pathotypes 6 and 8 (Table 2.12). 'CR 2748' and 'CR 3279' were the two *B. nigra* genotypes showed no resistance to any of the Canadian *P. brassicae* pathotype.

Table 2.11: Resistance response of *Brassica nigra* subsp. *hispida* var. *rigida* Sinskaya and var. *orientales* Sinskaya genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm[1]	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
CR 2138	100	77.8	77.8	100	66.7
CR 2129	44.4	100	100	100	100
Sv76-39011	100	100	100	100	100
CR 2762	100	100	100	100	100
CR 2120	100	100	100	100	100
CR 2128	22.2	00	22.2	00	77.8

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.
[1]The genotype, CR 2128 belongs to *Brassica nigra* subsp. *hispida* var. *orientales* Sinskaya and all other genotypes belong to *B. nigra* subsp. *hispida* var. *rigida* Sinskaya.

Of the 77 B-genome genotypes evaluated in Experiment 1, 10 *B. nigra* subsp. *nigra* var. *nigra* genotypes *viz*., 'CR 1199', Primus', 'Sizaja', 'CR 2143', 'CR 2144', 'CR 2716', 'CR 2719', 'CR 340', 'Alsaska' and 'Balkan', one genotype from sub-species *hispida viz*., 'CR 2120', and 10 genotypes from this species with no taxonomic classification at the sub-species level *viz*., 'CR 1216', 'CR 2094', 'CR 2095', 'CR 2097', 'CR 2098', 'CR 2101', 'CR2102', 'CR 2122', 'Gibera' and 'CR 2725' were selected based on their resistance to all five Canadian pathotypes for further evaluation against pathotypes 3 and 5 in Experiment 2.

Table 2.12: Resistance response of *Brassica nigra* (taxonomic information at sub species level was not available) genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	Plasmodiophora brassicae pathotypes				
	2	3	5	6	8
CR 1216	33.3	100	33.3	44.4	88.9
CR 2094	22.2	55.6	55.6	88.89	100
CR 2095	100	55.6	55.6	100	88.9
CR 2096	44.4	66.7	33.3	100	88.9
CR 2097	22.2	100	77.8	88.9	100
CR 2098	88.9	100	77. 8	100	100
CR 2101	100	44.4	44.4	55.6	88.9
CR 2102	100	88.9	100	100	100
CR 2104	11.1	77.8	22.2	55. 6	88.9
CR 2105	44.4	55.6	77.8	100	100
CR 2106	00	55.6	100	55.6	100
CR 2107	11.1	11.1	55.6	11.1	44.4
CR 2109	22.2	55.5	44.4	100	100
CR 2110	00	66.7	44.4	11.1	66.7
CR 2112	00	44.4	22.2	00	88.9
CR 2121	88.9	100	88.9	100	88.9
CR 2122	100	100	100	100	100
CR 2123	88.9	66.7	66.7	11.1	100
CR 2124	100	100	100	100	100
CR 2126	100	88.9	44.4	100	100
CR 2135	88.9	00	00	00	100
CR 2136	22.2	88.9	88.9	88.9	100
CR 2137	88.9	77.8	66.7	100	100
Pavlikenski	00	00	00	88.9	88.9
CR 2703	22.2	100	88.9	100	88.9
Gibera	100	100	77.8	100	88.9
CR 2723	77.8	100	100	100	33.3
CR 2725	100	100	100	100	100
CR 2748	00	00	00	00	00
CR 3279	00	00	00	00	00
CR 2731	100	88.9	88.9	100	100

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

2.3.1.4 *Brassica napus*

Forty four *B. napus* genotypes were evaluated of which rutabaga showed better resistance to Canadian *P. brassicae* pathotypes than oilseed rape genotypes. The rutabaga genotypes ‘Wilhelmsburger’, ‘Brookfield-9005’ and ‘Polycross-

9006' were found to carry resistance to all five pathotypes. 'York' showed resistance to pathotypes 2, 5, 6 and 8, but not to the pathotype 3. On the other hand, one rutabaga genotype from Seedway, USA showed resistance to pathotypes 2, 6 and 8 (Table 2.13).

Table 2.13: Resistance response of *Brassica napus* subsp. *napobrassica* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
Wilhelmsburger	100	88. 9	100	100	100
BrookField-9005	100	88.9	100	100	100
PolyCross-9006	100	88.9	100	100	100
York	71.4	00	22.2	100	100
Unknown	88.89	00	00	100	100

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

Of these five rutabaga genotypes, 'Wilhelmsburger', 'Brookfield-9005' and 'Polycross-9006' were selected for experiment 2.

The five spring type *B. napus* subsp. *napus* var. *napus* genotypes provided by USDA, showed resistance to *P. brassicae* pathotypes 2 and 8. The genotype 'Legend' and 'Lindora-00' also showed resistance to pathotype 6. However, none of them showed resistance to pathotypes 3 and 5 (Table 2.14). Resistance to only pathotypes 2 and 8 was also observed in 'Giant Rape Commercial', 'Bina Sorisha-4' and 'Bina Sorisha-5'. On the other hand, 'Fodder Nevin', 'Giant Rape Selection' and 'New Zealand Resistant Rape' showed resistance to pathotypes 2, 5, 6 and 8 but not to pathotype 3. The Bangladeshi spring type *B. napus* cultivar 'Bina Sorisha-3' was found to possess resistance to *P. brassicae* pathotype 3 in addition to the resistance to pathotypes 2 and 8.

Most of the Swedish spring type rapeseed genotypes *viz*., 'BRA 1276', 'Ww1273', 'Ww1286', 'Korall', 'Granit', 'Puma', 'CR 3021', 'CR 3086', 'Olivia', 'CR 638', 'CR 999' and 'K7967' and the single Russian genotype 'CR

1886' and the Romanian genotype 'CR 3189' showed resistance to *P. brassicae* pathotypes 2, 6 and 8 (Table 2.14).

Table 2.14: Resistance response of *Brassica napus* subsp. *napus* var. *napus* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	***Plasmodiophora brassicae* pathotypes**				
	2	**3**	**5**	**6**	**8**
Legend	44.4	00	00	100	100
Global	22.2	00	00	00	100
Capricorn	22.2	00	00	00	100
Winfield	22.2	00	00	00	100
Lindora-00	11.1	00	00	11.1	44.4
Fodder Nevin	33.3	00	11.1	100	100
Giant Rape Commercial	22.2	00	00	00	33.3
Giant Rape Selection	33.3	00	11.1	100	100
New Zealand Resistant Rape	11.1	00	22.2	55.6	88.9
Bina Sorisha-3	33.3	11.1	00	00	66.7
Bina Sorisha-4	22.2	00	00	00	11.1
Bina Sorisha-5	11.1	00	00	00	100
BRA 1276	11.1	00	00	44.4	100
Ww 1273	11.1	00	00	22.2	22.2
Ww 1286	22.2	00	00	22.2	22.2
Ww 1289	55.6	11.1	00	33.3	88.9
CR 1886	44.4	00	00	22.2	100
Kajsa	44.4	00	00	00	100
Korall	55.6	00	00	33.3	88.9
Granit	44.4	00	00	11.1	100
Puma	22.2	00	00	11.1	66.7
Conny	00	00	00	00	0
CR 3021	11.1	00	00	66.7	100
CR 3086	11.1	00	00	11.1	100
CR 3090	00	00	00	33.3	77.8
CR 3100	00	00	00	33.3	55.6
CR 3189	11.1	00	00	55.6	55.6
Olivia	44.4	00	00	22.2	100
CR 634	33.3	00	00	22.2	100
Hanna	00	00	00	00	100
Olga	100	00	00	22.2	NT
Omega	22.2	00	00	33.3	NT
Regina	22.2	00	11.1	77.8	100
Sv 716	33.3	11.1	00	11.1	11.1
CR 999	100	00	00	77.8	100
K 7967	66.7	00	00	33.3	100
Apollo	00	00	00	100	100
Altex	00	00	00	100	100

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

The Swedish genotype 'Kajsa' showed resistance to pathotypes 2 and 8, 'CR 3090' and 'CR 3100' to pathotypes 6 and 8, and the cultivar 'Hanna' showed resistance to only pathotype 8. The cultivar 'Conny' did not show any resistance to any of the Canadian *P. brassicae* pathotype. Cultivar 'Olga' and 'Omega' were tested against Canadian *P. brassicae* pathotypes 2, 3, 5 and 6 and showed resistance to only pathotypes 2 and 6. Three Canadian spring type cultivar 'Apollo', 'Altex' and 'AWP-F647' were found to carry resistance to only pathotypes 6 and 8. Thus, resistance to pathotypes 2, 6 and 8 was found to be frequent in these *B. napus* genotypes (Table 2.14).

On the other hand, single seedlings of Swedish rapeseed cultivars 'Ww 1289' and 'Sv 716' showed resistance to pathotypes 3 and a seedling of the cultivar 'Regina' was resistant to pathotype 5 (Table 2.14).

Of the 24 Swedish spring type *B. napus* ssp. *napus* var. *napus* genotypes tested in Experiment 1, the cultivars 'Ww 1289', 'Sv 716' and 'Regina' were selected for further evaluation against pathotype 3 and 5 in Experiment 2.

2.3.1.5 *Brassica juncea* and *Brassica carinata*

A total of 48 genotypes of *B. juncea* (Table 2.15) and 24 of *B. carinata* (Table 2.16) were evaluated for resistance to five Canadian *P. brassicae* pathotypes. However, none of them showed resistance to any of these pathotypes.

