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Review Paper

Clubroot on Oilseed Rape / Canola : A Review

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Abstract

Plasmodiophora brassicae causes the disease clubroot in cruciferous plants, and has been a threat to brassica crops production all over the world since its identification. Clubroot has become a major economically important disease of oilseed rape (OSR) in many countries, especially in areas where cool, cloudy, and humid weather persists. Further, the importance of the disease can be determined by its spread in about 88 countries globally on >3700 species of plants, with > 60 vernacular names accountable for 10-100 per cent losses to brassica vegetables, and oil yielding crops. The pathogen *P. brassicae* survives in soil as the long time survival structures commonly known as resting spores, and are viable up to 17 years. The resting spores of *P. brassicae* are highly robust and are well protected by five spore walls. These spore walls are composed of fungal chitin, and carbohydrates that makes the spores resilient to degradation by extra-cellular enzymes produced by predatory soil organisms. Root galls are the characteristics symptoms of the disease clubroot, which function as strong metabolic sinks in infected plants by drawing nutrients, and other resources away from the shoots, and leaves contributing to above ground symptoms development. There have been extensive investigations on pathological, physiological, and molecular characteristics of *P. brassicae* causing clubroot on OSR/ canola and over 300 brassica hosts that gives the researchers a scope to work on this pathogen and its management across the world. In management of the disease clubroot lack of chemical control option coupled with the need for longer crop rotations forced the deployment of host plant resistance as a major option for clubroot management. Effective and repeatable controlled-environment and field-screening techniques have been developed for identification of host plant resistance. *P. brassicae* genome is small in size (24.2-25.5 Mb) with high gene density. The pathogen contains genes potentially involved in host hormone metabolism and metabolic pathways. Mapping of quantitative and qualitative traits loci (QTL's) have revealed several (12) dominant loci in *B. rapa* over 22 QTL's in *B. oleracea* and more than 19 QTL's in *B. napus* for clubroot resistance to different pathotypes of *P. brassicae*. Eventually several genes governing quantitative and qualitative resistance to *P. brassicae* have been identified and characterized in different *Brassica* species. However, some of genes have been demonstrated to be related to the pathogenicity of *P. brassicae*. Extensive use of the resistant cultivars in shorter crop rotations has resulted in emergence of the pathotypes in OSR/canola. The pathotypes of *P. brassicae* display great variations with tendencies for overcoming resistance sources from *Brassica* species in a short period of time. Soil amendments with different types of lime based on pH of the soil were helpful in small scale applications or in patches. However, the interactions of physical, chemical, and biological, properties between *P. brassicae*, and *Brassica* hosts and non-hosts in the soil environment are very complex, and pose challenges to determine conduciveness and suppressiveness of the disease or the pathogen. Integrated disease management (IDM) of clubroot has proved more effective than any of the individual disease management components in large-scale and in on-farm studies conducted in Canada and in the USA. Further information on the biology of *P. brassicae* and epidemiology of the disease is needed to strengthen the IDM programs. Current manuscript summarizes on recent studies into the; geographic distribution, morphology, molecular biology, taxonomy, life cycle, symptoms, ecology, host range, genetic basis of resistance, pathotypes, soil pH, quantification of resting spores from soil and available management practices of clubroot on OSR/canola have been reviewed with a mention of future research priorities.

Keywords: *Brassica hosts, canola, Clubroot, oilseed rape, Plamodiophora brassicae*

Introduction

Clubroot disease caused by *Plasmodiophora brassicae* Woronin offers abundant challenges to agricultural and biological scientists. This pathogen acclimatizes faster to the environment which it inhabits. The pathogen *P. brassicae* survives in soil as microscopic well protected resting spores, and then grows actively, and reproduces while shielded inside the roots of host plants under favorable conditions. The pathogen is active outside the host for only short periods in the form of zoospore in thin film of water. Complete symptom expression happens solely in members of the Brassicaceae family. The characteristic and distinguishing symptoms of clubroot are the galls or clubs that are visible only after uprooting the infected plants. The complex nature of the pathogen consequently, leads to scientific studies much challenging in terms of biological context of the host, and the pathogen. Likewise, the technology required to investigate and understand that relationship. Management of clubroot disease is a challenge for growers, crop consultants, commodity groups and plant pathology practitioners because of the inadequate alternatives for its management approaches. Over the decades quite a success has been achieved in production of genetically resistant cultivars to clubroot in various brassica host crops and the search for higher levels of disease resistance is continuous. Currently, only a few genes expressing strong resistance to *P. brassicae* are known, and readily available. Agrochemical control is similarly limited by difficulties in molecule formulation which combines efficacy with environmental acceptability. Manipulation of soil husbandry encouraging practices such as soil structure, texture, nutrient composition, and moisture content can reduce populations of *P. brassicae*. Integrating such strategies with rotation, sanitation and genetic resistance will reduce but not eliminate this disease. There are indications that forms of biological competition may be mobilized as additions to integrated control strategies.

Taxonomy of *Plasmodiophora brassicae*

Plasmodiophora brassicae Woronin is an obligate parasite, can survive in soil over 17 years in soil as resting spores without the presence of the host (Wallenhammar, 1996). The taxonomical classification of *P. brassicae* has been through several changes over the years because of its complex nature of evolution. Initially it was considered in the phylum, Plasmodiophoromycota, which largely consists a monophyletic group that has members of the Fungi (Waterhouse, 1972), Protozoa (Cavalier-Smith and Chao, 2003) or Protoctista (Margulis *et al*., 1989). However, the database in the Index Fungorum has classified the organism *Plasmodiophora* in the Kingdom *Protozoa*, phylum *Cercozoa*, class *Phytomyxea*, order *Plasmodiophora* and family *Plasmodiophoridae* (Cavalier-Smith and Chao, 2003; Ricarova *et al*., 2016). The commonly and well-known recognized plant pathogens of this group are *P. brassicae*, the causal agent of clubroot of brassica crops (Hwang *et al*., 2011a), *Spongospora subterranea* (Wallroth) Lagerheim *f. sp. subterranea* Tomlinson, the causal organism of powdery scab of potato (Kole, 1954) and crook root of watercress (*Nasturtium officinale*, R.Br. and *N. officinale* × *N. microphyllum*, Boenn. ex Rchb.) caused by *S. subterranea* (Wallroth) *Lagerheim* f. sp. *nasturtii* Tomlinson (Tomlinson, 1958). The two genus of this group *S. subterranea*, *Polymyxa betae* Keskin, and *P. graminis* Ledingham, commonly serve as vectors for plant pathogenic viruses (Sarwar et al. 2020).