Table 2.15: Resistance response of *Brassica juncea* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	*Plasmodiophora brassicae* pathotypes				
	2	3	5	6	8
BARI Sorisha -6	00	00	00	00	00
BARI Sorisha -7	00	00	00	00	00
BARI Sorisha -8	00	00	00	00	00
BARI Sorisha -9	00	00	00	00	00
BARI Sorisha -10	00	00	00	00	00
BARI Sorisha -11	00	00	00	00	00

Table 2.15: (Continued)

Germplasm	*Plasmodiophora brassicae* pathotypes				
	2	**3**	**5**	**6**	**8**
BARI Sorisha -12	00	00	00	00	00
BARI Sorisha -13	00	00	00	00	00
BARI Sorisha -14	00	00	00	00	00
BARI Sorisha -11	00	00	00	00	00
Daulat	00	00	00	00	00
SS-75	00	00	00	00	00
Cutlass	00	00	00	00	00
CD: 4-1	00	00	00	00	00
Yoi Li Ku	00	00	00	00	00
Long Jiano	00	00	00	00	00
Brown mustard	00	00	00	00	00
Oriental mustard	00	00	00	00	00
Ornamental rai	00	00	00	00	00
321083	00	00	00	00	00
Ma Wei	00	00	00	00	00
Zhou Yu 801	00	00	00	00	00
Sambal	00	00	00	00	00
Rai-5	00	00	00	00	00
Blaze	00	00	00	00	00
Kobu Takana	00	00	00	00	00
Ib 1692	00	00	00	00	00
Secus	00	00	00	00	00
Domo	00	00	00	00	00
Ib 1434	00	00	00	00	00
CR 481	00	00	00	00	00
Hei-ye-mi-tou-gai	00	00	00	00	00
CR 2611	00	00	00	00	00
Budakalaszi Fekete	00	00	00	00	00
Murasaki Takana	00	00	00	00	00
CR 2485	00	00	00	00	00
CR 2492	00	00	00	00	00
Yamashiona	00	00	00	00	00
Ha Karashina	00	00	00	00	00
Ib 1632	00	00	00	00	00
Gielva	00	00	00	00	00
CR 104	00	00	00	00	00
CR 137	00	00	00	00	00
Ha Karashina	00	00	00	00	00
Karashi Hakusai	00	00	00	00	00
CR 342	00	00	00	00	00
CR 341	00	00	00	00	00
Einjahrig	00	00	00	00	00

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

Table 2.16: Resistance response of *Brassica carinata* genotypes to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Germplasm	*Plasmodiophora brassicae* pathotypes				
	2	3	5	6	8
BRA 2569	00	00	00	00	00
BRA 2452	00	00	00	00	00
BRA 1028	00	00	00	00	00
BRA 2481	00	00	00	00	00
BRA 2125	00	00	00	00	00
BRA 2109	00	00	00	00	00
BRA 2137	00	00	00	00	00
BRA 2108	00	00	00	00	00
BRA 2127	00	00	00	00	00
BRA 2483	00	00	00	00	00
BRA 1030	00	00	00	00	00
BRA 1029	00	00	00	00	00
BRA 2607	00	00	00	00	00
BRA 2543	00	00	00	00	00
BRA 2237	00	00	00	00	00
BRA 2427	00	00	00	00	00
BRA 1043	00	00	00	00	00
Parental line	00	00	00	00	00
391078	00	00	00	00	00
3-1	00	00	00	00	00
Unknown	00	00	00	00	00
YS	00	00	00	00	00
17	00	00	00	00	00
36	00	00	00	00	00

Note: Number under *P. brassicae* pathotype indicates percent of the seedlings showed resistance.

2.3.2 Experiment 2

Fifty nine *Brassica* genotypes possessing resistance to most of the pathotypes, selected from Experiment 1, were evaluated in three replications against the most virulent Canadian *P. brassicae* pathotype 3 and the second most virulent pathotype 5. In each replication the Chinese cabbage cultivar ‘Granaat’, commonly known as ECD-5, was used as susceptible control. The Index of Disease (ID) for the susceptible control was always 100.

Brassica germplasm showed significant ($p<0.0001$) variation for resistance to the Canadian *P. brassicae* pathotypes. The two pathotypes also

showed significant virulence (p = 0.0312) to the tested germplasms. Statistical analysis confirmed highly significant interaction (p<0.001) between *P. brassicae* pathotypes and *Brassica* germplasm (Table 2.17).

Table 2.17 Analysis of Variance (ANOVA) for statistical significance of *Brassica* germplasm and *Plasmodiophora brassicae* pathotypes for mean Index of Disease (ID)

Effect	Numerator df	Denominator df	F value	Pr > F
P. brassicae pathotype	1	4	10.6	0.0312
Brassicae germplasm	58	232	146.19	<0.0001
P. brassicae pathotype * *Brassicae* germplasm	58	232	3.26	<0.0001

2.3.2.1 Resistance patterns in *Brassica rapa* genotypes

Among 17 diploid A-genome genotypes of *Brassica*, the European turnip (*B. rapa* var. *rapifera*) genotypes *viz*., 'Debra', 'Gelria', 'ECD-3' and 'ECD-4', showed no to mild reactions to pathotypes 3 and 5, indicating higher genomic homozygosity in these genotypes for resistance to these pathotypes (Table 2.18). The genotypes 'Debra' and Gelria' constantly had ID value of 0.00% for both pathotypes 3 and 5; 'ECD-4' had ID value of 0.00% for pathotype 5 and 3.09% for pathotype 3; while 'ECD-3' had ID value of 5.56% and 1.86% for pathotypes 3 and 5 respectively. However, infection of pathotypes 3 and 5 on these four turnip genotypes was not statistically significant (Table 2.18) and they did not differ from each other for resistance to either of the pathotypes (Table 2.26 and Table 2.27). The American turnip cultivar 'Royal Gam' was significantly infected by both pathotypes. But, ID value of 59.26% and 51.85% respectively for pathotypes 3 and 5 indicated presence of resistance in some of the individual plants of this genotype (Table 2.18). The difference for infection between these two pathotypes on this genotype was not statistically significant (Table 2.19).

Table 2.18: Disease severity index of *Brassica rapa* genotypes caused by single spore-isolates of *Plasmodiophora brassicae* pathotypes 3 and pathotype 5

Germplasm	*P. brassicae* pathotypes	Index of Disease (ID)	t value	Pr > |t|
Debra	Pathotype 3	0.00	0.00	1.0000
	Pathotype 5	0.00	0.00	1.0000
Gelria	Pathotype 3	0.00	0.00	1.0000
	Pathotype 5	0.00	0.00	1.0000
ECD-3	Pathotype 3	5.56	1.16	0.2465
	Pathotype 5	1.85	0.39	0.6987
ECD-4	Pathotype 3	3.09	0.65	0.5193
	Pathotype 5	0.00	0.00	1.0000
Royal Gam	Pathotype 3	59.26	12.39	< 0.0001
	Pathotype 5	51.85	10.84	< 0.0001
BARI Shak-1	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Bilko (F_1)	Pathotype 3	4.32	0.90	0.3673
	Pathotype 5	0.00	0.00	1.0000
Mirako (F_1)	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Largo	Pathotype 3	58.03	12.13	< 0.0001
	Pathotype 5	72.22	15.10	< 0.0001
Prisma	Pathotype 3	83.95	17.55	< 0.0001
	Pathotype 5	77.78	16.26	< 0.0001
JSv01-11403	Pathotype 3	83.95	17.55	< 0.0001
	Pathotype 5	87.04	18.20	< 0.0001
JSv01-13102	Pathotype 3	86.42	18.07	< 0.0001
	Pathotype 5	90.74	18.97	< 0.0001
JSv01-11449	Pathotype 3	75.31	15.75	< 0.0001
	Pathotype 5	68.52	14.33	< 0.0001
JSv01-13051	Pathotype 3	47.53	9.94	< 0.0001
	Pathotype 5	68.52	14.33	< 0.0001
JSv01-13084	Pathotype 3	89.50	18.71	< 0.0001
	Pathotype 5	77.78	16.26	< 0.0001
JSv00-13426	Pathotype 3	85.18	17.81	< 0.0001
	Pathotype 5	87.04	18.20	< 0.0001
JSv00-15588	Pathotype 3	49.38	10.33	< 0.0001
	Pathotype 5	61.11	12.78	< 0.0001

[1]The genotype, BARI Shak-1 belongs to *Brassica rapa* var. *pekinensis*, Bilko and Mirako belong to *B. rapa* var. *chinensis*, Debra, Gelria, ECD-3, ECD-4 and Royal Gam belongs to *Brassica rapa* var. *rapifera* and all other genotypes belongs to *B. rapa* var. *oleifera*.

Of the two hybrid Chinese cabbage (*B. rapa* var. *chinensis*) cultivars tested, ‘Bilko’ was significantly resistant to both pathotype 3 and 5 with ID value of 4.32% and 0.00% respectively (Table 2.18). On the other hand, ‘Mirako’ showed no resistance (ID value of 100%) to either of the pathotypes (Table 2.18). All

winter type *B. rapa* genotypes were significantly infected by both pathotypes 3 and 5 and had high ID value. However, difference in ID value among these nine winter rape genotypes for pathotypes 3 and 5 was not statistically significant except for 'Largo' and 'JSv01-13051' (Table 2.19). These two genotypes were found to possess significantly higher resistance to pathotype 3 than pathotype 5 (Table 2.19).

Table 2.19: Comparative resistance of *Brassica rapa* genotypes to the single spore-isolates of *Plasmodiophora brassicae* pathotypes 3 and pathotype 5

Germplasm	Index of Disease (ID)		t value	Pr > |t|
	Pathotype 3	Pathotype 5		
Debra	0.00	0.00	0.00	1.0000
Gelria	0.00	0.00	0.00	1.0000
ECD-3	5.56	1.85	0.55	0.5845
ECD-4	3.09	0.00	0.46	0.6485
Royal Gam	59.26	51.85	1.10	0.2746
BARI Shak-1	100.00	100.00	0.00	1.0000
Bilko (F_1)	4.32	0.00	0.64	0.5236
Mirako (F_1)	100.00	100.00	0.00	1.0000
Largo	58.02	72.22	-2.20	0.0369
Prisma	82.01	77.78	0.91	0.3621
JSv01-11403	83.95	87.04	-0.46	0.6485
JSv01-13102	86.42	90.10	-0.64	0.5236
JSv01-11449	75.3	68.50	1.00	0.3167
JSv01-13051	47.54	68.52	-3.10	0.0022
JSv01-13084	89.50	77.78	1.73	0.0843
JSv00-13426	85.18	87.04	-0.27	0.7843
JSv00-15588	49.40	61.11	-1.73	0.0842

[1]The genotype, BARI Shak-1 belongs to *B. rapa* var. *pekinensis*, Bilko and Mirako belong to *B. rapa* var. *chinensis*, Debra, Gelria, ECD-3, ECD-4 and Royal Gam belongs to *B. rapa* var. *rapifera* and all other genotypes belongs to *B. rapa* var. *oleifera*.

2.3.2.2 Resistance patterns in *Brassica oleracea* genotypes

All 13 *B. oleracea* genotypes studied in Experiment 2 were significantly affected by *P. brassicae* pathotypes 3 and 5 (Table 2.20). However, resistance to these pathotypes was found in individual plants of two cabbage genotypes, 'Badger Shipper' and 'Bindsachsenn' as indicated by their ID values were significantly lower than ID value of 100% for the susceptible control. The genotype 'Badger Shipper' had significantly ($p<0.0001$) higher resistance to

pathotype 3 (ID value 57.41%) than pathotype 5 (ID value 85.18%); whereas, 'Bindsachsenn' was more resistant (p=0.0001) to pathotype 5 (ID value 33.95) compared to pathotype 3 (ID value 41.97%). The cabbage genotype 'Jersey Queen', 'Emblem', 'Augusta', 'Balbro', 'Head Start' and 'Blue Thunder' did not show resistance to either of these two virulent pathotypes (Table 2.20).

Table 2.20: Disease severity index of *Brassica oleracea* genotypes caused by single-spore isolates of *Plasmodiophora brassicae* pathotype 3 and pathotype 5

Germplasm	*P. brassicae* pathotypes	Index of Disease (ID)	t value	Pr > |t|
Badger Shipper	Pathotype 3	57.40	12.00	< 0.0001
	Pathotype 5	85.19	17.81	< 0.0001
Bindsachsenn	Pathotype 3	60.49	12.65	< 0.0001
	Pathotype 5	33.95	7.10	< 0.0001
Jersey Queen	Pathotype 3	98.15	20.52	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Emblem	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Augusta	Pathotype 3	96.91	20.26	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Balbro (F_1)	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Head Start	Pathotype 3	97.53	20.39	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Blue Thunder	Pathotype 3	98.15	20.52	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Hybrid Spouket	Pathotype 3	98.77	20.65	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Diablo (F_1)	Pathotype 3	82.10	17.17	< 0.0001
	Pathotype 5	98.15	20.52	< 0.0001
Monterey	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Premium Crop (F_1)	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
BRA 1896	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001

[1]The genotypes Badger Shipper, Bindsachsenn, Jersey Queen, Emblem, Augusta and Balbro are *B. oleracea* var. *capitata*, Head Start and Blue Thunder are white cabbage, Hybrid Spouket and Diablo belongs to *B. oleracea* var. *gemmifera*, Monterey and Premium Crop belong to *B. oleracea* var. *italica* and the genotype BRA 1896 belongs to *B. oleracea* var. *villosa*.

On the other hand, some plants of the brussels sprout (*B. oleracea* var. *gemmifera*) cultivar 'Diablo' were resistant to pathotype 3 (ID value 82.10%) but were totally susceptible to pathotype 5 (ID value 98.14%). Resistance to either

pathotype 3 or 5 was totally absent in the two broccoli (*B. oleracea* var. *italica*) cultivars as well as in the single *B. oleracea* var. *villosa* genotype (Table 2.20). *P. brassicae* pathotype 5 was significantly more virulent on 'Badger Shipper' and 'Diablo', whereas pathotype 3 showed more virulence on 'Bindsachsenn' than pathotype 5 (Table 2.21).

Table 2.21: Comparative resistance of *Brassica oleracea* genotypes to the single-spore isolates of *Plasmodiophora brassicae* pathotype 3 and pathotype 5

Germplasm	Index of Disease (ID)		t value	Pr > |t|
	Pathotype 3	Pathotype 5		
Badger Shipper	57.41	85.18	-4.11	< 0.0001
Bindsachsenn	41.97	33.95	3.92	< 0.0001
Jersey Queen	98.15	100	-0.27	0.7843
Emblem	100	100	0.00	1.0000
Augusta	96.91	100	-0.46	0.6485
Balbro F_1	100	100	0.00	1.0000
Head Start	97.53	100	-0.37	0.7153
Blue Thunder	98.14	100	-0.27	0.7843
Hybrid Spouket	98.76	100	-0.18	0.8555
Diablo F_1	82.1	98.14	-2.37	0.0185
Monterey	100	100	0.00	1.0000
Premium Crop (F_1)	100	100	0.00	1.0000
BRA 1896	100	100	0.00	1.0000

[1]The genotypes Badger Shipper, Bindsachsenn, Jersey Queen, Emblem, Augusta and Balbro are *B. oleracea* var. *capitata*, Head Start and Blue Thunder are white cabbage, Hybrid Spouket and Diablo belongs to *B. oleracea* var. *gemmifera*, Monterey and Premium Crop belongs to *B. oleracea* var. *italica* and the genotype, BRA 1896 belongs to *B. oleracea* var. *villosa*

2.3.2.3 Resistance patterns in *Brassica nigra* genotypes

Of the three genomic groups of *Brassica*, the B-genome species *B. nigra* generally showed high resistance to the Canadian *P. brassicae* pathotypes. Twenty one *B. nigra* genotypes were evaluated against pathotypes 3 and 5 in replicated experiments (Table 2.22). All of them were found to possess very high to moderate levels of resistance to these two pathotypes. Among them, the *B. nigra* subsp. *nigra* var. *nigra* genotypes *viz*., 'CR 2144' and 'CR 2716' and *B. nigra* subsp. *hispida* var. *rigida* genotype 'CR 2120' showed no reaction to either of the pathotypes.

Table 2.22: Disease severity index of *Brassica nigra* genotypes caused by single-spore isolates of *Plasmodiophora brassicae* pathotype 3 and pathotype 5

Germplasm	*P. brassicae* pathotypes	Index of Disease (ID)	t value	Pr > |t|
CR 1199	Pathotype 3	24.69	5.16	< 0.0001
	Pathotype 5	1.24	0.26	0.7951
Primus	Pathotype 3	1.85	0.39	0.6987
	Pathotype 5	1.85	0.39	0.6987
Sizaja	Pathotype 3	3.09	0.65	0.5193
	Pathotype 5	1.85	0.39	0.6987
CR 2143	Pathotype 3	0.00	0.00	1.0000
	Pathotype 5	1.85	0.39	0.6987
CR 2144	Pathotype 3	0.00	0.00	1.0000
	Pathotype 5	0.00	0.00	1.0000
CR 2716	Pathotype 3	0.00	0.00	1.0000
	Pathotype 5	0.00	0.00	1.0000
CR 2719	Pathotype 3	17.90	3.74	0.0002
	Pathotype 5	1.85	0.39	0.6987
CR 340	Pathotype 3	22.22	4.65	< 0.0001
	Pathotype 5	16.67	3.48	0.0006
Alsaska	Pathotype 3	14.19	2.97	0.0033
	Pathotype 5	2.47	0.52	0.6060
Balkan	Pathotype 3	48.15	10.07	< 0.0001
	Pathotype 5	1.85	0.39	0.6992
CR 2120	Pathotype 3	0.00	0.00	1.0000
	Pathotype 5	0.00	0.00	1.0000
CR 1216	Pathotype 3	38.89	8.13	< 0.0001
	Pathotype 5	27.16	5.68	< 0.0001
CR 2094	Pathotype 3	57.41	12.00	< 0.0001
	Pathotype 5	33.33	6.97	< 0.0001
CR 2095	Pathotype 3	39.51	8.26	< 0.0001
	Pathotype 5	27.78	5.81	< 0.0001
CR 2097	Pathotype 3	0.00	-0.00	1.0000
	Pathotype 5	10.49	2.19	0.0292
CR 2098	Pathotype 3	35.18	7.36	< 0.0001
	Pathotype 5	17.28	3.61	0.0004
CR 2101	Pathotype 3	50.00	10.45	< 0.0001
	Pathotype 5	51.85	10.84	< 0.0001
CR 2102	Pathotype 3	37.04	7.74	< 0.0001
	Pathotype 5	29.63	6.20	< 0.0001
CR 2122	Pathotype 3	8.64	1.81	0.0721
	Pathotype 5	8.02	1.68	0.0947
Gibera	Pathotype 3	12.96	2.71	0.0072
	Pathotype 5	13.58	2.84	0.0049
CR 2725	Pathotype 3	4.94	1.03	0.3027
	Pathotype 5	3.71	0.78	0.4391

Several *B. nigra* subsp. *nigra* var. *nigra* type genotypes *e.g.*, 'Primus', 'Sizaja' and 'CR 2143' had ID value up to 3.1% to pathotypes 3 or 5; however, they were statistically similar to 'CR 2144' and 'CR 2716' for resistance to these pathotypes. Some of the genotypes *e.g.*, 'CR 1199', 'CR 2719', 'Primus' and 'Sizaja' were significantly infected by pathotype 3 but not by pathotype 5; while *B. nigra* (no information on taxonomic classification at sub species level) genotype 'CR 2097' was infected by pathotype 5 but not by pathotype 3 (Table 2.22). On the other hand, 'CR 340', 'CR 1216', 'CR 2094', 'CR 2095', 'CR 2098', 'CR 2101', 'CR 2102' and 'Gibera' were significantly infected by both pathotypes.

Table 2.23: Comparative resistance of *Brassica nigra* genotypes to the single-spore isolates of *Plasmodiophora brassicae* pathotype 3 and pathotype 5

Germplasm	Index of Disease (ID)		t value	Pr > |t|
	Pathotype 3	Pathotype 5		
CR 1199	24.69	1.2	3.47	0.0006
CR 1204	1.86	1.86	-0.00	1.0000
CR 1206	3.1	1.86	0.18	0.8555
CR 2143	0.00	1.86	-0.27	0.7843
CR 2144	0.00	0.00	0.00	1.0000
CR 2716	0.00	0.00	0.00	1.0000
CR 2719	17.9	1.86	2.37	0.0185
CR 340	22.22	16.67	0.82	0.4124
Alsaska	14.18	2.5	1.73	0.0843
Balkan	48.15	1.86	6.85	< 0.0001
CR 2120	0.00	0.00	0.00	1.0000
CR 1216	38.89	27.16	1.73	0.0842
CR 2094	57.41	33.33	3.56	0.0004
CR 2095	39.51	27.78	1.73	0.0842
CR 2097	0.00	10.49	-1.55	0.1221
CR 2098	35.18	17.28	2.65	0.0087
CR 2101	50.00	51.85	-0.27	0.7843
CR 2102	37.04	29.63	1.10	0.2744
CR 2122	8.64	8.02	0.09	0.9274
Gibera	12.96	13.58	-0.09	0.9270
CR 2725	4.94	3.70	0.18	0.8555

2.3.2.4 Resistance patterns in *Brassica napus* genotypes

The amphidiploid rutabaga (*B. napus* subsp. *napobrassica*) genotypes *viz.*, 'Wilhelmsburger', 'Brookfield-9005' and 'Polycross-9006' were highly resistant to pathotypes 3 and 5. 'Wilhelmsburger' and 'Polycross-9006' had no reaction to either of the pathotype, whereas 'Brookfield-9005' had ID value 1.21% to pathotype 3 only (Table 2.24). On the other hand, the three spring type *B. napus* subsp. *napus* var. *napus* cultivar *viz.*, 'Bina Sorisha-3', 'Ww 1289', 'Sv 716' and the fodder rape genotype 'New Zealand Resistant Rape' (Table 2.24) were significantly infected by both pathotypes 3 and 5, as indicated by ID values similar to susceptible control (100%).