Morphology of *Plasmodiophora* in the host

The detailed informations of morphological structures of plasmodium, zoospores, and resting spores from early stages of infection to maturity have been observed in the diseased root cells through scanning electron microscopy. On receiving chemical signals from root exudates of brassica crops the resting spores of *P. brassicae* germinate and produce flagellated zoospores. These zoospores swim towards the roots of the host plant and infect roots and root hairs of the brassica hosts. In the initial stage of infection, *P. brassicae* in an infected cell appear as spherical bodies of 3-5µm in diameter. These young plasmodia appears in different shapes when advance microscopic equipment used, mostly spherical with or without strand-like material, with smooth or rough surfaces, and cluster-like bodies. The strand-like material connecting the young plasmodia appears to be the common shape (Dekhuijzen, 1976) as observed with transmission electron micrographs. Diversity in morphology of young plasmodia is presumably depends on the nutritional status of the host cell, and the portion of root from which the cell is sampled. Thus, stressing the significance of detailed studies of the initial phase of infection by using scanning, and transmission electron microscopy. Young plasmodial bodies often coalesce to a large multinucleate plasmodium may perhaps be observed due to the fusions among them. Studies reported that the vegetative plasmodia grew progressively in an infected cell resulting in the formation of a large fungal mass in the cell. Once penetration of root tissue has occurred, *P. brassicae* induces a suite of changes in its host in primary, and secondary metabolism of cell which eventually result in gross physical distortion of the infected root.

Diverse growth rates of plasmodia were observed in the infected cells that were split out of tissues of galls/clubs from 45- to 60 days old plants. A young plasmodium, a vegetative plasmodium, or a resting spore mass were observed in the same section when most of the studied cells when excited from the infected old plants. Plasmodia are assumed to be unable to mature into resting spores with in the infected host cells could be due to lack of an enveloping membrane around the cell. However, the plasmodial membrane is presumably disintegrated over the time in the process of the resting spores maturing from vegetative plasmodium. In contrast, Williams *et al*. (1967) described that the outer membrane of the plasmodial envelope disintegrates on the initiation of sporogenesis. A great number of resting spores are easily released into the soil by rotten clubbed roots, these serve as a formidable inoculum of future clubroot disease on brassica hosts. The number of resting spores quantified were 4 x 10^8 in average per g of fresh club of both turnip, and Chinese cabbage (Ikegami *et al*., 1978).

The pathogen *P. brassicae* to complete its life cycle produces following morphological structures for its survival, infection, development, and multiplication in the infected host tissues of brassicas/ crucifers.

a. Resting spores: The resting spore of *P. brassicae* average about 3 µm in size, and sub-spherical to spherical in shape (Buczacki and Cadd, 1976). The outer wall of each resting spore mainly covered with spines (Williams and McNabola, 1967; Ikegami *et al*., 1978). Young resting spores are usually connected by fine filamentous material, and mature resting spores have spines (Ikegami *et al*., 1978).

b. Zoospores: Zoospores of *P. brassicae* are spindle or pyriform in shape, 2.8- 5.9 µm long, and biflagellate, a shorter flagellum with a blunt end, and a longer flagellum with a whiplash or tail piece (Ayers, 1944). In contrast, McDonald *et al*. (2014) reported that the zoospores are 9.6 to 14.4 µm in size, and uninucleate. In general, visually the primary, and secondary zoospores cannot be differentiated; as both are ovate, with two whiplash flagella of unequal length.

c. Plasmodium: Young plasmodium remains spherical in shape with smooth surface of around 3.7 µm in diameter, with a range of 2.7 to 5 µm in size. These strands connect the nearby spherical bodies. These connected plasmodia increases in size with a range of 3.3-4.5 µm and averages 4.2 µm in diameter. Each plasmodium individually enveloped by a membranous material with a smooth surface. However, young round plasmodia with partially rough surface with average diameter of 8 µm were found in the host tissues. Vegetative plasmodia existed in clusters and add considerable large mass in infected cells with a size of about 45 µm in diameter. The vegetative plasmodia fill most of the host cells volume and provided the appearance of sponge-like structure to the root gall (Ikegami *et al*., 1978).

Molecular biology of *Plasmodiophora brassicae:* The genus *Plasmodiophora* descended from a common evolutionary ancestor, hence considered in a monophyletic group with uncertain systematic similarities. The species belonging to *Plasmodiophora* genus are characterized by features of cruciform nuclear division, parasitism, obligate nature, biflagella, heterocont zoospores, and environmentally resistance, and long-living resting spores (Braselton, 1995). The disease clubroot caused by *P. brassicae* is considered as the most economically significant in terms of yield losses in *Brassica* species and has shown a wide biological range, usually its populations consist of a mixture of different pathotypes. The biological, physical, and chemical properties of soil influence the survival of some physiological races of the pathogen differentially. Isolates obtained from field collected root galls caused by *P. brassicae* display great variation and have the capability to overcome different resistance sources from Brassica species (Dixon, 2009). It is widely accepted that life cycle of *P. brassicae* consists of two infectious phases (Karling, 1968; Ingram and Tommerup, 1972). Inside the host cell cytoplasm of the *P. brassicae* amoeba under goes endomitotic division forming a multinucleate primary plasmodium. The primary plasmodium cleaves into sporoangiosori which give rise to the secondary oospores. Secondary zoospores fuse, and form binucleate zoospores. Meiotic division in the multinucleate secondary plasmodium takes place just after karyogamy and before sporangiogenesis (Braselton, 1981).