Table 2.24: Disease severity index of *Brassica napus* genotypes caused by single-spore isolates of *Plasmodiophora brassicae* pathotype 3 and pathotype 5

Germplasm	*P. brassicae* pathotypes	Index of Disease (ID)	t value	Pr > |t|
New Zealand Resistant Rape	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Bina Sorisha- 3	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	100.00	20.91	< 0.0001
Ww 1289	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	98.15	20.52	< 0.0001
Regina	Pathotype 3	68.52	14.33	< 0.0001
	Pathotype 5	26.54	5.55	< 0.0001
Sv 716	Pathotype 3	100.00	20.91	< 0.0001
	Pathotype 5	99.37	20.78	< 0.0001
Wilhelmsburger	Pathotype 3	00.00	0.00	1.0000
	Pathotype 5	00.00	0.00	1.0000
Brookfield-9005	Pathotype 3	1.21	0.26	0.7951
	Pathotype 5	00.00	0.00	1.0000
Polycross-9006	Pathotype 3	00.00	0.00	1.0000
	Pathotype 5	00.00	0.00	1.0000

[1]The genotypes Wilhelmsburger, Brookfield-9005 and Polycross-9006 belong to the rutabaga (*Brassica napus* subsp. *napobrassica*) and all other genotypes belong to *B. napus* subsp. *napus* var. *napus.*

The Swedish spring type *B. napus* subsp. *napus* var. *napus* cultivar 'Regina' showed moderate reaction to pathotype 5 (ID value 26.54%) but higher reaction to pathotype 3 (ID value 68.52%) (Table 2.24); thus, the level of infection by these two pathotypes was significantly ($p < 0.0001$) different on this genotype (Table

2.25). There was no variation among the three rutabaga genotypes for resistance to either of the two Canadian *P. brassicae* pathotypes (Table 2.26 and Table 2.27)

Table 2.25: Comparative resistance of *Brassica napus* genotypes to the single-spore isolates of *Plasmodiophora brassicae* pathotype 3 and pathotype 5

Germplasm[1]	Index of Disease (ID)		t value	Pr > |t|
	Pathotype 3	Pathotype 5		
New Zealand Resistant Rape	100	100	-0.00	1.0000
Bina Sorisha-3	100	100	0.00	1.0000
Ww 1289	100	98.17	0.27	0.7843
Regina	68.52	26.54	6.21	< 0.0001
Sv 716	100	99.37	0.09	0.9255
Wilhelmsburger	0.00	0.00	0.00	1.0000
Brookfield-9005	1.21	0.00	0.18	0.8543
Polycross-9006	0.00	0.00	-0.00	1.0000

[1]The genotypes Wilhelmsburger, Brookfield-9005 and Polycross-9006 belong to the rutabaga (*Brassica napus* subsp. *napobrassica*) and all other genotypes belong to *B. napus* subsp. *napus* var. *napus.*

2.4 Discussion

The aim of this research was to explore the *Brassica* A-, B- and C-genome species and their amphidiploids to identify genotypes with superior resistance to clubroot disease, based on greenhouse screening, for use in breeding oilseed *Brassica* crops with durable resistance to Canadian *P. brassicae* pathotypes. Several investigations have been done by researchers in the past to identify resistance to different *P. brassicae* pathotypes or races in the diploid A- and C-genome species and in their amphidiploids (AACC-genome) (Tjallingii, 1965; Ayers and Lelacheur, 1972; Toxopeus and Janssen, 1975; Miller and Williams, 1986; Voorrips and Visser, 1993; Dias *et al*., 1993). However, none of these studies included all diploid and amphidiploid *Brassica* species in order to get a comparative view on the occurrence of resistance to the specific pathotype(s). Furthermore, resistance expression of a genotype can be different depending on the pathotype/race with which it is being challenged. In this context, it is therefore extremely important to challenge the materials under investigation against the pathotype of interest for the geographical location. To the best of my knowledge, no extensive study so far been conducted to identify clubroot resistance in the

Brassica B- genome species – a gene reservoir which can be used for breeding of *B. juncea* and *B. carinata* cultivars. Data presented in this thesis from Experiment 1 indicated that *B. napus* genotypes often lack resistance to Canadian *P. brassicae* pathotypes 3 and 5. According to Strelkov *et al.* (2006a) these two pathotypes are also the most virulent of all Canadian pathotypes. Therefore, study in Experiment 2 was concentrated on pathotypes 3 and 5 only. Any genotype which had resistance to either pathotype 3 and/or 5 often showed resistance to the other three less virulent pathotypes too.

Tinline *et al*. (1989) noted that a species, when evolving from its progenitors at the centre of origin in constant interaction with the ever evolving pathogen(s), will evolve together with the pathogen to achieve a biological balance; and these plant species are rich in diversity and often serve as a good source of resistance. European Clubroot Differential (ECD) genotype 'Debra', 'Gelria', 'ECD-3' and 'ECD-4' are the selections from genetically diverse European turnip genotypes (Buczacki *et al*., 1975; reviewed by Diederichsen *et al*., 2009) also evolved in Mediterranean region (for detailed review, see Gomez-Campo and Prakash, 1999) along with *P. brassicae* for centuries. Therefore, it can be assumed that these four turnip genotypes could possess genetic diversity for resistance to Canadian *P. brassicae* pathotypes.

On the 36 diploid A-genome (*B. rapa*) genotypes investigated in this study, the turnip group was found to carry resistance to all five Canadian *P. brassicae* pathotypes. Strelkov *et al*. (2006a) also observed a high level of resistance in four European turnip type genotypes, *viz.* 'Debra', 'Gelria', 'ECD-3' and 'ECD-4' against all Canadian pathotypes. In addition to these resistance sources, the present study also disclosed the existence of resistance in American turnip cultivar 'Royal Gam'. This cultivar appeared to be heterogeneous for resistance to pathotypes 3 and 5, which is not surprising in case of a cross-pollinated crop. It is plausible that strict selection has not been applied during breeding of this cultivar for resistance to these Canadian pathotypes. However, it would be possible to

develop homozygous clubroot resistant lines(s) from this cultivar through repeated self pollination of the resistant plants. Whether the resistance gene(s) in this cultivar is the same as in the ECD genotypes would need further investigation.

None of the Chinese cabbage genotypes was resistant to any of the pathotypes except the hybrid cultivar ‘Bilko’ from Noresco, Laval, Quebec. ‘Bilko’ did not show susceptibility to any of the pathotypes. This suggests occurrence of one or more dominant gene(s) in this hybrid cultivar, originating from its parental genotype (s), for resistance to all five *P. brassicae* pathotypes. It is also possible that resistance in ‘Bilko’ is conferred by recessive genes; and in this case both parents of this hybrid must have been in homozygous condition for resistance. Further study would be needed to unveil the nature of resistance in this genotype. These leafy vegetable forms evolved in South China (Sun, 1946; Denford and Vaughan, 1977; Song *et al.*, 1988) where clubroot pathogen was introduced during colonial period thereby explaining the absence of clubroot resistance in them in general.

High level of resistance among the winter type *B. rapa* genotypes to the less virulent pathotypes (6 and 8) compared to lower resistance to the virulent pathotypes (3 and 5) was apparently due to high homogeneity among them for resistance to these less virulent pathotypes. However, it is not possible to draw any conclusion from this experiment on the genetic nature of this resistance in these genotypes against the pathotypes 6 and 8. These winter *B. rapa* genotypes appeared to be heterogeneous for resistance to pathotypes 3 and 5, and thus suggest the possibility of isolating lines resistant to these pathotypes through selection.

In contrast to the A-genome species, the C-genomic group of *Brassica* has rarely been reported to possess resistance to clubroot disease (as reviewed by Piao *et al*., 2009). Results of this study were of no exception from the previous reports.

Only two cabbage genotypes 'Badger Shipper' and 'Bindsachsenn' were found to carry some resistance to different pathotypes; and few plants of the hybrid brussels sprout cultivar 'Diablo' showed resistance to pathotype 3. The cultivar 'Diablo' was not evaluated against the pathotypes 2, 6 and 8 due to unavailability of sufficient number of seeds. On the other hand, the broccoli and cauliflower genotypes completely lacked resistance to these pathotypes. Crisp *et al.* (1989) also reported similar results based on evaluation of 1000 *B. oleracea* genotypes representing different subspecies and varieties.

On the other hand, wide genetic diversity has been reported to be present in the B- genome species *B. nigra* (Negi *et al*., 2004). This species has been used as valuable source of resistance to leaf spot (*Alternaria brassicicola*), black rot (*Xanthomonas campestris* pv. *campestris*) (Westman *et al*., 1999) and black leg (*Leptosphaeria maculans*) (Gerdemann-Knorck *et al*., 1995) diseases. To my knowledge, there is no extensive study on *B. nigra* for resistance to different *P. brassicae* pathotypes. In this study, 77 *B. nigra* genotypes were evaluated against five Canadian *P. brassicae* pathotypes, of which 60 were found to possess high level of resistance against these pathotypes. Only two genotypes (*e.g.*, CR '2748' and 'CR 3279') did not show any resistance to any of the pathotypes. To date, there is no report on the genetic control of this B- genome resistance to different pathotypes. However, these resistant *B. nigra* can be used as valuable genetic resources for introgression of resistance into *B. juncea* or *B. carinata* through interspecific hybridization or resynthesis of these species. Furthermore, *B. nigra* can also be used as a source to introduce resistance into *B. napus*, if needed. In this case, there is a limited chance of introgression of resistance from the B-genome chromosome of *B. nigra* into A- or C-genome chromosome of *B. napus,* due to low homoeology between the B chromosomes with the A/C chromosomes (Attia and Röbbelen, 1986; Attia *et al*., 1987). This needs to be taken into consideration while designing an experiment for introgression of resistance from the B-genome into the A/C-genome of *B. napus*.

Although the A-, B- and C-genome diploid species were found to carry resistance to different *P. brassicae* pathotypes, different results was obtained in case of the amphidiploid species. So far, clubroot disease resistance has been reported only in the amphidiploid species *B. napus* (AACC) (Colhoun, 1958; Karling, 1968; Johnston, 1970; Ayers and Lelacheur, 1972; Buczacki *et al*., 1975; Crute *et al.*, 1983; Gustafsson and Fält, 1986); but not in *B. juncea* (AABB) and *B. carinata* (BBCC) (as reviewed by Diederichsen *et al.*, 2009). The results of this study were of no exception from the previous reports. None of the 48 *B. juncea* and 24 *B. carinata* genotypes studied in this experiment was found to carry resistance to any of the Canadian *P. brassicae* pathotypes; while different *B. napus* genotypes showed various degrees of resistance to different pathotypes. The rutabaga genotypes 'Wilhelmsburger', 'Brookfield-9005' and 'Polycross-9006' showed high level and broad-spectrum resistance to Canadian *P. brassicae* pathotypes. Also, some of the Swedish spring type oilseed rape cultivars and fodder rape genotypes showed some resistance to multiple pathotypes. These findings are in agreement with the previous reports where race-specific to broad-spectrum resistance to clubroot pathotype has also been reported in rutabaga and fodder rape (Colhoun, 1958; Karling, 1968; Johnston, 1970; Ayers and Lelacheur, 1972; Buczacki *et al*., 1975; Crute *et al.*, 1983; Gustafsson and Fält, 1986). Several Swedish spring type rapeseed genotypes also showed race-specific to broad-spectrum resistance to multiple Canadian *P. brassicae* pathotypes; even this type of rapeseed (*B. napus* subsp. *napus* var. *napus*) is known to have narrow genetic diversity (Hasan *et al*., 2006). The pedigree of these Swedish cultivars is not known. It might be possible that these cultivars have received resistance gene(s) from their resistant ancestors and/or from rutabaga or fodder type *B. napus*. Occurrence of clubroot in oilseed *Brassica* crops in Sweden has already been reported in 1980's (Wallenhammar, 1996) and breeding for clubroot resistant oilseed *B. napus* cultivar has been one of the research objectives for the Swedish breeders. Some of the Swedish spring *B. napus* lines, evaluated in field trials in Quebec, found to carry resistance to clubroot (Pageau *et al*., 2006).

The occurrence of resistance in *B. napus* (AACC) but not in *B. juncea* (AABB) and *B. carinata* (BBCC) is difficult to explain since resistance was found to occur in all diploid *Brassica* species. It is reported that the amphidiploid species *B. juncea* evolved in the Middle East region of Asia to Central China from its progenitor species *B. rapa* (A-genome) and *B. nigra* (B- genome) (Olsson, 1960; Mizushima and Tsunoda, 1967) and *B. carinata* evolved in the Ethiopian plateau (Dixon, 2006) from wild or semi-domesticated *B. nigra* (B genome) and land races of *B. oleracea* (C genome). To my knowledge, there is no report of occurrence of clubroot disease in the Middle East and in the Ethiopian plateau or existence of clubroot resistance in *B. rapa* and *B. oleracea* from these regions. However, this does not answer the question since most of the *B. nigra* genotypes in this study were found to carry resistance and this diploid species has been involved in the evolution of these two amphidiploids. If resistant *B. nigra* have been involved in the evolution of these two amphidiploid species in nature, it might be possible that the B-genome resistance in these two amphidiploids is hypostatic (≈ recessive) to clubroot susceptibility of the A- or C-genome. Resynthesis of *B. juncea* and *B. carinata* from different combinations of resistant and susceptible diploid species might answer some of these questions.

On the other hand, the amphidiploid species *B. napus* have evolved in the South-Western Europe of Mediterranean region (for additional review see Gomez-Campo and Prakash, 1999). This region is also the centre of origin of its diploid parental species *B. rapa* (A- genome) (Sun, 1946; Denford and Vaughan, 1977; Song *et al.*, 1988) and *B. oleracea* (C genome) (for additional review see Gomez-Campo and Prakash, 1999) and clubroot resistance was identified in these species. Resistance is frequently reported especially in turnip type *B. rapa* which has evolved in the Mediterranean region (Sun, 1946; Denford and Vaughan, 1977; Song *et al.*, 1988; for additional review see Gomez-Campo and Prakash, 1999), and possibly evolved with constant interaction with the ever evolving clubroot pathogen(s) in this region. Thus, it is might be possible that clubroot resistance in *B. napus* (AC genome) is inherited from either one or both of the progenitor

species during its evolution in nature. Furthermore, resynthesis of *B. napus* from its diploid parents has been done by several researchers (Johnston, 1974; Gowers, 1982; Kraling, 1987; Akbar, 1989; Lu *et al*., 2001; Abel *et al*., 2005; Werner *et al*., 2008) for widening the genetic diversity in this species including resistance to clubroot disease.

The *Plasmodiophora brassicae* pathotypes 3 and 5 showed similar levels of infection on most of the genotypes evaluated in Experiment 2. The Index of Disease (ID) due to pathotype 3 was higher than the pathotypes 5 on *B. napus* genotype 'Regina' and some of the *B. nigra* genotypes ('CR 1199', 'CR 2719', 'Sizaja', 'CR 2094' and 'CR 2098'); but the ID of pathotype 3 was lower on two winter *B. rapa* ('JSv 01-13051' and 'Largo') and three *B. oleracea* ('Badger Shipper', 'Bindsachsenn' and 'Diablo') genotypes. Most of these *Brassica* genotypes are cross-pollinated in nature, where genetic heterogeneity is very common. The genetic makeup of individual plants in such a population can vary significantly; where the frequency of resistance alleles for a locus can be significantly different from the frequency of resistance alleles of the other locus. For example, in such a heterogeneous population the frequency of A_1 (resistance) and A_2 (susceptibility) alleles of locus '*A*' can be significantly different from the frequency of B_1 (resistance) and B_2 (susceptibility) alleles of locus '*B*'. As the experiment in the present study was based on different sets of seedlings challenged with these two pathotypes, as opposed to use of genetically uniform population (*e.g*., DH line), it is therefore, not possible to deduce from the data whether the observed difference in ID for pathotypes 3 and 5 is due to differences in their virulence or due to difference in the frequency of the resistance alleles for these two pathotypes in these genotypes. Further study using either DH or highly inbred lines from these genotypes would be needed to answer this question.

Table 2.26: Comparisons of the mean Index of Disease of *Brassica* genotypes inoculated with *Plasmodiophora brassicae* pathotype 3. Groups of common letters are not significantly different (P < 0.05)

Scientific Name	Germplasm	ls means	Groups of significance							
B. rapa var. *rapifera*	Debra	0.00	a							
B. rapa var. *rapifera*	Gelria	0.00	a							
B. napus subsp. *napobrassica*	Wilhelmsburger	0.00	a							
B. napus subsp. *napobrassica*	Polycross-9006	0.00	a							
Brassica nigra	CR 2097	0.00	a							
B. nigra subsp. *hispida* var. *rigida*	CR 2120	0.00	a							
B. nigra subsp. *nigra* var. *nigra*	CR 2143	0.00	a							
B. nigra subsp. *nigra* var. *nigra*	CR 2144	0.00	a							
B. nigra subsp. *nigra* var. *nigra*	CR 2716	0.00	a							
B. napus subsp. *napobrassica*	Brookfield-9005	1.21	a	b						
B. nigra subsp. *nigra* var. *nigra*	Primus	1.86	a	b						
B. nigra subsp. *nigra* var. *nigra*	Sizaja	3.10	a	b						
B. rapa var. *rapifera*	ECD-4	3.09	a	b						
B. rapa var. *chinensis*	Bilko (F_1)	4.32	a	b						
Brassica nigra	CR 2725	4.94	a	b	c					
B. rapa var. *rapifera*	ECD-3	5.56	a	b	c					
Brassica nigra	CR 2122	8.64	a	b	c					
Brassica nigra	Gibera	12.96	a	b	c	d				
B. nigra subsp. *nigra* var. *nigra*	Alaska	14.19		b	c	d				
B. nigra subsp. *nigra* var. *nigra*	CR 2719	17.90			c	d				
B. nigra subsp. *nigra* var. *nigra*	CR 340	22.22				d	e			
B. nigra subsp. *nigra* var. *nigra*	CR 1199	24.69				d	e	f		
Brassica nigra	CR 2098	35.18					e	f	g	
Brassica nigra	CR 2102	37.04						f	g	
Brassica nigra	CR 1216	38.89							g	
Brassica nigra	CR 2095	39.51							g	
B. oleracea var. *capitata*	Bindsachsenn	41.97								h
B. rapa var. *oleifera*	JSv01-13051	47.54								h
B. nigra subsp. *nigra* var. *nigra*	Balkan	48.15								h
B. rapa var. *oleifera*	JSv00-15588	49.40								h

Table 2.26: (Continued)

Scientific Name	Germplasm	ls means	Groups of significance
Brassica nigra	CR 2101	50.00	h
B. oleracea var. *capitata*	Badger Shipper	57.40	h i
Brassica nigra	CR 2094	57.41	h i
B. rapa var. *oleifera*	Largo	58.02	h i
B. rapa var. *rapifera*	Royal Gam	59.26	h i
B. napus subsp. *napus* var. *napus*	Regina	68.51	i j
B. rapa var. *oleifera*	JSv01-11449	75.30	j k
B. rapa var. *oleifera*	Prisma	82.01	k l
B. oleracea var. *gemmifera*	Diablo F_1	82.10	k l
B. rapa var. *oleifera*	JSv01-11403	83.95	k l
B. rapa var. *oleifera*	JSv00-13426	85.18	k l m
B. rapa var. *oleifera*	JSv01-13102	86.42	k l m n
B. rapa var. *oleifera*	JSv01-13084	89.50	l m n o
B. oleracea var. *capitata*	Augusta	96.91	m n o
B. oleracea var. *capitata*	Head Start	97.53	m n o
B. oleracea var. *capitata*	Blue Thunder	98.15	n o
B. oleracea var. *capitata*	Jersey Queen	98.15	n o
B. oleracea var. *gemmifera*	Hybrid Spouket	98.77	n o
B. rapa var. *chinensis*	Mirako (F_1)	100.00	o
B. rapa var. *pekinensis*	BARI Shak-1	100.00	o
B. oleracea var. *capitata*	Emblem	100.00	o
B. oleracea var. *capitata*	Balbro F_1	100.00	o
B. oleracea var. *italica*	Monterey	100.00	o
B. oleracea var. *italica*	Premium Crop (F_1)	100.00	o
B. oleracea var. *villosa*	BRA 1896	100.00	o
B. napus subsp. *napus* var. *napus*	CR 1101	100.00	o
B. napus subsp. *napus* var. *napus*	Bina Sorisha-3	100.00	o
B. napus subsp. *napus* var. *napus*	New Zealand Resistant Rape	100.00	o
B. napus subsp. *napus* var. *napus*	Sv 716	100.00	o

[1]ls means indicate least square means of the Index of Disease (ID) value.