Over the years thorough understanding of the pathogenicity factors of *P. brassicae* causing clubroot disease on different *Brassica* hosts has been possible through use of several advance molecular techniques and other tools that have been employed to determine *P. brassicae* genome size, structure, and number of possible functional genes in the whole genome. The use of pulse-field gel electrophoresis (PFGE) in several studies have enabled to study that the karyotyping in *P. brassicae*. Sheroplast studies differentiated 13 chromosomal bands in the range of 1.9 Mb to 750 kb (Ito *et al*., 1994). Molecular studies on isolated plasmodia differentiated six chromosomal bands in the range of 1.7 Mb to 680 kb (Bryan *et al*., 1996). Likewise, Graf *et al*. (2001) distinguished 16 chromosomal bands in the range of 2.2

Mb to 680 kb. These studies have estimated that the *P. brassicae* total genome size can be approximately 18–20.3 Mb (Graf *et al*., 2004). Several molecular markers were employed to determine the virulence pattern of the *P. brassicae* population derived from single-spore isolate and from field isolates. However, the numbers of determined distinguishing pattern was very low, and were not correlated to the virulence patterns of *P. brassicae*. Further efforts have led to identification of two Random Amplified Polymorphic (RAPD) markers, and one Sequence Characterized Amplified Region (SCAR) marker, and were correlating to isolates of pathotype 1 (Some *et al*., 1996). Availability of molecular marker sets that can distinguish other pathotypes from field isolates and can help clubroot resistance brassica breeding has not been reported for a long time (Buhariwalla *et al*., 1995; Moller and Harling, 1996; Manzanares-Dauleux *et al*., 2000, 2001).

The *Plasmodiophora brassicae* genome: *P. brassicae* genomes of pathotypes 2,3,5,6 and 8 are available with public domain in GenBank (Rolfe *et al*., 2016) from Canada, also one reference genome from a European isolate, and of one Chinese isolates. Often to compare these pathotype classifications of these isolates pose difficulties in comparison as they have been characterized using different differential hosts sets. These studies have described the main characteristics of the *P. brassicae* genome as of having small size (24.2-25.5 Mb) with high gene density. Thirteen chitin synthases were found to be playing an important role in this gene family in resting spore formation (Bi *et al*., 2019; Bi *et al*., 2016; Rolfe *et al*., 2016; Schwelm *et al*., 2015). Further studies on secreted proteins described to play a significant role in plant pathogenic organisms in suppressing the plant defense responses, and modifying the host metabolism. *P. brassicae* genome studies also revealed that it contains genes potentially to be involved in host hormone regulation; such as the auxin-responsive Gretchen Hagen 3, isopentenyl-transferases, methyltransferase, and cytokinin oxidase. The clubroot genome found to be lacking several metabolic pathways, as observed as the prominent characteristic feature of the eukaryotic biotrophic plant pathogens. The information of missing genes encoding proteins involved in sulfur, and nitrogen uptake, as well as the arginine, lysine, thiamine, and fatty acid biosynthesis pathways was found. Further, only a few carbohydrate active enzymes (CAZymes), involved in the synthesis, metabolism, and transport of carbohydrates were observed (Spanu *et al*., 2010). Schwelm *et al*. (2016) summarized the current life stage-specific transcriptomics data on correlating to the molecular events in the life cycle of *P. brassicae* revealed that during spore germination, and at the primary zoospore stage, the chitinous cell wall digestive enzymes were highly upregulated. Numerous metabolic pathways such as the citrate cycle, starch, pentose phosphate pathway, as well as pyruvate, and trehalose metabolism were found to be very active. Despite none of the molecular techniques could demonstrate the mechanisms involved in primary root hair infection. However, the secondary phase has been better described where the genes involved in basal, and lipid metabolism were highly expressed during re-infection by the secondary zoospores in root hairs or the root cortex. Galls on the root are the characteristic clubroot symptoms on brassica host are the result of hyperplasia and hypertrophy of the infected tissues and were found as the result of the deregulation of the host plant primary, and secondary metabolism genes as well as due to the modification of plant hormone homeostasis by the pathogen *P. brassicae*. During the process of infection the main plant hormone pathways that are modified were cytokinin biosynthesis, auxin homeostasis, and salicylic acid and jasmonic acid metabolism. However, the mechanisms of the pathogen *P. brassicae* that influence changes in plant hormone homeostasis are still not completely studied. The end of the *P. brassicae* life cycle nears on secondary plasmodia transforming into resting spores. In these structures presumably trehalose could play an important role for their long-term survival capacity. Description of the recent transcriptomics data indicates that some crucial steps of *P. brassicae* developmental stages that are still not well-characterized at the molecular level.

A de *novo*-sequence from a second European isolate of *P. brassica* was used to generate further structural and functional knowledge of its genome (Daval *et al*., 2018). These findings revealed that the genome sequence of the European *P. brassicae* isolate belonging to a eH and is widespread in France but different to e3, the reference clubroot isolate. The mitochondrial genome of the clubroot pathogen was further described with an aim to use these improved genome annotation findings to predict new metabolic pathways and to develop Clubroot Cyc, a metabolic pathway database for *P. brassicae*. This methodology as a tool helps researchers in getting access to a repertoire of *P. brassicae* metabolic, and transport pathways as well as to an Omics viewer in analyzing expression data, in turn can help to be updated with latest data. The genome of eH, of the second European isolate has been sequenced. The data of these sequences of the eH genome led to the identification of the mitochondrial genome sequence, which was found to be larger in size than that of *S. subterranea*, the phylogenetically close relative plant parasitic Plasmodiophorid to *P. brassicae*. Novel pathways of synthesis of spermidine, a polyamine

up-regulated in clubbed regions of roots were also predicted. A very informative *P. brassicae* pathway genome database was created to facilitate the functional study of metabolic pathways in transcriptomics approaches. With the available arsenals and their use can help in understanding the diverse constraints of *P. brassicae* regulation and metabolism during infection (Daval *et al*., 2018).