Table 2.27: Comparisons of the mean Index of Disease of *Brassica* genotypes inoculated with *Plasmodiophora brassicae* pathotype 5. Groups of common letters are not significantly different (P <0.05)

Scientific Name	Germplasm	ls means	Groups of significance						
B. rapa var. *rapifera*	Debra	0.00	a						
B. rapa var. *rapifera*	Gelria	0.00	a						
B. rapa var. *rapifera*	ECD-4	0.00	a						
B. rapa var. *chinensis*	Bilko (F_1)	0.00	a						
B. nigra subsp. *nigra* var. *nigra*	CR 2144	0.00	a						
B. nigra subsp. *nigra* var. *nigra*	CR 2716	0.00	a						
B. nigra subsp. *hispida* var. *rigida*	CR 2120	0.00	a						
B. napus subsp. *napobrassica*	Wilhelmsburger	0.00	a						
B. napus subsp. *napobrassica*	Brookfield-9005	0.00	a						
B. napus subsp. *napobrassica*	Polycross-9006	0.00	a						
B. nigra subsp. *nigra* var. *nigra*	CR 1199	1.24	a	b					
B. rapa var. *rapifera*	ECD-3	1.85	a	b					
B. nigra subsp. *nigra* var. *nigra*	Balkan	1.85	a	b					
B. nigra subsp. *nigra* var. *nigra*	Primus	1.85	a	b					
B. nigra subsp. *nigra* var. *nigra*	Sizaja	1.85	a	b					
B. nigra subsp. *nigra* var. *nigra*	CR 2143	1.85	a	b					
B. nigra subsp. *nigra* var. *nigra*	CR 2719	1.85	a	b					
B. nigra subsp. *nigra* var. *nigra*	Alaska	2.47	a	b					
Brassica nigra	CR 2725	3.71	a	b	c				
Brassica nigra	CR 2122	8.02	a	b	c	d			
Brassica nigra	CR 2097	10.49	a	b	c	d	e		
Brassica nigra	Gibera	13.58		b	c	d	e	f	
B. nigra subsp. *nigra* var. *nigra*	CR 340	16.67			c	d	e	f	
Brassica nigra	CR 2098	17.28				d	e	f	g
B. napus subsp. *napus* var. *napus*	Regina	26.54					e	f	g
Brassica nigra	CR 1216	27.16						f	g
Brassica nigra	CR 2095	27.78						f	g
Brassica nigra	CR 2102	29.63							g
Brassica nigra	CR 2094	33.33							g
B. oleracea var. *capitata*	Bindsachsenn	33.95							g

Table 2.27: (Continued)

Scientific Name	Germplasm	ls means	Groups of significance
B. rapa var. *rapifera*	Royal Gam	51.85	h
Brassica nigra	CR 2101	51.85	h
B. rapa var. *oleifera*	JSv00-15588	61.11	h i
B. rapa var. *oleifera*	JSv01-11449	68.50	i j
B. rapa var. *oleifera*	JSv01-13051	68.52	i j
B. rapa var. *oleifera*	Largo	72.22	i j k
B. rapa var. *oleifera*	Prisma	77.78	j k l
B. rapa var. *oleifera*	JSv01-13084	77.78	j k l
B. oleracea var. *capitata*	Badger Shipper	85.19	k l
B. rapa var. *oleifera*	JSv00-13426	87.04	l m n
B. rapa var. *oleifera*	JSv01-11403	87.04	l m n
B. rapa var. *oleifera*	JSv01-13102	90.10	l m n
B. oleracea var. *gemmifera*	Diablo F_1	98.15	l m n
B. napus subsp. *napus* var. *napus*	CR 1101	98.15	l m n
B. napus subsp. *napus* var. *napus*	Sv 716	99.37	m n
B. napus subsp. *napus* var. *napus*	Bina Sorisha-3	100.00	n
B. napus subsp. *napus* var. *napus*	New Zealand Resistant Rape	100.00	n
B. rapa var. *pekinensis*	BARI Shak-1	100.00	n
B. rapa var. *chinensis*	Mirako (F_1)	100.00	n
B. oleracea var. *capitata*	Jersey Queen	100.00	n
B. oleracea var. *capitata*	Emblem	100.00	n
B. oleracea var. *capitata*	Augusta	100.00	n
B. oleracea var. *capitata*	Balbro F_1	100.00	n
B. oleracea var. *capitata*	Head Start	100.00	n
B. oleracea var. *capitata*	Blue Thunder	100.00	n
B. oleracea var. *gemmifera*	Hybrid Spouket	100.00	n
B. oleracea var. *italica*	Monterey	100.00	n
B. oleracea var. *italica*	Premium Crop (F_1)	100.00	n
B. oleracea var. *villosa*	BRA 1896	100.00	n

[1]ls means indicate least square means of the Index of Disease (ID) value

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Chapter 3

Conclusions

3.1 Conclusions

Since suitable measures to control the clubroot disease in canola are yet to be discovered, cultivation of resistant cultivars is the only reliable strategy to avoid economic loss of the crop for this disease as well as to prevent this pathogen from multiplying in the infested fields. Resistant cultivars are also imperative to prevent evolution of rare pathotypes into predominant ones, since *P. brassicae* populations in Alberta have been reported to be fairly diverse (Xue *et al*., 2008). A pre-requisite for resistance breeding is to identify natural sources of resistance based on host-pathogen interactions and the findings from this study will certainly fulfill this requirement. The findings of this study can be summarized as follows:

- The rutabaga or swede (*B. napus* subsp. *napobrassica*) genotypes 'Wilhelmsburger', 'Brookfield-9005' and 'Polycross-9006' showed broad-spectrum resistance to all Canadian *P. brassicae* pathotypes and they appeared to be homogeneous for this resistance against all these pathotypes. These germplasm can immediately be used as a reliable source to introduce clubroot resistance into Canadian canola quality *B. napus* cultivars as well as to breed new canola cultivars for broad-spectrum resistance to all Canadian *P. brassicae* pathotypes.
- Swedish spring type *B. napus* subsp. *napus* var. *napus* genotype, 'Regina' also showed resistance to all five pathotypes. This genotype was highly homogenous for resistance to less virulent pathotypes 6 and 8 but heterogeneous for virulent pathotypes. An inbred line resistant to the virulent pathotype(s) can be developed from this genotype through self-pollination of the resistant plants, and thereby can be used as germplasm for clubroot resistance breeding.
- Among the diploid A-genome species germplasm, European turnip (*B. rapa* var. *rapifera*) genotypes 'Debra', Gelria, 'ECD-3' and 'ECD-4' and the hybrid Chinese cabbage (*B. rapa* var. *chinensis*) cultivar 'Bilko' were

homogeneous for resistance to all five *P. brassicae* pathotypes. These germplasm can immediately be used for resynthesis of clubroot resistant *B. napus* genotypes. The cultivar 'Bilko' can also be a suitable choice for vegetable growers to cultivate in the already clubroot infested home or market garden, or source as a genetic material for breeding clubroot resistant Chinese cabbage cultivar for Canada.

- American turnip genotype 'Royal Gam' showed heterogeneity for resistance to all five *P. brassicae* pathotypes. Selfing of the resistant plants, challenged against the most virulent pathotype 3 could be expected to yield a homozygous clubroot resistant line for use in breeding and research.
- In contrast to the A-genome species, the C-genome species *B. oleracea* have rarely shown resistance to clubroot pathogen. Only two cabbage genotypes, 'Badger Shipper' and 'Bindsachsenn' showed some resistance to different pathotype. These genotypes can be used with resistant *B. rapa* genotypes to resynthesize clubroot resistant *B. napus*.
- Broad-spectrum resistance to wide range of *P. brassicae* pathotypes has been confirmed in the diploid B-genome species *B. nigra* for the first time in this study. Resistant genotypes of this species can be used as a valuable germplasm to resynthesize clubroot resistant *B. juncea* or *B. carinata* cultivars. These germplasm can also be used to introduce resistance into *B. napus*, if needed.
- *Brassica* species *e.g.*, turnip type *B. rapa*, rutabaga type *B. napus* and *B. nigra* evolved from their ancestors or wild forms at their centre of origin with constant interaction to the ever evolving *P. brassicae* pathotypes in that region were found to have resistance against *P. brassicae* pathotypes. This knowledge can be used to narrow down the search for the appropriate group of germplasm from Gene Bank collections to identify the natural source of resistance for a disease.

So far, five pathotypes of *P. brassicae* have been identified in Canada (Xue *et al.*, 2008), of which pathotypes 2, 3, 6 and 8 were reported in the canola fields

of Alberta (Strelkov *et al*., 2007; Xue *et al.*, 2008) and pathotype 5 in vegetable field of Ontario (Xue *et al*., 2008). By the end of 2008, clubroot disease has been reported in more than 400 canola fields in Alberta region (Cao *et al.*, 2009). Pathotypic classification of the *P. brassicae* populations from these fields is urgently needed to determine proper management strategy for the control of this disease. To my knowledge, there is no molecular technique available for classification of a wide variety of *P. brassicae* populations. Several techniques have been described by researches for physiological classification of this pathogen based on their reactions to different groups of *Brassica* genotypes, of which Williams (1966), Buczacki *et al.* (1975) and Somé *et al.* (1996) systems are widely used. However, all these systems require both resistant and susceptible reactions of the *P. brassicae* populations to a group of *Brassica* genotypes in different combinations along with complex mathematical calculations of the reactions. Moreover, heterozygosity present in the genotypes used in those systems creates intermediate reactions which cause ambiguity in accurate classification of the pathotype(s).

Meanwhile, several *Brassica* germplasms used in this study showed interesting pattern of reactions to different Canadian *P. brassicae* pathotypes, which can be used to develop a differential set to identify the physiological classes of *P. brassicae* populations from the Canadian prairies. I propose the differential set as outlined in Table 3.1, of which “one *Brassica* genotype can be used to identify a specific *P. brassicae* pathotype”. In this differential set, the *B. nigra* subsp. *nigra* var. *nigra* genotype ‘CR 2734’ can be used for identification of pathotype 2, as this genotype showed positive reaction only to pathotype 2. Similarly, *B. napus* subsp. *napobrassica* genotype ‘York’, *B. nigra* subsp. *nigra* var. *nigra* genotypes ‘CR 2704’ and ‘CR 2093’ can be used to identify *P. brassicae* pathotype 3, 5 and 6, respectively. On the other hand, *B. nigra* subsp. *nigra* var. *nigra* genotype, ‘CR 2115’ can be used to identify pathotype 8 as it is resistant to only this pathotype. Any field populations showing different reactions to these *Brassica* genotypes other than the pattern mentioned will indicate

occurrence of new pathotype in the region. The proposed genotypes 'York' and 'CR 2704' showed heterogeneity for resistance to pathotypes 2, 5 and pathotypes 3, 5 respectively. Selfing of these genotypes would be needed to increase homogeneity for resistance to the pathotypes.

Table 3.1 Reaction patterns of *Brassica* genotypes proposed to develop a new clubroot differential set for physiological classification of *P. brassicae* pathotypes in Canadian situation

Scientific name	Varietal name/ Accession no.	*P. brassicae* pathotypes					*P. brassicae* pathotype indicate
		2	3	5	6	8	
B. nigra subsp. *nigra* var. *nigra*	CR 2734	+	-	-	-	-	2
B. napus subsp. *napobrassica*	York	-	+	-	-	-	3
B. nigra subsp. *nigra* var. *nigra*	CR 2704	-	-	+	-	-	5
B. nigra subsp. *nigra* var. *nigra*	CR 2093	-	-	-	+	-	6
B. nigra subsp. *nigra* var. *nigra*	CR 2115	+	+	+	+	-	8

Note: '+' sign indicate compatible reaction (susceptibility) and '-' sign indicate incompatible reaction (resistance) of the genotype to the *P. brassicae* pathotype

The differential set outlined above is based on their reaction against single-spore derived isolates. However, multiple *P. brassicae* pathotypes have been reported to coexist under field conditions (Ayers, 1972; Jones *et al*., 1982; Xue *et al.*, 2008). Therefore, further experiment is needed to study their reaction pattern against field population of the pathogen. However, the genotypes characterized in the present research project will form the basis in the development of a new clubroot differential set under the Canadian situation.

3. 2 REFERENCES

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Appendix A

Table A-1: *Brassica rapa* germplasm evaluated for resistance to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Name of the species	Germplasm	Origin	Source of collection[1]
B. rapa var. *rapifera*	Debra	Europe	Green Gene International, UK
	Gelria	Europe	Green Gene International, UK
	ECD-3	Europe	Green Gene International, UK
	ECD-4	Europe	Green Gene International, UK
	Royal Gam	New York, USA	USDA-ARS
B. rapa var. *chinensis*	Granaat	China	Green Gene International, UK
	Qui Lu 75	China	Canola program, University of Alberta
	Mirako (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/ Dr. Howard
	Bilko (F_1)	Noresco, Quebec	AAFRD/ Dr. Howard
	Feng Quig Choi	Sakata, USA	AAFRD/ Dr. Howard
	Blues	Stokes, Japan	AAFRD/ Dr. Howard
	Kasumi	Stokes, The Netherlands	AAFRD/ Dr. Howard
	Nikko (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/ Dr. Howard
B. rapa var. *pekinensis*	Nai Bai Cai	China	Canola program, University of Alberta
	Feng Rang-70	China	Canola program, University of Alberta
	Qingmayc	China	Canola program, University of Alberta
	BARI Chinashak-1	Bangladesh	BARI, Bangladesh
	BARI Shak-1	Bangladesh	BARI, Bangladesh
B. rapa var. *oleifera* (Winter type)	Largo	Finland	Canola program, University of Alberta
	Prisma	Finland	Canola program, University of Alberta
	JSv01-11403	Finland	Canola program, University of Alberta
	JSv01-13102	Finland	Canola program, University of Alberta
	JSv01-11449	Finland	Canola program, University of Alberta
	JSv01-13051	Finland	Canola program, University of Alberta
	JSv01-13084	Finland	Canola program, University of Alberta
	JSv00-13426	Finland	Canola program, University of Alberta
	JSv00-15588	Finland	Canola program, University of Alberta
B. rapa var. *oleifera* (Spring type)	CR 25-7	Canada	Canola program, University of Alberta
	YS 49	Bangladesh	Canola program, University of Alberta
	TR 7/94-6792	Canada	Canola program, University of Alberta
	TR 4-3-3-1	Canada	Canola program, University of Alberta
	3.0026.027	Canada	Canola program, University of Alberta
	Parkland	Canada	Canola program, University of Alberta
	Horizon	Canada	Canola program, University of Alberta
	Tobin	Canada	Canola program, University of Alberta
	Tori-7	Bangladesh	BARI, Bangladesh

[1]Green Gene International, UK: Green Gene International, Hill Rising, Horse Castles Lane, Sherborn, United Kingdom.
USDA-ARS: United States Department of Agriculture, Agricultural Research Service, North Central Regional Plant Introduction Station, Iowa State University, Regional Plant Introduction Station, Ames, Iowa, USA, 50011-1170
AAFRD/ Dr. Howard: Alberta Agriculture, Food and Rural Development, Alberta, Canada/ Dr. Ronald J. Howard.
BARI, Bangladesh: Bangladesh Agricultural Research Institute, Gazipur, Bangladesh

Table A-2: *Brassica oleracea* germplasm evaluated for resistance to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Name of the species	Germplasm	Origin	Source of collection[1]
B. oleracea var. *capitata*	Badger Shipper	Europe	Green Gene International, UK
	Bindsachsenn	Europe	Green Gene International, UK
	Jersey Queen	Europe	Green Gene International, UK
	Septa	Europe	Green Gene International, UK
	Supreme Vantage	Sakata Seed, America Inc.	AAFRD/Dr. Howard
	SCB 7322	Sakata Seed, America Inc.	AAFRD/Dr. Howard
	Silver Dynasty	Asgrow Vegt. Seeds, USA	AAFRD/Dr. Howard
	Optiko	Seedway, The Netherlands	AAFRD/Dr. Howard
	Manoko	Seedway, The Netherlands	AAFRD/Dr. Howard
	Blue Thunder	Stokes, USA	AAFRD/Dr. Howard
	Joi Choi	Seedway, New York	AAFRD/Dr. Howard
	Loughton (F_1)	Seedway, The Netherlands	AAFRD/Dr. Howard
	Lennox (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Balaton (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Farao (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Head start	Stokes, Chile	AAFRD/Dr. Howard
B. oleracea var. *botrytis*	Dok Elgon	The Netherlands	USDA, ARS
	Nimba Meda	The Netherlands	USDA, ARS
	BARI Cauliflower-1	Bangladesh	BARI, Bangladesh
	BARI Cauliflower-2	Bangladesh	BARI, Bangladesh
	Symphony	Rogers	AAFRD/Dr. Howard
	Amazing	Seedway, The Netherlands	AAFRD/Dr. Howard
	Fremont	Seedway, Chile	AAFRD/Dr. Howard
	Cortes	Seedway, The Netherlands	AAFRD/Dr. Howard
	Shasta	Unknown	AAFRD/Dr. Howard
	Concept	Unknown	AAFRD/Dr. Howard
	Concert (F_1)	West Coast Seeds, Canada	AAFRD/Dr. Howard
	Arctic	Noresco, Laval, Quebec	AAFRD/Dr. Howard
	Tower (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Hateman	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard

Table A-2: (Continued)

Name of the species	Germplasm	Origin	Source of collection[1]
B. oleracea var. *italica*	Iron	Seminis, Oxnard, Canada	AAFRD/Dr. Howard
	Premium Crop (F_1)	Seedway, Hall, NY. USA	AAFRD/Dr. Howard
	Major (F_1)	Seedway, Hall, NY. USA	AAFRD/Dr. Howard
	Surveyor (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Captain	Seminis, Oxnard, California, USA	AAFRD/Dr. Howard
	Coroado	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Acadia	Seedway, Hall, NY. USA	AAFRD/Dr. Howard
	Windsor	Rogers Seed Co., USA	AAFRD/Dr. Howard
	SBC 93 11	Sakata Seed, America Inc.	AAFRD/Dr. Howard
	Sessantina Grossa	Johnny's Selected Seeds, USA	AAFRD/Dr. Howard
	Lucky	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Belstar (F_1)	Bejo Zaden BV, The Netherlands	AAFRD/Dr. Howard
	Alborado (F_1)	Seedway, Hall, NY. USA	AAFRD/Dr. Howard
B. oleracea var. *gemmifera*	Hybrid Spouket	Noresco, Laval, Quebec	AAFRD/Dr. Howard
	Oliver	Stokes, Buffalo, USA	AAFRD/Dr. Howard
	Diablo (F_1)	Seedway, Hall, NY. USA	AAFRD/Dr. Howard
B. oleracea var. *alboglabra*	CD-2	Canada	Canola program, University of Alberta
B. oleracea var. *villosa*	BRA 1896	Italy	IPK, Germany

[1]Green Gene International, UK: Green Gene International, Hill Rising, Horse Castles Lane, Sherborn, United Kingdom.
USDA-ARS: United States Department of Agriculture, Agricultural Research Service, North Central Regional Plant Introduction Station, Iowa State University, Regional Plant Introduction Station, Ames, Iowa, USA, 50011-1170
AAFRD/ Dr. Howard: Alberta Agriculture, Food and Rural Development, Alberta, Canada/ Dr. Ronald J. Howard.
IPK, Germany: Leibniz Institute of Plant Genetics and Crop Plant Research, Department of Gene Bank, Gatersleben, Germany

Table A-3: *Brassica nigra* germplasm evaluated for resistance to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Name of the species	Germplasm	Origin	Source of collection[1]
B. nigra subsp. *nigra* var. *nigra*	CR 1198 (Alsaska)	Unknown	IPK, Germany
	CR 1199	Bulgaria	IPK, Germany
	CR 1201 (Hneda Z Danska Hneda)	Unknown	IPK, Germany
	CR 1204 (Primus)	Unknown	IPK, Germany
	CR 1206 (Sizaja)	USSR	IPK, Germany
	CR 1214	Unknown	IPK, Germany
	CR 132 (Lu De No. 2)	Unknown	IPK, Germany
	CR 2093	Greece	IPK, Germany
	CR 75 (Alsaska)	India	IPK, Germany
	CR 2115	Italy	IPK, Germany
	CR 2127	Unknown	IPK, Germany
	CR 2130	Unknown	IPK, Germany
	CR 2142	Unknown	IPK, Germany
	CR 2143	Unknown	IPK, Germany
	CR 2144	Unknown	IPK, Germany
	CR 2696 (Black Mustard)	Unknown	IPK, Germany
	CR 2697	Unknown	IPK, Germany
	CR 2704 (Balkan)	Unknown	IPK, Germany
	CR 2706	Unknown	IPK, Germany
	CR 340	Unknown	IPK, Germany
	CR 91 (Gibera)	Germany	IPK, Germany
	CR 2707 (Alaska)	Unknown	IPK, Germany
	CR 2709	Unknown	IPK, Germany
	CR 2713 (Junius)	Germany	IPK, Germany
	CR 2714	Unknown	IPK, Germany
	CR 2715	Unknown	IPK, Germany
	CR 2716	Unknown	IPK, Germany
	CR 2717	Italy	IPK, Germany
	CR 2718	Unknown	IPK, Germany
	CR 2719	Unknown	IPK, Germany
	CR 77 (Balkan)	Czechoslovakia	IPK, Germany
	CR 2721	Unknown	IPK, Germany
	CR 2724	Unknown	IPK, Germany
	CR 2727	Italy	IPK, Germany
	CR 2734	The Netherlands	IPK, Germany
	CR 2735	Greece	IPK, Germany
	CR 2746	Ethiopia	IPK, Germany
	CR 2755	India	IPK, Germany
B. nigra subsp. *nigra* var. *pseudocampestris*	CR 2141	Unknown	IPK, Germany
	CR 1210	Unknown	IPK, Germany

Table A-3: (Continued)