Geographical distribution

Clubroot spread and widely distributed way earlier before the time of Brassica oil seed introduction in many countries around the world (Olsson, 1939; Wallenhammer *et al.*, 2014). Currently, clubroot is reported to be distributed in over 88 countries around the world. For instance, in the early 1980s, heavy infections were reported in oilseed rape from the central western and central eastern parts of Sweden (Hedene, 1986). In Canada the first report of clubroot was made when 12 clubroot-infested commercial fields (< 800 ha) were found infected in the central part of the Province of Alberta on an important oilseed rape (OSR) predominantly called as canola (*B. napus* L.) in 2003 (Tewari *et al*., 2005). The first report of clubroot on the canola in the United States was made in 2013 (Chittem *et al*., 2014). However, historical reports of clubroot are date back to the 13th century in Europe. Clubroot was first reported in the United States of America in 1852 on vegetables and believed to be found in China and Japan about 100 years ago prior to that report. Likewise, reports indicate that the clubroot pathogen *P. brassicae* was first detected in Europe in the 1870s, however, was assumed to be present there for centuries. Woronin, a Russian scientist reported the cause of club root as a "plasmodiophorous", a living organism in 1875, and named as *Plasmodiophora brassicae* for the first time. The disease has been reported in all brassica crops around the world, and getting worse due to tighter rotations and favorable (moist) conditions for higher yield. In Canada and in the United States, the impact of clubroot historically has been greatest on cruciferous vegetables, with the most significant outbreaks of the disease restricted to vegetable production regions of the countries (Howard *et al*., 2010; Chapara *et al*., 2019). High yielding cultivars, in combination with an attractive price, made OSR/canola, one of the most profitable crops in the world. Oilseed rape is now superior as a cash crop to the traditional wheat in the Canada, Europe, Australia, South America and in the State of North Dakota in the United States. Within the last couple of decades, clubroot has emerged as a paralyzed disease of OSR/canola with 30-100% of yield losses recorded in Alberta, Canada (Hwang *et al*., 2011b; Holtz *et al*., 2021) and overwhelming reports of new fields with clubroot throughout Canada. Recent reports of clubroot were documented in the internal clubroot works by indicating an increase in clubroot infected fields in the countries such as the United States, Canada, Germany, Poland, France, Japan, South Korea, China, Columbia, India, Nepal, and Czech Republic in the International Clubroot Workshop (Anonymous, 2018).

Life cycle, symptoms and ecology

The complex life cycle of Plasmodiophorids includes along with its long term survival structures, the resting spores, followed by different zoosporic stages, formation of plasmodia inside host cells, and completes with resting spore formation and releasing it to the soil. The obligate parasite *P. brassicae* survives in the soil as dormant cysts commonly referred as resting spore. Resting spores can survive in soil for several years in the absence of a brassica host, and will germinate in response to the presence of brassica root exudates. The germinating resting spores releases primary zoospores that infect host root hairs by encysting on the root surface. Later, makes its way in through developing epidermal cells after forming in to amoeba like cell. In brassica hosts infection occurs through root hairs and rhizodermal cells and the pathogen subsequently induces local hypertrophy, resulting in gall formation. The infected roots become friable, decompose and release the resting spores in large quantities into the soil, where they can survive up to 17 years (Wallenhammar, 1996). Older roots with wounds get infected that acts as an entry way to the pathogen. Amoeboid cells of the pathogen cluster to form a multinucleate plasmodium in the root hairs. This plasmodium undergoes division and forms multiple secondary zoospores that are released into the soil on maturity. After maturity, these secondary zoospores infect the healthy root hairs of the brassicas and nearby host plants by transforming into amoeboid cells that migrate into the cortical cells of the host. Once in the cortex, the amoeboid cells multiply and coalesce with other amoeboid cells to form a plasmodium. The developing plasmodium releases plant hormones (Indole Acetic Acid) that triggers the host cells to multiply and enlarge nearly 20 times of its normal size. During the growth the plasmodium divides and infects neighboring cells causing them to swell. These swollen cells in the roots appear as the clubs or galls, hence the name clubroot (Fig.1). These galls are diagnostic of *P. brassicae* infections. Several groups of pathogens that infect roots produce similar galling, such as root knot nematode, gall inducing fungi and bacteria and herbicide injury from a specific group. With the application of proper staining techniques the presence of *P. brassicae* structures in the form of

Figure 1: Characteristic galls found on clubroot infected roots of oil seed rape/canola

plasmodia or ameba within a host can be confirmed through slide mounts of clubbed roots. Symptoms vary slightly from host to host. Fresh infections start as small bladder like galls on roots, whereas advanced infections display long spindle shaped clubs on primary roots and root hairs. Day wilting is the first conspicuous above ground symptom. Likewise, healthy looking plants of brassica crops growing in fields with saturated moisture appear wilting on hot day light and recover once the temperatures cool after sunset. On the advancement of the disease, leaves turn yellow and plants die. Another significant on field symptom of the diseased plants is stunting compared to non-infected plants and will often be in patches particularly in low, wet areas of the field. On digging, roots exhibit galled/clubbed symptoms. Eventually late season symptomatic plants show the rotted or friable galls. Moisture content of the soil and higher temperature influence the onset of infections on oilseed rape (Gossen *et al*., 2015). Every individual gall of clubroot has the capability of releasing approximately 8 x 10^8 resting spores back to the soil, indicating that the field will never be free of clubroot resting spores if brassica hosts are exposed to the soil quite often (Hwang *et al*., 2011b).

Pathogen dissemination

Dispersal of the clubroot pathogen occurs from one field to another through various agencies.

a. Seed: The possibility of seed contamination and dispersal is low and rare but considered one of the important modes of dissemination to long distances across continents. Mostly, imported seed with chunks of infected soil is the main cause of seed dispersal. In Sweden Eriksson (1930) described an occurrence of clubroot on turnips (*B. rapa* var. *rapa*) that was reportedly caused by seed-borne inoculum, Likewise, Gibbs (1931) attributed several cases of clubroot to seed-borne infestations. Using quantitative PCR to measure DNA of *P. brassicae*, the seed-borne dissemination of *P. brassicae* is possible (Rennie *et al*., 2011). Similarly, a report of seeds of white mustard (*Sinapis alba* L.) imported from Canada related to the source of two cases of clubroot in New South Wales, Australia (Hind-Lanoiselet and Parker, 2005).

b. Infected plant material: Transmission of *P. brassicae* to long distances occur quite often via the movement of resting spores in infected plant material in the form of galls. Dissemination of clubroot is often associated with the spread of infested soil from a clubroot infested field to a non-infested field on the roots of the vegetables. The planting time of Brassica crops varies in different regions of the world. Domestic transportation of infected plants and the movement of equipment from one area to the other is the main cause of transmission of *P. brassicae* (Li *et al*., 2010). The common practice of transporting non-seasonal vegetables infested with clubroot among different provinces, states and countries created the chances for rapid spread of the pathogen has been reported in many countries. Transplanting of cruciferous vegetables from clubroot-infested fields onto non-infested fields could introduce the pathogen to new areas or increase existing inoculum levels in the soil.

c. Soil: Infected soil plays a major role in *P. brassicae* transmission around the world**.** This soil-borne pathogen, spreads via the movement of infested soil on farm and other machinery (Howard *et al*., 2010). The infested soil with *P. brassicae* resting spores known to be carried to uninfected plants through the movement of infested soil and water (Dixon, 2009; Kageyama and Asano, 2009), likewise, by windborne dust (Rennie *et al*., 2012). The occurrence of resting spore dispersal through the wind is quite possible when the resting spores are picked up along with light, dry, dusty soil particles over to far longer distances. Soil dwelling animals such as earthworms and possibly moles, root nematodes, and other insects may

be vectors for *P. brassicae* in the soil (Dixon, 2009). The character of the resting spores of *P. brassicae* of extreme longevity, contributes to the infections and severity of clubroot. Inspite of the half-life of 3.6 to 4.4 years of *P. brassicae* resting spores they are known to survive in the soil for nearly 20 years (Wallenhammar, 1996; Dixon, 2009). Using real time PCR for identification and quantification of *P. brassicae* in soil samples, a population density of 10^4– 10^6 resting spores g^{-1} soil was found in naturally infested soil samples (Wallenhammar *et al.*, 2012). Based on the correlation between the population density of resting spores and gall formation, the minimum population density for gall formation was calculated to be 3.5 resting spores g^{-1} soil (Naiki *et al.*, 1978). In Canada, the resting spore population was estimated up to 80,000 spores per gram of soil where OSR/canola start showing symptoms as galls or clubs on roots. However, several reports of single resting spores found to induce gall formation (Scott, 1985; Narisawa *et al.*, 1996). Considering that primary infection results in the production of millions of resting spores, the population density of *P. brassicae* increases substantially after continuous cropping with susceptible plants.

d. Rain and irrigation water: Rain and flood water disseminate *P. brassicae* over considerable distances, especially on sloping land. Often, irrigation water contaminated with resting spores of *P. brassicae* could potentially infest large areas of fields. *P. brassicae* has been quantified in irrigation water, pond sediments (Datnoff *et al.*, 1984), from dams and bore wells (Faggian *et al.*, 1999). The viability of resting spores in the irrigation water was estimated to be 34 months and on repeated irrigation with water containing as few as 10 spores/ml results in root galling (Donald, 2005). It was estimated that the resting spores settles in undisturbed columns of water at a rate of 25 cm per day. In dams of a farm scenario, most of the resting spore population were expected to be concentrated in the sediments at the bottom. The risk of spreading clubroot through irrigation water using contaminated water from farm dams can be reduced by finding the irrigation intake pipe in the stillest part of the dam and by mounting on a float to collect water from the surface (Donald and Porter, 2009). Contaminated water with *P. brassicae* spores from ponds or creeks resulting from runoff from infested fields should not be used for irrigation of brassica vegetables. As observed in southern China, most of the mountainous regions of Yunnan, Hubei, Hunan, Sichuan and other provinces. Where, brassica crops are planted on slopes or terraced fields, Likewsie planted in fields at lower elevations are easily infested by rain or irrigation water contaminated with *P. brassicae* resting spores coming from infested fields located at higher elevations (Chai *et al.*, 2014).

e. Animal fodder and manure: Spread of clubroot on animal fodder or from manure from livestock fed with *P. brassicae*-infested fodder was also reported (Gibbs, 1931). Spores may spread in from the manure and from the moving farm animals and further found that the resting spores are capable of withstanding the highly acidic gut environment of the animals (Li, 2013). A case study indicated that the movement of the clubroot pathogen from Europe occurred on transporting diseased fodder turnips and swedes that were the major feed of the livestock was moved by early European settlers to America, Australasia and other centers of settlement (Dixon, 2009). One of the earliest report indicated that the clubroot as an agricultural problem in the fourth century AD, when the Roman Pallidus described the development of spongy roots on rape, turnip and radishes grown in soil fertilized with manure in Italy (Watson and Baker, 1969). This report could be the first record of clubroot transmission by livestock manure (Howard *et al.*, 2010). In 1964, an epidemic of clubroot in new fields or the fields with no prior reports of clubroot was linked to the spread of manure from animals fed on diseased roots (Creelman, 1965).

Lately, from Hubei Province in China reported a sharp rise in clubroot spread in five years (2007–2012). Over 70% of the fields were infested in these 5 years with an estimation of 60% yield loss (Li, 2008). A key survey found that livestock were raised on a free-range, where a mix of *P. brassicae*-infected material was present. The contaminated livestock manure was used by the local farmers directly without composting to fertilize the field which appears to be a common practice in this province. Further, it was reported that the resting spores of *P. brassicae* can withstand the digestive tracts of cattle, pigs and chickens, thus acted as vectors of *P. brassicae* (Li, 2013).

Host range

Plasmodiophora brassicae can be found worldwide in brassica crops. Reports of over 300 species in 64 genera of crucifers have been found infected with *P. brassicae* in both cultivated and wild brassicas. The most important economic hosts are OSR/canola (*B. napus* L), Brussels sprouts (*B. oleracea* L. var. *gemmifera* DC.), cabbage (*B. oleracea* L. var. *oleracea*), cauliflower (*B. oleracea* L. var. *botrytis*), chinese cabbage (*B. rapa* L. subsp. *pekinensis*), kale (*B. oleracea* L. var. *acephala*), arugula/ taramira (*Eruca sativa* Mill.), false flax/camelina (*C. sativa* (L.) Crantz), radish daikon (*Raphanus sativus* L. var. *longipinnatus*), radish round (*R. raphanistrum* subsp. *sativus* (L.) Domin), rutabaga (*B. napus* L. var.

napobrassica), shepherd's purse (*Capsella bursa pastoris* (L) Medic.), turnip (*B. rapa* L. var. *rapifera*), and wild mustard (*Sinapis arvensis* (L.) Rabenh (Chapara *et al*., 2021; Ren *et al*., 2016).

Surveys

The clubroot pathogen *P. brassicae* is widespread throughout the world. More than 60 countries reported its occurrence that resulted in yield losses ranging from 10-100 per cent reduction on a global scale (Dixon, 2009). Jing *et al*. (2008) quantified losses of oil seed rape based on the growth stage at which the infections took place in China; there were 17% loss of plant stand at very young stage, and around 15% at maturity with recorded losses of 10.2% in the yield. Disease surveys played a very useful role in managing this difficult to control disease the clubroot and were proved very crucial worldwide. Research reports of canola survey in central Sweden indicated that 78% of 190 fields surveyed were infested with clubroot (Wallenhammer, 1996). Yearly survey report from Manitoba indicated that significant and fast spread of clubroot infected fields. In Manitoba, it was identified in two fields in 2012, by 2014 it was in more than 600 fields and more over the following years. Likewise, in another province survey report of clubroot in Alberta, Canada confirmed over 2000 fields that were infected with clubroot (Strelkov *et al*., 2018). An annual survey conducted in North Dakota State in the United States indicated clubroot presence and rapid spread from one to 33 new fields by 2018 in a single County and the infected clubroot fields has pH ranged from 4.5 to 6.7 (Chapara *et al*., 2019). All these studies prove the importance of continues survey.

Soil pH and types

Clubroot disease incidence and development is favored by acidic soils (Tewari *et al*., 2005). Subsequent research proved that clubroot on canola is not only limited to acidic soils but can also occur in alkaline soils (Strelkov *et al*., 2007). Although, Palm (1963) observed the maximum clubroot disease infestation was at pH 6.6. Reports from clubroot infected fields indicated that soils with light and sandy soils *P. brassicae* was less widespread compared with loamy soils and the infection level was significantly influenced by soil type, content of sand and soil pH value: soil types with a sand content of 30 – 35 % and a pH values lower than 5.6 showed high clubroot severity and disease incidences.

Quantification of resting spores of *P. brassicae* from soil

Baiting brassica plants has been considered the most reliable identification method for assessing for the presence of *P. brassicae* from soil and is most commonly used. The availability of more modern techniques may help in faster results. Real time PCR provides faster results than the traditional soil bioassays, these assays allows to quantity *P. brassicae* DNA from infested soil samples that has to be diagnosed. The quantitative PCR (Q-PCR) assays developed offered farmers quantitative, fast and accurate results of *P. brassicae* amounts in individual fields or in patches with in a field. The constant increase in the acreage of OSR/canola around the world that is grown for food, feed and fuel and also the introduction of clubroot susceptible brassica crops as cover crops demands routine testing of soils. Determination of the threshold levels of *P. brassicae* inoculum from soils using the advanced techniques helps the possibilities for detection of clubroot infected fields way early and in adopting the necessary management practices such as use of brassica cultivars that are resistant to clubroot and the information on the length into crop rotations to be followed (Holtz *et al*., 2021; Wallenhammer *et al*., 2012).

Clubroot management

With almost no or limited data available on effectiveness of pesticides that are available to control clubroot, it is certain that the pathogen *P. brassicae* cannot be eradicated from a field. The clubroot management alternatives that are readily available so far are practicing longer crop rotations, using disease resistant cultivars, soil amendments and sanitation. Integrated disease management (IDM) of clubroot on OSR/canola has proved more effective than any of the individual disease management components in large-scale, on-farm studies conducted in Canada and in the USA.

Crop rotation

A longer break from brassica hosts is the better management option for clubroot control. Longer rotations will help to lower *P. brassicae* resting spores in soil likewise, tight rotations can increase the resting spores/ inoculum of the pathogen once clubroot is present in a field. Along with longer crop rotations, limiting soil movement, controlling clubroot host weeds and volunteers of OSR/canola is considered as an important long-term management option to manage Clubroot. On top of it using the available OSR/canola cultivars with resistance gene on those fields infested with clubroot spores is considered as the cheap and best option. The two-year break reduced *P. brassicae* resting spore concentrations by 90 per cent relative to growing continuous OSR or a one-year break in heavily infested field plots (Peng *et al*., 2015)

Resistant cultivars in OSR/canola

Effective management requires use of clubroot-resistant varieties. Right now this practice is widely in use to manage clubroot of brassicas. However, clubroot resistant cultivars will be helping once or twice and farmers cannot rely to use them as a continuous option, and the only way to keep resistant varieties effective is through a 4 year rotation, at minimum. The resistant mechanism is apparent when root hairs of both resistant and susceptible OSR cultivars are infected by the primary zoospores that are released from resting spores in to the soil and the secondary zoospores can infect only susceptible cultivars and cannot infect the roots of resistant cultivars so the symptoms and yield loss do not occur (Peng *et al*., 2015). Piao *et al*. (2004) have reported the sources of *P. brassicae* resistance genes (R-genes) and genetic mapping studies and later have been reviewed by Rahman *et al*. (2014). Around 19 R-genes/QTL have been mapped for clubroot resistance in *B. napus*, by Werner *et al*. (2007) to date. Out of which two clubroot resistance genes were cloned, *Crr1a* and *CRa* from *B. rapa* (Table 1).

Soil amendments

Chemicals and soil ameliorating substances have been used for several years to treat fields infested with *P. brassicae;* among them, few studies have been promised significant control (Anonymous, 2018). In contrast, there are studies that have given inconsistent management of clubroot (Donald and Porter, 2009). Soil reclamation with calcium carbonate, calcium cynamide helped showed potential in managing clubroot of oilseed rape (Graham *et al*., 2016). Application of calcium cyanimide and lime has been promising in effective management of clubroot (Donald *et al*., 2006). Likewise, Quintzene (PCNB) and high levels of wood ash found reducing clubroot severity on canola in Edmonton, Canada (Hwang *et al*., 2011b). Adjuvant such as non-ionic surfactants has been reported to be having significant effect in managing clubroot pathogen (Hildebrand and McRae, 1998). Three products (beet lime, wood ash and pellet lime has lower clubroot disease severity index when tested on OSR (Anonymous, 2018).

Soil pH and liming

Clubroot incidences diminishes rapidly when the soil pH is at 7.0 or above and is correlated to the inhibition of spore germination. However, the highest degree of clubroot infection was observed at soil pH of 6.6. Application of lime before planting influences the soil acidity. Incorporating lime of different kinds into the soil followed by light irrigation has proven promising success in lowering clubroot disease index. However, should be done precariously as raising the soil pH too high invites other imbalances in soil such as nutrient deficiencies or diseases of other crops may become more prominent, for example powdery scab of potatoes. Avoid adding lime if the expected increase in soil pH has not observed. Reports indicated that clubroot infections in acidic soils can be controlled with lime application (Chapara, 2018) and more likely can be controlled in the fields with lighter infections than the severe ones or those on alkaline soils (Myers and Campbell, 1985).

Control of weed hosts and volunteers

Controlling brassica weeds in all fields of the crops is very important to prevent the further increase in spores. Weeds should be controlled within three weeks of emergence so they aren't able to produce new resting spores. Known clubroot hosts including canola volunteers, *rassica* weeds (ex. flixweed, stinkweed, shepherds purse, wild mustard), tame mustard, camelina, and other *Brassica* crops as well as vegetables (Chapara, 2021).

Tillage

A lower clubroot severity was observed under reduced tillage. Reduced tillage is a practice where a direct seeding of the crop is sown into the field without any previous tillage operations. In general research reports indicate that fewer tillage operations help in preventing the movement of contaminated soil within a field and between the fields. Further has been shown that the fields with the largest affected areas have been fields where intensive tillage has been used (Ekeberg and Riley, 1997).

Sanitation

One of the most important ways to reduce clubroot spread from one field to the other and to the entire OSR growing region is sanitation. A well-known fact of *P. brassicae* propagules spreads is through the movement of contaminated soil. The most usual way of soil movement is from field to field on farm machinery and on vehicle tires of tractor, drill, combine and any other equipment that is used in the infected soils can pick up the inoculum. Preliminary cleaning by scraping all loose soil off of the machinery and their tires while working in between the fields can stop 90% spread or more of clubroot spores that are present on the machinery. Overall sanitation research done in the laboratory, greenhouse and from the field trials report that preliminary cleaning, followed by pressure wash and disinfection of farm and industrial equipment thereafter was found to be an effective measure in limiting the spread of the clubroot pathogen. Reports indicated that a few spores can turn into billions of spores

Table 1: Brassica crops and R genes/QTL identified and genetically mapped or cloned against *Plasmodiophora brassicae* (Neik *et al*., 2017)

R- genes/ QTLs	Chromosome	Lines	Pathotype	Crop	Reference
CRa and *CRb*^{Kato}	chromosome A3	"1CA0591.323" and "1CA0591.263"	Williams pathotype 3	*B. napus (canola)*	Zhang *et al*., 2014; Fredua-Agyeman and Rahman, 2016
New CR gene, act singly or combined with *CRa*	-	"12-3" (R) × inbred line "12-1" (S)	pathotype 3	*B. napus*	Zhang *et al.,* 2016
CRa/*CRb*^{Kato} Crr1	Chrosome A03 Chromosome A08	ECD 02	Pathotypes 3H, 5X and 5G	*B. rapa*	Fredua-Agyeman *et al*., 2020
CRk and *Crr3;* 19 QTL mapped	Chromosome A2, A3, A8, A9, C3, C5, C6, and C9	(DH "263/11" (R) × Cultivar "Express" (S)	7 different isolates from Sweden, Germany, and France	*B. napus*	Werner *et al*., 2007
One single dominant locus from *B. rapa* ("ECD-04"), two recessive loci from *B. oleracea* ("ECD-15")	-	"Mendel"(R) × Breeding line (S)	Isolate 1 which is highly virulent on *B. oleracea*	*B. napus*	Diederichsen *et al*., 2006
Dominant gene *Pb-Bn1*	-	DH progeny from Darmor-*bzh* (R) × Yudal (S)	pathotypes 4 and 7	*B. napus*	Some *et al*., 1996
DY4	-	-	-	-	Manzanares-Dauleux *et al*., 2000
Two dominant, unlinked genes	-	ECD-15" (R) × B. rapa" ECD-04" (R)	virulent field isolates	*B. napus*	Diederichsen and Sacristan, 1996
Rcr2	chromosome A3	Jazz" (R) × DH ACDC (S)	pathotype 3	*B. rapa*	Huang *et al*., 2017
Rcr4, Rcr8, Rcr9	chromosome A3, A2, A8	T19" originate from cultivar "Pluto" (R) × DH line "ACDC" (S)	Williams pathotype 2,3,5,6,8, 5x	*B. rapa*	Yu *et al*., 2017
single dominant gene	-	CCR13685" (R) × Pak choi "GHQ11021" (S)	unknown	*B. rapa*	Chen *et al*., 2016
Rpb1 (TIR-NBS-LRR)	LGA3	-	pathotype 3		Chu *et al*., 2014; Yu *et al*., 2017
CRa and CRb^{Kato}	chromosome A3	Pak choi "702-5" (S) × DH line "CR Shinki" (R)	-	*B. rapa*	Fredua-Agyeman and Rahman, 2016; Zhang *et al.,* 2014

Locus *QS_B3.1* on corresponding to *CRa* and *CRb*	chromosome A3	turnip "Siloga" (R) × Chinese cabbage "BJN3" (S)	Williams race 4	*B. rapa*	Pang *et al*., 2014
linkage group N3	chromosome A3	cabbage cultivars (R) × *B. rapa* oilseed accession (S)	Canadian field isolate	*B. rapa*	Gao *et al*., 2014
CRb gene	tightly linked with *CRa* and *CRb*^{Kato}	DH line "CR Shinki" (R) × Chinese cabbage "702-5" (S)	pathotype 4	*B. rapa*	Zhang *et al*., 2014
Crr1a with major effect and *Crr1b* with minor effect at *Crr1* locus	-	DH "G004" (R) x DH "A9709" (S)	field isolates Ano-01, Wakayama-01, and Nos. 5, 7, 9, and 14	*B. rapa*	Hatakeyama *et al*., 2004, 2013
PbBa1.1 PbBa3.2 and PbBa3.3 PbBa8.1	*A1* A3 A8	Inbred turnip line "ECD04" (R) × Inbred Chinese cabbage line "C59-1" (S)	Pb2, Pb4, Pb7, and Pb10 isolates	*B. rapa*	Chen *et al*., 2013
Rpb1	LGA3	Pak choi "FN" (R) x DH "ACDC" (S)	Williams pathotype 2, 3, 5, 6, 8	*B. rapa*	Chu *et al*., 2013
Single dominant gene linked to *CRb*	-	Akiriso" and "CR Shinki	isolate No. 14 or pathotype group 3	*B. rapa*	Hatakeyama *et al*., 2004, 2017; Kato *et al*., 2012
Crr1	-	*Arabidopsis*	-	*B. rapa*	Suwabe *et al*., 2012
CRk is very near or identical to	-	one F2 progeny from DH "K10" (R) × DH "Q5" (S), the other DH "C9" (R) × DH "6R" (S)	-	*B. rapa*	Matsumoto *et al*., 1998; Hayashida *et al.,* 2008
CRb	chromosome A3	F2 populations	isolate M85	*B. rapa*	Sakamoto *et al*., 2008
CRc	chromosome A2 or R2		Williams race 2 and K04 with unknown pathotype/race	*B. rapa*	
Crr1 and *Crr2*	-	DH "G004" (R) × DH "A9709" (S)	isolate Wakayama-01 close to Williams race 4	*B. rapa*	Kuginuki *et al*., 1997; Suwabe *et al*., 2003
Crr1 and *Crr2*	chromosome A8 of *B. rapa* and chromosome 4 in *Arabidopsis*	*A. thaliana* and *B. rapa*	-	*B. rapa*	Suwabe *et al*., 2006
Crr3	chromosome 3	Map position of on *B. rapa* and *Arabidopsis*	-	*B. rapa*	Hirai *et al*., 2004; Saito *et al*., 2006
CRb	Chromosome A3	DH Chinese cabbage "CR Shinki" (R) × "94SK" (S)	Williams race 4	*B. rapa*	Piao *et al*., 2004; Diederichsen *et al*., 2009; Saito *et al*., 2006

Crr4 loci on LG 2	chromosome A6	DH "G004" (R) × DH "A9709" (S)	isolate Wakayama-01 and Ano-01	*B. rapa*	Suwabe *et al*., 2006
Crr3, major dominant gene	-	Inbred line "N-WMR-3" containing cultivar "Milan White" (R) × DH "A9709" (S)	isolate Ano-01 Williams race 3	*B. rapa*	Hirai *et al*., 2004
CRa Single dominant major gene	chromosome A3	DH "T136-8" (R) × DH "Q5" (S), both Chinese cabbage	isolate Williams race 2	*B. rapa*	Matsumoto *et al*., 1998
Single dominant	-	European fodder turnip "Siloga S2" (R) × DH Chinese cabbage"Homei"(S)	isolate Williams race 2	*B. rapa*	Kuginuki *et al*., 1997

over the course of one season, so this level of sanitation is reasonably easy and effective. This practice can be mandated for all farmers in areas where clubroot has been detected and where equipment or custom operators that often work in known clubroot-positive fields, and to farmers who feel they are at risk of getting clubroot pathogen in their fields (Porter and Cross, 1995).

Resistance breakdown in OSR/canola cultivars by *P. brassicae*

Resistance breakdown

The emergence of physiologic specialization is well documented in the clubroot pathogen, with multiple pathotypes identified based on their virulence patterns on various sets of differential hosts (Strelkov *et al*., 2007 & 2018). One clubroot gall produces millions to billions of resting spores and that population of spores can contain multiple *P. brassicae* pathotypes. However, one or two pathotypes tend to be dominant across a field, with other pathotypes present only at low levels. With repeated use of same resistant cultivars leads natural selection, mutations and recombination resulting in the development of new pathotypes and sometimes mutated pathotypes. Implementation and growing of clubroot resistant cultivars in tight rotations lead to selection pressure on the pathogen, in turn they co evolve and there by leading to overcome the resistance (Diederichsen *et al.*, 2009; Hwang *et al.*, 2011a). It is advisable to practice judicious use of the available resistant cultivars in order to maintain its durability for longer time regardless of the nature of resistance the cultivar possess.

Conclusion

Clubroot, caused by the obligate parasite *P. brassicae*, is one of the most significant soilborne diseases of the Brassicaceae around the world. The most characteristic symptoms of clubroot on the OSR/canola are the malformation of the tap and lateral roots, resulting in the development of clubs or galls. Severe galling hinders the uptake of water and nutrients and the affected plants wilt even at saturated soil moisture eventually clubroot infections result in stunting, premature plant senescence, accelerated flowering, and ultimately major yield losses. In less than a decade, clubroot of OSR/canola has emerged as one of the most important diseases of this crop in Canada and in the state of North Dakota of the United States. Surveys indicated that from a dozen *P. brassicae* infested fields, representing less than 800 ha, the pathogen has spread to more than to 560 fields, representing over 35000 ha, in central Alberta province of Canada in a very short time, and has also been identified in other provinces. Somewhat similar trend has been observed in the United States on Oilseed rape/canola. A concrete research effort and implementation of the results in grower's fields in Canada has contributed to an accurate understanding of the pathogen *P. brassicae*, the disease clubroot and its management with high success. Integrated approaches using cultural, chemical, biological, and host resistance may extremely effective as clubroot management strategy to obtain high yield of brassica crops. Finally, successful control of *P. brassicae* will require an integrated and multifaceted approach with making use of all the available tools and knowledge to lower the devastating impact of this pathogen on Brassica crops such as OSR/canola.

Future research scope: Determination of resting spores of *P. brassicae* from soil and pathotyping to identify mutants or new pathotypes through molecular markers can be a major breakthrough if pursued further. Likewise, many more management options can be tested with the currently available novel technologies and will undoubtedly facilitate additional research at a faster pace and progress in this important area in OSR/canola. In this process sequencing of the *P. brassicae* genome, the development of molecular markers and constant search for the detection of resistance genes in host genotypes needed to be adopted and incorporated in clubroot research protocols. A recent clubroot tracking app is an open database to record the presence of clubroot and *P. brassicae* reported by researchers, industry and growers has been developed in Canada can be very helpful to researchers and OSR/canola growers around the world and needs to be popularized.

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