Name of the species	Germplasm	Origin	Source of collection[1]
B. nigra subsp. *hispida* var. *rigida*	CR 2138	Germany	IPK, Germany
	CR 2129	Unknown	IPK, Germany
	CR 2726 (Sv76-39011)	Unknown	IPK, Germany
	CR 2762	Denmark	IPK, Germany
	CR 2120	Unknown	IPK, Germany
B. nigra subsp. *hispida* var. *orientales*	CR 2128	Unknown	IPK, Germany
Brassica nigra (Taxonomic information at the sub-species level was unavailable)	CR 1216	Poland	IPK, Germany
	CR 2094	Greece	IPK, Germany
	CR 2095	Greece	IPK, Germany
	CR 2096	Greece	IPK, Germany
	CR 2097	Unknown	IPK, Germany
	CR 2098	Italy	IPK, Germany
	CR 2101	Greece	IPK, Germany
	CR 2102	Greece	IPK, Germany
	CR 2104	Greece	IPK, Germany
	CR 2105	Greece	IPK, Germany
	CR 2106	Greece	IPK, Germany
	CR 2107	Greece	IPK, Germany
	CR 2109	Greece	IPK, Germany
	CR 2110	Greece	IPK, Germany
	CR 2112	Greece	IPK, Germany
	CR 2121	Unknown	IPK, Germany
	CR 2122	Unknown	IPK, Germany
	CR 2123	Unknown	IPK, Germany
	CR 2124	France	IPK, Germany
	CR 2126	Unknown	IPK, Germany
	CR 2135	Italy	IPK, Germany
	CR 2136	Unknown	IPK, Germany
	CR 2137	Unknown	IPK, Germany
	CR 2698 (Pavlikenski)	Unknown	IPK, Germany
	CR 2703	Germany	IPK, Germany
	CR 2722 (Gibera)	Unknown	IPK, Germany
	CR 2723	Unknown	IPK, Germany
	CR 2725	Unknown	IPK, Germany
	CR 2748	Unknown	IPK, Germany
	CR 3279	Unknown	IPK, Germany
	CR 2731	Unknown	IPK, Germany

[1]IPK, Germany: Leibniz Institute of Plant Genetics and Crop Plant Research, Department of Gene Bank, Gatersleben, Germany

Table A-4: *Brassica napus* germplasm evaluated for resistance to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Name of the species	Germplasm	Origin	Source of collection[1]
B. napus subsp. *napus* var. *napus* (fodder type)	Fodder Nevin	Europe	Green Gene International, UK
B. napus subsp. *napus* var. *napus* (oilseed type)	Legend	Sweden	USDA-ARS
	Global	Sweden	USDA-ARS
	Capricorn	England	USDA-ARS
	Winfield	Canada	USDA-ARS
	Lindora-00	Germany	USDA-ARS
	Giant rape commercial	Europe	Green Gene International, UK
	Giant rape selection	Europe	Green Gene International, UK
	New Zealand resistant rape	New Zealand	Green Gene International, UK
	Bina Sorisha-3	Bangladesh	BINA, Bangladesh
	Bina Sorisha-4	Bangladesh	BINA, Bangladesh
	Bina Sorisha-5	Bangladesh	BINA, Bangladesh
	BRA 1276	Sweden	IPK, Germany
	Ww 1273 (CR 1099)	Sweden	IPK, Germany
	Ww 1286 (CR 1100)	Sweden	IPK, Germany
	Ww 1289 (CR 1101)	Sweden	IPK, Germany
	CR 1886	USSR	IPK, Germany
	Kajsa (CR 2012)	Sweden	IPK, Germany
	Korall (CR 2013)	Sweden	IPK, Germany
	Granit (CR 2014)	Sweden	IPK, Germany
	Puma (CR 2015)	Sweden	IPK, Germany
	Conny (CR 235)	Sweden	IPK, Germany
	CR 3021	Sweden	IPK, Germany
	CR 3086	Sweden	IPK, Germany
	CR 3090	Sweden	IPK, Germany
	CR 3100	Sweden	IPK, Germany
	CR 3189	Romania	IPK, Germany
	Olivia (CR 3301)	Sweden	IPK, Germany
	CR 634	Sweden	IPK, Germany
	Hanna (CR 639)	Sweden	IPK, Germany
	Olga (CR 812)	Sweden	IPK, Germany
	Omega (CR 814)	Sweden	IPK, Germany
	Regina (CR 882)	Sweden	IPK, Germany
	Sv 716 (CR 994)	Sweden	IPK, Germany
	CR 999	Sweden	IPK, Germany
	K 7967	Sweden	IPK, Germany
	Apollo	Canada	University of Manitoba
	Altex	Canada	Canola program, University of Alberta
	AWP-F 647	Canada	Canola program, University of Alberta

Table A-4: (Continued)

Name of the species	Germplasm	Origin	Source of collection[1]
B. napus var. *rapifera*	Wilhelmsburger	Europe	Green Gene International, UK
	BrookField-9005	Newfoundland, Canada	Dr. Dean Spaner, University of Alberta
	PolyCross-9006	Newfoundland, Canada	Dr. Dean Spaner, University of Alberta
	York	Veseys, Canada	AAFRD/ Dr. Howard
	Unknown	Seedway, Hall, NY. USA	AAFRD/ Dr. Howard

[1]Green Gene International, UK: Green Gene International, Hill Rising, Horse Castles Lane, Sherborn, United Kingdom.

AAFRD/ Dr. Howard: Alberta Agriculture, Food and Rural Development, Alberta, Canada/ Dr. Ronald J. Howard.

IPK, Germany: Leibniz Institute of Plant Genetics and Crop Plant Research, Department of Gene Bank, Gatersleben, Germany

Dr. Dean Spaner, Univ. of Alberta: Dr. Dean Spaner, Professor, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.

USDA-ARS: United States Department of Agriculture, Agricultural Research Service, North Central Regional Plant Introduction Station, Iowa State University, Regional Plant Introduction Station, Ames, Iowa, USA, 50011-1170.

BINA, Bangladesh: Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh.

Table A-5: *Brassica juncea* germplasm evaluated for resistance to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Name of the species	Germplasm	Origin	Source of collection[1]
Brassica juncea (Information at sub-species level was not available)	BARI Sorisha -6	Bangladesh	BARI, Bangladesh
	BARI Sorisha -7	Bangladesh	BARI, Bangladesh
	BARI Sorisha -8	Bangladesh	BARI, Bangladesh
	BARI Sorisha -9	Bangladesh	BARI, Bangladesh
	BARI Sorisha -10	Bangladesh	BARI, Bangladesh
	BARI Sorisha -11	Bangladesh	BARI, Bangladesh
	BARI Sorisha -12	Bangladesh	BARI, Bangladesh
	BARI Sorisha -13	Bangladesh	BARI, Bangladesh
	BARI Sorisha -14	Bangladesh	BARI, Bangladesh
	BARI Sorisha -11	Bangladesh	BARI, Bangladesh
	Daulat	Bangladesh	BARI, Bangladesh
	SS-75	Bangladesh	BARI, Bangladesh
	Cutlass	Unknown	Canola program, University of Alberta
	CD: 4-1	Unknown	Canola program, University of Alberta
	Yoi Li Ku	China	Canola program, University of Alberta
	Long Jiano	China	Canola program, University of Alberta
	Brown mustard	Unknown	Canola program, University of Alberta
	Oriental mustard	Unknown	Canola program, University of Alberta
	Ornamental rai	Unknown	Canola program, University of Alberta
	321083	Unknown	Canola program, University of Alberta
	Ma Wei	Unknown	Canola program, University of Alberta
	Zhou Yu 801	China	Canola program, University of Alberta
	Sambal	Bangladesh	BAU, Bangladesh
	Daulat	Bangladesh	BAU, Bangladesh
	Rai-5	Bangladesh	BAU, Bangladesh

Table A-5: (Continued).

Name of the species	Germplasm	Origin	Source of collection[1]
B. juncea subsp. *integrifolia*	Blaze	Canada	IPK, Germany
	Kobu Takana	Japan	IPK, Germany
	Ib 1692	India	IPK, Germany
	Secus	The Netherlands	IPK, Germany
	Domo	Canada	IPK, Germany
	Ib 1434	India	IPK, Germany
	CR 481	Romania	IPK, Germany
	Hei-ye-mi-tou-gai	Japan	IPK, Germany
	CR 2611	Denmark	IPK, Germany
	Budakalaszi Fekete	Hungary	IPK, Germany
	Murasaki Takana	Japan	IPK, Germany
	CR 2485	Italy	IPK, Germany
	CR 2492	Poland	IPK, Germany
	Yamashiona	Japan	IPK, Germany
	Ha Karashina	Japan	IPK, Germany
	Ib 1632	India	IPK, Germany
	Gielva	The Netherlands	IPK, Germany
	CR 104	India	IPK, Germany
	CR 137	China	IPK, Germany
	Ha Karashina	Japan	IPK, Germany
	Karashi Hakusai	Japan	IPK, Germany
	CR 342	The Netherlands	IPK, Germany
	CR 341	The Netherlands	IPK, Germany
	Einjahrig	Unknown	IPK, Germany

[1]BARI, Bangladesh: Oil Crop Division, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh.
BAU, Bangladesh: Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh.
IPK, Germany: Leibniz Institute of Plant Genetics and Crop Plant Research, Department of Gene Bank, Gatersleben, Germany

Table A-6: *Brassica carinata* germplasm evaluated for resistance to clubroot disease caused by Canadian *Plasmodiophora brassicae* pathotypes

Name of the species	Germplasm	Origin	Source of collection[1]
Brassica carinata A. Braun	Parental line	Unknown	Canola program, University of Alberta
	391078	Unknown	Canola program, University of Alberta
	3-1	Unknown	Canola program, University of Alberta
	3-2	Unknown	Canola program, University of Alberta
	Unknown	Bangladesh	BINA, Bangladesh
	YS	Bangladesh	BINA, Bangladesh
	17	Bangladesh	BINA, Bangladesh
	36	Bangladesh	BINA, Bangladesh
	BRA 2569	Ethiopia	IPK, Germany
	BRA 2452	Ethiopia	IPK, Germany
	BRA 1028	Ethiopia	IPK, Germany
	BRA 2481	Ethiopia	IPK, Germany
	BRA 2125	Ethiopia	IPK, Germany
	BRA 2109	Ethiopia	IPK, Germany
	BRA 2137	Ethiopia	IPK, Germany
	BRA 2108	Ethiopia	IPK, Germany
	BRA 2127	Ethiopia	IPK, Germany
	BRA 2483	Ethiopia	IPK, Germany
	BRA 1030	Zambia	IPK, Germany
	BRA 1029	Zambia	IPK, Germany
	BRA 2607 (M 12)	Ethiopia	IPK, Germany
	BRA 2543	Ethiopia	IPK, Germany
	BRA 2237	Ethiopia	IPK, Germany
	BRA 2427	Ethiopia	IPK, Germany
	BRA 1043	Sweden	IPK, Germany

[1]IPK, Germany: Leibniz Institute of Plant Genetics and Crop Plant Research, Department of Gene Bank, Gatersleben, Germany

BINA, Bangladesh: Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh.