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Proto-oncogenes in a eukaryotic unicellular organism play essential roles in plasmodial growth in host cells K. BI, T. CHEN, Z.C. HE, Z.X. GAO, Y. ZHAO, Y.P. FU, J.S. CHENG, J.T. XIE AND D.H. JIANG *State Key Laboratory of Agriculture Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei Province, People's Republic of China*

The eukaryotic unicellular protist *Plasmodiophora brassicae* is an endocellular parasite of cruciferous plants. In host cortical cells, this protist develops a unicellular structure that is termed the plasmodium. The plasmodium is actually a multinucleate cell, which subsequently splits and forms resting spores. The mechanism for the growth of this endocellular parasite in host cells is unclear. Here, combined *de novo* genome sequence and transcriptome analysis of *P. brassicae* strain ZJ-1 identified the top five significantly enriched KEGG pathways of differentially expressed genes as Translation, Cell growth and death, Cell communication, Cell motility and Cancers. We detected 171 proto-oncogenes from the genome of *P. brassicae* that were implicated in cancer-related pathways. Three predicted proto-oncogenes (*Pb-Raf1*, *Pb-Raf2*, and *Pb-MYB*), which showed homology to the human proto-oncogenes *Raf* and *MYB*, were specifically activated during plasmodial growth in host cortical cells, suggesting their involvement in the multinucleate developmental stage of the unicellular protist organism. Gene networks involved in the tumorigenic-related signaling transduction pathways and the activation of 12 core genes were identified. Inhibition of phosphoinositol-3-kinase relieved clubroot symptoms and significantly suppressed the developmental process of plasmodia. The results suggest that proto-oncogene-related regulatory mechanisms play an important role in the plasmodial growth of *P. brassicae*.

Effect of *Plasmodiophora brassicae* inoculum density on yield of canola (*Brassica napus*) A. BOTERO-RAMÍREZ, S.F. HWANG AND S.E. STRELKOV *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada; (S.F.H.) Crop*

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Clubroot, caused by *Plasmodiophora brassicae* Wor., is a major threat to canola (*Brassica napus* L.) production on the Canadian Prairies. However, reliable estimates of the relationship between yield and *P. brassicae* inoculum density under western Canadian conditions are lacking. This research aimed to evaluate the effect of clubroot disease on the yield of two canola hybrids with different clubroot resistance levels under field conditions in Alberta. The susceptible canola hybrid '45H31' and the resistant hybrid '45H29' were grown in soil inoculated with pathotype 5X at rates equivalent to 10^7, 10^6, 10^5, 10^4 and 10^3 resting spores of *P. brassicae* per plant. Clubroot incidence and severity, along with yield parameters including seed weight, productive branches and pods per plant, were measured at 79 and 122 days after planting. It was found that as *P. brassicae* inoculum density increased, so did the incidence and severity of clubroot on both the resistant and susceptible hybrids, although no statistical differences were observed at the lower inoculum densities (10^3 - 10^5 resting spores per plant). Yield was not affected by *P. brassicae* inoculum density in the resistant hybrid, but was reduced by 31-51% in the susceptible hybrid. Regression analysis indicated that a second-grade polynomic equation best described the inoculum density effect over the yield in the susceptible hybrid.

Clubroot status in Colombia A. BOTERO-RAMÍREZ, F. L. PADILLA-HUERTAS, L. TARAZONA, J. C. GÓMEZ, J. S. NAVAS AND C. GARCÍA *Agricultural Sciences Faculty, Universidad Nacional de Colombia, Bogotá, (Avenue Quito # 40-03), Colombia; (A.B.R) Present address: Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada*

Clubroot, caused by *Plasmodiophora brassicae* Woron, is a major disease in Colombia. This first nation-wide survey for clubroot showed that it is spread along the main cruciferous productive areas with the exception of the southernmost part of the country, with prevalence ranging from 29.4-88.9%. Under Colombian conditions, clubroot occurrence is favored by high contents in the soil of calcium, boron, phosphorus and copper. Given the importance of the disease, yield losses, inoculum sources and in-field distribution of the pathogen have been evaluated to better

understand its behavior in the country. When yield losses were evaluated, inoculum densities of 10^3 and 10^6 resting spores·gram of soil^{-1} reduced yield by 43.9% and 74.5% in cabbage, 42.5% and 61.2% in broccoli, 33.3% and 70.1% in cauliflower, respectively. Open irrigation systems can be a source of viable *P. brassicae* inoculum, howeverk infection risks have not been assessed. A patchy distribution of *P. brassicae* was observed, accompanied by a reduction in inoculum density as depth increased. The status of clubroot in Colombia suggests that additional efforts are required to better understand the behavior of the causal agent and provide effective solutions for its management in the short- and long-term.

Impact, management and control of clubroot disease in the UK F. BURNETT AND J. SMITH *SRUC (Scotland's Rural College), West Mains Road, King's Buildings, EH9 3JG, Edinburgh, UK; (J.S.) ADAS Rosemaund, Preston Wynne, HR1 3PG, Hereford, UK*

The UK has a long history of vegetable crop production and of crop losses to clubroot, caused by *Plasmodiophora brassicae*. The introduction on oilseed rape and its intensive inclusion in arable rotation has exacerbated this soil-borne problem. Survey work shows that over 50% of arable soils in the UK are infested with clubroot. The practice of testing soils and selecting a negative field is popular with growers but has proved unsustainable in the long term. In areas of intensive production, clean fields are a diminishing resource and for arable farmers moving oilseed rape production to clean land is not practical since infected fields have to be managed within the individual farming units concerned. In this latter example, the use of resistant varieties of oilseed rape has been the preferred strategy. Historically in the UK, these have been lower yielding and only deployed where infection levels are high enough to justify their use. Trial results show that in fields where resistance has not been deployed before it is highly effective but in fields where it has been previously deployed 2-3 times, efficacy has been eroded through the selection of pathotypes virulent against the only available resistance mechanism ‘Mendel’. Yield loss measurements in susceptible and resistant varieties are similar at comparable infection levels. The use of soil amendments is also practiced with some efficacy in vegetable crop production, but their use in oilseed rape crops has proved less effective and is seldom cost effective. Longer rotations are helpful but as oilseed rape is the most profitable crop after wheat in arable rotations as well as a useful break crop, rotations of one susceptible crop in three (or less) are common.

Field mapping shows useful information on the patchy nature of the disease and the difficulties this poses in terms of setting economic decision thresholds for fields and offers some potential going forward that available solutions could be targeted to infected areas. Significant knowledge gaps exist, particularly on the long term cost benefits of management decisions, the resolution of which could inform long term rotational and treatment decisions in fields and would also inform higher level policy strategies on optimal, sustainable management of clubroot.

Effect of canola cultivar rotation on *Plasmodiophora brassicae* pathotype population dynamics TIESEN CAO, VICTOR P. MANOLII, QIXING ZHOU, SHEAU-FANG HWANG AND STEPHEN E. STRELKOV *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada; (Q.Z. AND S.F.H.) Alberta Agriculture and Forestry, 17507 Fort Road NW, Edmonton, AB T5Y 6H3, Canada*

In Alberta, Canada, clubroot (*Plasmodiophora brassicae* Wor.) is managed mainly by planting clubroot resistant (CR) canola (*Brassica napus* L.) cultivars. However, multiple new pathotypes of *P. brassicae* have emerged recently which are virulent on CR canola. To understand the impact of cultivar rotation on pathotype population dynamics, greenhouse experiments were conducted in which different canola rotations were grown in a soil mix containing equal amounts of pathotypes 5X and 3, which are virulent and avirulent, respectively, on CR canola. Three treatments were assessed: T1, the same susceptible cultivar planted over 4 cycles; T2, the same CR cultivar planted over 4 cycles; and T3, different CR cultivars planted in each cycle. Clubroot severity increased from cycles 1 to 4 in all treatments, with the exception of one CR cultivar in T3 that may carry a different resistance source. Pathogen populations were recovered with a susceptible bait crop and pathotyped on the differentials of Williams plus a CR host. The proportion of galls classified as pathotype 5X in T1 declined to 6.7% over the course of the experiment. In contrast, the proportion of pathotype 5X increased to 66.7% and 70% in T2 and T3, respectively. Pathotype 5X-specific quantitative PCR analysis of the soil mix indicated a significantly higher amount of 5X-DNA in T2 vs T1. The results suggest that continual planting of CR canola favours the proliferation of virulent pathotypes of *P. brassicae,* as evidenced by the increase in pathotype 5X observed in this study.

Evaluation of various soil amendments to manage Clubroot on Canola in field condition V. CHAPARA *9280, 107th Avenue, Langdon Research Extension Center, Langdon, ND-58249*

Clubroot of canola (*Brassica napus* L.), caused by *Plasmodiophora brassicae* W., is a primitive living organism with characteristics of fungi, plasmodium and slime mold. These characteristics make the pathogen difficult to control with any pesticide group. Research indicates that soil amendments that increase soil pH show significant control of clubroot. A field study was conducted to evaluate the efficiency of amendments that alter soil pH, plant defense mechanisms and fungicidal properties against clubroot pathogen. The experimental design consisted of a randomized complete block with four replications. Treatments of wood ash, pelletized lime, beet lime and gypsum were applied seven days before planting. Likewise, cyazofamid, fluazinam, penta chloro nitro benzene, zinc nano-particle, and a non-ionic surfactant were applied just prior to planting. The treatments were mixed thoroughly into soil with a rototiller to a 10-cm depth after each application. A clubroot rating scale of 0-3 described by Strelkov was used to assess symptom development. Soil samples were collected before and after the application of treatments for pH assessments. Clubroot incidence and severity were significantly lower in the wood ash treatment followed by beet lime and pellet lime compared with the other treatments tested. Likewise, an increase of soil pH from acidity to alkalinity in the treatments amended with wood ash, beet lime and pelletized lime was observed.

Transferring clubroot resistance by intergeneric hybridizations between *Brassica napus* and *Raphanus sativus* E. DIEDERICHSEN, N. GOLLINGE, J. MADER, J. SCHONDELMAIER, K. LOHGALL AND M. SCHLATHÖLTER *Institut für Biologie - Angewandte Genetik, Freie Universität Berlin, Albrecht- Thaer-Weg 6, D-14195 Berlin, Germany; (J.M., M.S.) P.H.PETERSEN SAATZUCHT LUNDSGAARD GmbH, Streichmuehler Str. 8a, D-24977 Grundhof, Germany; (J.S., K.L.) Saaten-Union Biotec GmbH, Hovedisser Str. 94, 33818 Leopoldshöhe, Germany*

Raphanus crops are important vegetables and break crops to control nematodes and N leakage. Despite a relatively high resistance level, clubroot affects radish crops and even minor infections

in oil radish receive public attention. Clubroot resistance (CR) in oil radish needs strong improvement to support the use of this break crop without risking increases in clubroot inoculum. The RAPHKORE project aims at characterization and improvement of CR in Raphanus and studies different sources of CR. We have discovered a *P. brassicae* isolate from oil radish that shows strong virulence towards most Raphanus cultivars. On the other side, this isolate does not infect *B. napus* cultivars that were not considered to have clubroot resistance. This could indicate that virulence towards Raphanus is gained on the costs of virulence towards these *B. napus* cultivars. Combining these resistance sources could be a promising strategy to generate broad-spectrum resistance. We report on the results from intergeneric hybridizations between *B. napus* and *R. sativus*. To increase cross efficacy, a combination of ovary culture and ovule culture was applied. From 130 initial seedlings, 107 were confirmed as true hybrids using simple sequence repeat (SSR) markers. Clubroot tests showed the efficacy of resistance from both parents in nearly 50% of the hybrids. A backcross program has been initiated to further transfer clubroot resistance into oil radish.

Characterization of clubroot resistance in Raphanus E. DIEDERICHSEN, N. GOLLINGE AND M. SCHLATHÖLTER *Institut für Biologie - Angewandte Genetik, Freie Universität Berlin, Albrecht- Thaer-Weg 6, D-14195 Berlin, Germany; (M.S.) P.H.PETERSEN SAATZUCHT LUNDSGAARD GmbH, Streichmuehler Str. 8a, D-24977 Grundhof, Germany; Email: elked@zedat.fu-berlin.de*

Raphanus crops are important as vegetables and break crops for the management of N leakage and nematodes. While they have a relatively high resistance level of clubroot resistance (CR), Raphanus crops can nevertheless develop the disease. Clubroot resistance in oil radish needs to be improved to support the use of this break crop without increasing the clubroot risk for cash crops. Different sources of CR are studied in the framework of the RAPHKORE project. The known Raphanus CR that is effective against the prevailing pathotypes, which originates from Brassica crops, needs clarification of its inheritance and broadness. Newly collected *Plasmodiophora brassicae* isolates from oil radish crops were tested on different Raphanus and *B. napus* cultivars. So far, adaptation to this CR type is very seldom, and Raphanus cultivars usually show only a few infections. One isolate has been identified as being strongly virulent

towards Raphanus CR. This isolate originates from a farm focusing on potato production with oil radish as a break crop. We used this isolate to screen Raphanus gene bank accessions and found a small number of resistant accessions. Resistant individuals were selected and their S1-progeny were often significantly more resistant than the original population. Future studies on the inheritance of CR from Raphanus will reveal the genetic potential to control clubroot in this crop.

Screening of *Brassica oleracea* germplasm for resistance to *Plasmodiophora brassicae* *pathotype* 5X M. FARID, J. SI AND H. RAHMAN *Department of Agricultural, Food and Nutritional Science, 4-16 Agriculture/Forestry Centre, University of Alberta, Edmonton, Alberta, T6G 2P5, Canada, J.S. College of Horticulture and Landscape Architecture, Southwest University, Chongqing 400715, China*

Clubroot disease of brassica, caused by *Plasmodiophora brassicae* Woronin, is a concern to the canola (*Brassica napus* L.; 2*n* = 38, AACC) growers in Canada. Management of this disease through cultural practices is a challenging task; growing clubroot resistant cultivars in an appropriate crop rotation is, therefore, considered to be the cost-effective and environmentally friendly way of managing this disease. To date, several clubroot resistance genes have been identified in the A genome of *B. rapa* and used in the breeding of *B. napus* canola; however, very limited efforts have been made to use the resistance of the C genome of *B. oleracea*. Identification of resistance in the C genome and use in the breeding of *B. napus* canola will broaden the genetic base of resistance in this crop for sustainable production from a long-term perspective. To date, breeding efforts mainly focused on the use of the genotypes conferring resistance to pathotype 3. Recently, new virulent pathotypes have evolved in the canola fields in Alberta; among these, the pathotype 5X is a threat to canola production. The focus of this study was to evaluate a global population of the diploid species *B. oleracea* (CC) and a set of *B. napus* canola lines for resistance to *P. brassicae* pathotype 5X. Results from this research are expected to provide valuable information for the development of clubroot resistant canola cultivars.

Assessment of hydrated lime for the management of clubroot in canola N.M. FOX, S.F. HWANG, V.P. MANOLII, G. TURNBULL, AND S.E. STRELKOV *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada; (S.F.H., G.T) Crop Diversification Centre North, Alberta Agriculture and Forestry, Edmonton, AB, T5Y 6H3, Canada*

Clubroot, caused by *Plasmodiophora brassicae* Wor., is a soil-borne disease that has become a constraint to canola (*Brassica napus* L.) production in Alberta, Canada. The disease is managed primarily by the planting of clubroot resistant cultivars, but resistance has been overcome in >100 fields in the province. Clubroot development is favoured in acidic soils; therefore, increasing soil pH may reduce disease severity in infested soils and serve as another management tool. The efficacy of hydrated lime products in reducing clubroot was assessed in replicated field plot experiments in central Alberta in 2017. The addition of moderate to high rates of hydrated lime significantly reduced clubroot severity and increased above-ground biomass in a susceptible canola cultivar at 8 weeks after planting. At the highest application rate, lime treatment reduced the clubroot disease severity index by 35-91%, while increasing above-ground plant biomass by 58-116%. The field trails are being repeated in 2018. A greenhouse study also was conducted to assess the efficacy of hydrated lime in reducing clubroot severity in susceptible and moderately resistant canola cultivars, under different application rates and concentrations of inoculum. In the control treatments at all inoculum levels, the susceptible canola developed severe clubroot (92-100%) while the moderately resistant canola developed mild clubroot (9-13%). In contrast, neither cultivar developed visible symptoms of clubroot when treated with four rates of hydrated lime. Quantitative PCR analysis is underway to measure the impact of the treatments on *P. brassicae* inoculum levels in the soil and proliferation in host tissues.

Screening of *Brassica* accessions for resistance to 'old' and 'new' isolates of *Plasmodiophora brassicae* in Alberta, Canada. RUDOLPH FREDUA-AGYEMAN, SHEAU-FANG HWANG, STEPHEN E. STRELKOV, QIXING ZHOU, VICTOR P. MANOLII AND DAVID FEINDEL *Crop Diversification Center North, Alberta Agriculture and Forestry, 17507 Fort Road NW, Edmonton, AB, T5Y 6H3; (S.E.S., V.P.M.) Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada.*

Genetic resistance is the main tool used to manage clubroot of canola (*Brassica napus*) in Canada. However, the emergence of new virulent strains of the clubroot pathogen, *Plasmodiophora brassicae*, has complicated canola breeding efforts. In this study, 386 *Brassica* accessions were screened against five single-spore isolates and 17 field isolates of *P. brassicae* to identify resistance sources effective against these new strains. The results showed that one *B. rapa* accession [CDCNFG-046, mean index of disease (ID) = 3.3%] and two *B. nigra* accessions (CDCNFG-263, mean ID = 3.1%; and CDCNFG-262, mean ID = 4.7%) possessed excellent resistance to all 22 isolates evaluated. Fifty other accessions showed differential clubroot reactions (resistant, moderately resistant or susceptible), including 27 (one *B. napus*, two *B. rapa*, four *B. oleracea* and 20 *B. nigra*) accessions that were resistant to 8 - 21 *P. brassicae* isolates with mean IDs ranging from 5.3-29.6%. The remaining 23 accessions (two *B. napus*, one *B. rapa*, five *B. oleracea* and 15 *B. nigra*) were each resistant to 3 - 13 isolates, but developed mean IDs in the range of 30.3-47.0%. The three accessions which showed absolute resistance and the 50 accessions which showed differential clubroot reactions could be used to breed for resistance to the new *P. brassicae* strains.

Assessment of soils for the risk of clubroot disease of cruciferous vegetables using a simple dose response curve-dependent method A. FUKUNAGA, S. YOSHIDA AND S. TSUSHIMA *NARO Western Region Agricultural Research Center, 200 Ueno, Ueno-cho, Ayabeshi, Kyoto 623-0035, Japan; (S.Y) NARO Central Region Agricultural Research Center 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8666, Japan; (S.T) Tokyo University of Agriculture 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo, 156-8502, Japan*

The incidence of clubroot disease occurrence of cruciferous vegetables, caused by *Plasmodiophora brassicae*, has tended to increase in Japan. Development of methods for assessing soils for the risk of disease occurrence is important for reducing excessive soil sterilization using agricultural chemicals. Given the importance of evaluating the risk of disease occurrence in soils for the practice of Health checkup-based Soil-borne Disease Management (HeSoDiM), we developed a simple assessment system using plug tray-planted seedlings. Cabbage (indicator crop) seeds were sown into soils containing 0 to 10^6 g^{-1} soil resting spores of *P. brassicae* using a stepwise density distribution in plug trays (25 individual cells, 44-mm-

length × 44-mm-width × 49-mm-depth). After 5 weeks cultivation, the disease index of each seedling was determined to obtain a dose-response curve (DRC) pattern, requiring only half of the soil and planting area need with the conventional method using potted seedlings. Although this method was slightly sensitive to disease occurrence, comparing to that by conventional method, we obtained DRC patterns in the plug trays system similar to those obtained by the conventional system. Therefore, this simple assessment system is suggested to be applicable for the evaluation of soils for the risk of the disease occurrence.

Transcriptome changes in *Brassica napus* cultivars upon interaction with *Plasmodiophora brassicae* pathotype 5X L. GALINDO-GONZÁLEZ, S.F. HWANG AND S.E. STRELKOV *Department of Agricultural, Food & Nutritional Science, University of Alberta, AB, Canada, T6G 2P5*

With over 2,700 fields of canola (*Brassica napus* L.) infested by *Plasmodiophora brassicae* Wor. in Alberta, clubroot disease has become a major concern. Resistant cultivars are used to mitigate the impact of the pathogen, but this resistance was first broken in 2013 by a novel variant of pathotype 5 (Williams differentials) known as pathotype 5X. RNA-seq was used to identify key differentially expressed genes (DEGs) over a time-course in two *B. napus* cultivars with contrasting responses to this pathotype. At 7 days after inoculation (dai), the number of DEGs in ‘Laurentian’ (resistant cultivar) was much greater than in ‘Brutor’ (susceptible cultivar), and most genes were upregulated. This pattern was inverted at 14 and 21 dai. At 7 dai, ‘Laurentian’ activated more genes related to stress responses (dehydration, wounding, heat shock proteins), protein turnover and signalling compared with ‘Brutor’. At 14 dai the cultivars experienced a transition, with less DEGs but with some key genes (defense, hormones, cell wall, transcription factors) highly upregulated. Finally, at 21 dai, a strong downregulation in most genes was observed in ‘Brutor’, while ‘Laurentian’ still showed activity in numerous biotic stress-related genes. Candidate genes that can be modified by gene editing will be identified through this analysis to assess their roles in defense.

Identification of clubroot resistance QTLs in radish (*Raphanus sativus* L.) C. GAN, X. DENG, L. CUI, W. YUAN, X. YU, Y.P. LIM AND Z. PIAO *Institute of Economic Crops, Hubei Academy of Agricultural Sciences, Wuhan 430070, China; (Z.Y.P) College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China; (X.N.Y., Y.P.L.) Molecular Genetics and Genomics Lab, Department of Horticulture, Chungnam National University, Daejeon 305-764, Korea*

Clubroot is a devastating disease caused by *Plasmodiophora brassicae* and results in severe yield losses in cruciferous plants, including radish (*Raphanus sativus*). We first constructed a high-density linkage map using an F_2 population derived from a crossing between a partial clubroot resistant inbred line 'BJJ' and a susceptible inbred line 'XNQ'. The map consisting of 1148 SNPs was developed by restriction-site associated DNA sequencing (RAD-seq) technology and spanning 794.3 cM with an average distance of 0.7 cM between adjacent markers. Based on this high-density linkage map, we evaluated clubroot disease severity degree in F_3 progenies with two independent inoculation tests for QTL analysis. A total of five QTLs were identified and accounted for 5.23 to 7.65 of the LOD value, and 7.26 to 31.38% of phenotypic variance. The QTL *RsCr1* was on LG8 and another four QTLs were on LG9. Of these QTLs, *RsCr2* overlaps with *RsCr5*, detected in different tests. This high-density genetic map of radish could provide indispensable information for genetic and genomic research, and serve as a reference for effective gene exploration and for marker assisted-breeding programs.

Screening important secreted proteins during the process in *Brassica napus* infected by *Plasmodiophora brassicae* Z.X. GAO, Y. ZHAO, K. BI, J.T. XIE, J.S. CHENG, Y.P. FU AND D.H. JIANG *State Key Laboratory of Agriculture Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei Province, People's Republic of China*

It is of great value to understand the interaction of *Plasmodiophora brassicae* and its host at the early infection stage. In this research we constructed a hydroponic rapeseed inoculation method and inoculated the rapeseed with resting spores of *P. brassicae.* The spores were collected from the roots and total proteins were extracted 48 h after inoculation. The proteins were then analyzed by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

Proteins were annotated using the mascot search engine (Matrix Science, London, UK; version 2.3.02) against database containing genome sequences of *P. brassicae.* A total 860 proteins were identified in the treatment (spores collected from inoculated rapeseed roots) and 1237 proteins in the control (resting spores). Two hundred and seven unique proteins were found only in the treatment, among which 11 proteins that were predicted to be secretory proteins. From the whole genome, 865 putative secretory proteins were predicted, and combined with transcriptome data, 23 were chosen for further research. Expressing the genes by TRV in tobacco, we found that genes 01668 and 08125 could induce a strong HR and gene 08307 had a slight HR. Nine genes have been overexpressed in *Arabidopsis thaliana*. The study on the secreted proteins will help us to clarify the interactions between rapeseed and *P. brassicae*.

Decline in resting spores of *Plasmodiophora brassicae* in soil over 6 years B.D. GOSSEN, F. AL-DAOUD, T. DUMONCEAUX, J. DALTON, G. PENG, D. PAGEAU, AND M.R. MCDONALD *Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), 107 Science Place, Saskatoon, SK S7N 0X2, Canada; (F.A., J.D., M.R.M.) Department of Plant Agriculture, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada; and (D. P.) AAFC Research Farm, Normandin, QC G8M 4K3 Canada*

The concentration of resting spores of *Plasmodiophora brassicae* Wor. in a canola (*Brassica napus* L.) field has a major effect on clubroot severity and the risk of a breakdown in resistance. This study was carried out on soil samples collected from a long-term rotation study at Normandin, QC in 2014, with replicated plots representing a 0-, 1-, 2-, 3-, 5- or 6-year break following a susceptible canola crop heavily infested with clubroot. Five molecular techniques for estimating *P. brassicae* resting spores in soil were assessed, including quantitative polymerase chain reaction (qPCR), competitive internal positive control PCR (CIPC-PCR), droplet digital PCR (ddPCR), loop-mediated isothermal DNA amplification (LAMP), as well as propidium monoazide PCR (PMA-PCR) to assess spore viability. For several of the techniques, calibrations needed to be developed using spiked soil samples, where a known amount of resting spores were added. Each of the assays provided a similar pattern of spore decline over time. This result supported the conclusion of a previous study at this site, that resting spore numbers declined rapidly over the first 2 years after a susceptible crop, but the rate of decline was substantially

lower in subsequent years (type III survival curve). CIPC-PCR and ddPCR provided better estimates of resting spore numbers in soil compared with those from qPCR alone or LAMP. Estimates of viable spores from PMA-PCR were much lower than the estimates of spore numbers from the other techniques in the first year, but were similar thereafter.

A recipe for managing small patches of infestation of clubroot in canola B. D. GOSSEN, A. SEDAGHATKISH, S.F. HWANG AND M.R. MCDONALD *Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, 107 Science Place, Saskatoon, SK, S7N 0X2, Canada; (A.S., M. R. M.) Department of Plant Agriculture, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada, and (S. F. H.) Alberta Agriculture and Forestry, Crop Diversification Centre North, 17507 Fort Road, Edmonton, Alberta, T5Y 6H3, Canada*

Clubroot of canola (*Brassica napus*), caused by *Plasmodiophora brassicae* (Wor.), is spreading rapidly on the Canadian prairies. Genetic resistance can be extremely effective against clubroot but breaks down quickly under high disease pressure. A recipe for treating small areas of infestation has been proposed, as follows: mark the area (≥ 2× the area where symptoms occurred), apply lime (quick lime for rapid effect + standard lime for longer-term maintenance) to increase the soil pH to ~7.4, then seed a perennial grass crop. pH above 7.2 suppresses clubroot and grass crops such as perennial ryegrass (*Lolium perenne*) and smooth bromegrass (*Bromus inermis*) further reduced resting spores in soil. A grass cover also minimises the movement of spores from the treated area. Soil samples from the centre of the patch are used to determine when the resting spore levels drop below economic thresholds. Alternatives for reducing resting spore populations are solarisation or fumigation. Solarisation, achieved by covering the patch with totally impermeable film (TIF) for 16 days, increased mean soil temperatures by about 10 °C and reduced clubroot severity to 0% in 2016, but in 2017 only reduced severity from 81% to 35%. Addition of fumigants (chloropicrin or metam sodium) did not further reduce clubroot severity, but each was also effective on its own. However, application of fumigants requires specialized equipment and licences, and so is not available to most producers on the Canadian prairies. Crop rotation can also be effective, but takes longer.

Influence of nitrogen constraint on quantitative resistance to clubroot in *Brassica napus*
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Abiotic factors are known to influence quantitative resistance to plant pathogens, but underlying genetic and physiologic mechanisms are mostly unknown. In this work, we developed combined genetic and molecular physiology approaches to investigate the influence of nitrogen fertilization on quantitative resistance of *Brassica napus* to the clubroot causing agent *Plasmodiophora brassicae*. Disease response was studied in a panel of oilseed rape genotypes and *P. brassicae* isolates cultivated under low *vs* high nitrogen supplies. This work highlighted that lower nitrogen input can modulate disease symptoms (from strong symptom inhibition to no effect), depending on both plant genotype and *P. brassicae* isolate. QTL analysis conducted in a 'Darmor-*bzh*' x 'Yudal' doubled haploid progeny showed that nitrogen deficiency exerted a major switch between the effects of two QTL involved in resistance toward the isolate eH. One low-nitrogen-dependent QTL identified on chromosome C02 was found to exert a major effect on the resting spore content in infected roots, but moderately influencing club symptom development. By contrast, the effect of a major QTL involved in resistance towards the isolate K92-16 was unaffected by nitrogen fertilization. A combination of metabolomics and transcriptomics highlighted the putative role of nitrate transporter encoding genes, which are specially induced under the double biotic-abiotic stresses in the genotype 'Yudal' expressing low nitrogen-triggered resistance. Altogether, our results indicated that nitrogen fertilization influences clubroot disease in a QTL x isolate dependent manner. A better understanding of QTL x pathogen isolate x fertilization crosstalk may help to rationalize the use of clubroot quantitative resistance in breeding.

Molecular detection of *Plamodiophora brassicae*, the causal agent of clubroot of cruciferous crops
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Clubroot caused by *Plasmodiophora brassicae* is one of the most destructive diseases of cruciferous crops. The pathogen produces numerous resting spores that can survive in the soil for years. Pathogen dissemination is mainly through the movement of resting spores on seed, diseased plants, contaminated soil and farm equipment. In this study, a pair of PCR primers was designed based on the internal transcribed spacer (ITS) sequence of *P. brassicae*. This primer pair could be used in end-point PCR and quantitative PCR (qPCR). A specificity assay indicated that this primer pair could produce an amplicon against DNA extracted from *P. brassicae* but not from other plant pathogenic fungi, bacteria and nematodes as well as the endophytic bacteria isolated from the clubbed root. Sensitivity tests indicated that the end-point PCR could amplify a product from 1×10^{-6} ng of total *P. brassicae* DNA and DNA derived from 1×10^{3} resting spores. On the other hand, the sensitivity levels by qPCR analysis were 10^{-9} ng of total *P. brassicae* DNA and as low as 10 resting spores. This study provided an accurate and reliable method for detection of *P. brassicae* in host plants, soil and seed samples, which will be useful to forecast clubroot on cruciferous crops.

Evaluating chemical disinfectants for their ability to inactive *Plasmodiophora brassicae* resting spores using Evans blue staining M. W. HARDING, T. B. HILL, G. C. DANIELS, S. E. STRELKOV, S. F. HWANG AND J. FENG *Alberta Agriculture and Forestry, Crop Diversification Centre South, 301 Horticultural Station Road East, Brooks, Alberta T1R 1E6; (S.E.) University of Alberta, Agriculture Food and Nutritional Sciences, 410 Agriculture-Forestry Centre, Edmonton, Alberta T6G 2P5; (S.F.H., J.F.) Alberta Agriculture and Forestry, Crop Diversification Centre North, 17507 Fort Road NW, Edmonton, Alberta, T5Y 6H3*

Plasmodiophora brassicae Woronin, the causal agent of clubroot, has become an important disease on canola in Alberta. Its rapid spread across much of the Province has been due, in large part, to movement of infested soil on equipment. The most effective practice for avoiding the spread of *P. brassicae*-infested soil is equipment sanitization. Sanitization involves cleaning or washing away soil and plant material, followed by treatment with a chemical disinfectant.

However, the thick-walled resting spores produced by *P. brassicae* are known to survive exposures to physical and chemical treatments, making it challenging to predict which disinfectants may be effective. In order to make accurate recommendations for equipment sanitization it was important to know which disinfectants, if any, can quickly and effectively inactivate resting spores. Evans blue is a vital stain that can discriminate viable from non-viable resting spores. *P. brassicae* resting spores were treated with six concentrations (1%, 10%, 25%, 75% and 100%) of each of ten chemical disinfectants. After a 15 min exposure, the disinfectants were neutralized using a universal neutralizer solution. Spores were rinsed three times with sterile water and evaluated for their viability using the Evans blue staining method. Repeated experiments, and comparisons of spore staining results with paired plant bioassays, indicated that only two of the disinfectants tested were capable of achieving greater than 95% inactivation of resting spores; sodium hypochlorite and ethanol. Additional chemical disinfectants will be evaluated, but at the time of this presentation, a 2% sodium hypochlorite solution was found to be the most reliable, inexpensive, safe and effective treatment for inactivation of *P. brassicae* resting spores.

Molecular genetics of the clubroot resistance genes in Chinese cabbage (*Brassica rapa* L.)

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In *Brassica rapa* L., at least 11 clubroot resistance (CR) loci have been identified and two of them successfully cloned. However, uncertainties remain about the precise locations of the parts of CR loci and the relationships between the CR loci and pathotypes of the clubroot pathogen. Therefore, it is necessary to clone CR genes and evaluate their pathotype specificity. Recently we cloned and sequenced the genomic region of the *CRb* (CRb^{Kato}) locus identified in the cultivar 'Akiriso', demonstrating that this is a complex locus composed of at least six *R* genes in tandem with the same orientation, and that the gene with the *CRb* specificity and *CRa* are the same CR allele. Map-based cloning of the responsible genes for *CRk* and *Crr1b* is currently underway. Fine mapping of the *CRk* locus identified on *B. rapa* chromosome A03 revealed that two resistance loci were speculated to exist in the candidate region and showed different pathotype specificity. By sequencing of three BAC clones covering the *Crr1b*, the candidate region was

delimited to about 55 kb in the resistant line and at least four ORFs were tentatively identified. Cloning of CR genes will contribute to improving the pathotype classification system and marker-assisted selection in CR breeding.

Management of clubroot of canola in Alberta, Canada S.F. HWANG, H.U. AHMED, Q. ZHOU, H. FU, G.D. TURNBULL, R. FREDUA-AGYEMAN AND S.E. STRELKOV *Alberta Agriculture and Forestry, 17507 Fort Road NW, Edmonton, AB T5Y 6H3, Canada; (S.E.S.) Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB T6G 2P5, Canada.*

Plasmodiophora brassicae, the causal agent of clubroot of crucifers, poses a serious threat to Canadian canola production. Over the past 15 years, it has spread from 12 fields in central Alberta to over 2,700 fields across the province. The concentration of *P. brassicae* resting spores in the soil is a major determinant of clubroot severity, particularly for the new pathotypes. Control strategies have focused on reducing viable spore concentrations in the soil by rotating canola with non-host crops, planting bait crops, deploying resistant canola cultivars, and applying soil fumigants. Both non-host and bait crops reduced clubroot severity in bioassays relative to fallow treatments. A 2-3 year interval between canola crops reduced disease severity compared with a 1-year interval or no break between canola crops. Several 4-year rotation sequences with resistant canola cultivars reduced the severity of clubroot compared with continuous canola, although a 4-year non-host or fallow sequence reduced clubroot incidence to zero. Application of metam sodium or dazomet fumigants reduced clubroot severity and soil resting spore concentrations over a range of application rates (0.4–1. 6 mL L^{-1} soil), especially when applied into moist soil and incorporated with a rototiller. Covering the soil with a plastic moisture barrier after application enhanced fumigant efficacy.

Modulation of abscisic acid and ethylene levels in response to clubroot disease in *Brassica napus* C.P. JAYASINGHEGE, V.P. MANOLII, J.A. OZGA, S.F. HWANG AND S.E. STRELKOV *Department of Agricultural, Food and Nutritional Science, 410 Agriculture/Forestry Centre, University of Alberta, Edmonton, AB T6G 2P5, Canada; and*

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The plant defense mechanisms associated with clubroot (*Plasmodiophora brassicae* Wor.) disease of crucifers are not well understood. The plant hormones abscisic acid (ABA) and ethylene play direct and indirect roles in plant defense and stress responses. In this study, we compared the levels of ABA, ABA metabolites, and the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in the roots of a clubroot susceptible and a clubroot resistant cultivar of canola (*Brassica napus* L.). Hormone profiling was conducted by high-performance liquid chromatography-tandem mass spectrometry at 4, 14 and 21 days after inoculation (DAI). At 4 and 14 DAI, there were no or minimal differences in ABA and ACC levels between the two cultivars and inoculated and non-inoculated plants. By 21 DAI, however, the ABA level was 5-fold higher in the susceptible inoculated plants vs. the non-inoculated controls, indicating that the infected plants were experiencing drought stress conditions. Concomitantly, water demand in the susceptible, inoculated plants started to decline compared with the non-inoculated controls. Also at 21 DAI, the levels of ACC were 2.5- and 4-fold higher in inoculated plants of the susceptible and resistant cultivars, respectively, than in the non-inoculated controls. This suggests a role of ethylene in the clubroot-associated stress responses and disease resistance of *B. napus*.

Occurrence of *Plasmodiophora brassicae* in agricultural soils, pathotype variation and means of clubroot control in Poland M. JEDRYCZKA, M. KORBAS, A. CZUBATKA, K. MARZEC-SCHMIDT, A. NIEROBCA, J. KACZMAREK, E. JAJOR, N. RAMZI, AND W. IRZYKOWSKI *Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479, Poznan, Poland; (M.K., E.J.) Institute of Plant Protection – National Research Institute, Węgorka 20, 60-318 Poznan, Poland; (A.C.) Research Institute of Horticulture, Department of Vegetable and Ornamental Plants Protection, Kościuszki 2, 96-100 Skierniewice, Poland; (K.M.-S.) Department of General Botany, Institute of Experimental Biology, Faculty of Biology, University of Adam Mickiewicz, Umultowska 89, 61-614 Poznan, Poland; (A.N.) Institute of Soil Science and Plant Cultivation, State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland*

Plasmodiophora brassicae occurs in agricultural soils as well as in low and high peat bogs exploited for the production of vegetables and ornamentals. The primary inoculum attacks several crops, but the secondary inoculum is limited to the *Brassicaceae* family, causing the disease referred to as clubroot. Oilseed rape covers the highest acreage of *Brassica* crops and most current cultivars of oilseed rape are susceptible to clubroot. The disease occurrence has been monitored in Poland for the last several years and we have found clubroot in most regions of intensive cultivation of oilseed rape. The correlation between soil pH and the concentration of *P. brassicae* resting spores in the soil samples was weakly negative (−0.495). No spores were detected in three of four soils of pH ≤ 6.8, but a substantial amount of spores (4.7×10^6 resting spores/g soil) has been also found in soil of pH 6.9. Moreover, resting spores were also detected in soils of pH up to 7.6. Taking into account the longevity of resting spores and their high resistance to chemical treatments, an assessment of *P. brassicae* infestation levels before cultivation is one of the most effective ways to avoid yield losses. Early detection of the pathogen in soil facilitates disease control by avoiding the cultivation of susceptible crops. Therefore, our activities concentrated on detection of the pathogen in plants, soil and small water reservoirs in fields of oilseed rape and agricultural soils in general. The detection was done using bioassays as well as molecular approaches, including PCR, real-time PCR and LAMP techniques. Depending on the evaluation system used, we have detected up to nine pathotypes of *P. brassicae* in Poland.

Introgression of clubroot resistance and analyses of segregation distortion in two F_2 populations derived from *Brassica rapa* subsp. *rapifera* (ECD 02) JUNYE JIANG, RUDOLPH FREDUA-AGYEMAN, SHEAU-FANG HWANG AND STEPHEN E. STRELKOV *Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, T6G 2P5; (R.F.A, S.F.H.) Alberta Agriculture and Forestry, 17507 Fort Road NW, Edmonton, AB, T5Y 6H3, Canada*

Resistance breeding is the most effective way to manage clubroot, a soilborne disease of crucifers caused by *Plasmodiophora brassicae* Wor. In this study, *Brassica rapa* L. subsp. *rapifera* (European Clubroot Differential, ECD 02), which possesses broad spectrum resistance to many pathotypes of *P. brassicae*, was crossed with two clubroot-susceptible *B. rapa*

accessions to produce two F_2 populations of 1103 (Popl#1) and 464 (Popl#2) individuals. A Chi-square test for the goodness of fit for the F_2 plants of Popl#1 screened with pathotypes 5X (7R : 9S, χ^2 = 0.8372, *df* = 3, *P* = 0.8406) and 5G (6R : 10S, χ^2 = 5.1933, *df* = 3, *P* = 0.1578) indicated a variation of the 9:3:3:1 segregation ratio expected for control of resistance by two dominant genes. Similarly, a Chi-square test for the F_2 plants of Popl#2 screened with pathotype 5G (11R: 5S, χ^2 = 0.2402, *df* = 3, *P* = 0.9709) suggested a variation of the ratio expected for two dominant genes. In contrast, the 3:1 segregation ratio obtained for the F_2 plants of Popl#2 screened with pathotype 5X (χ^2 = 0.4099, *df* = 1, *P* = 0.5271) was consistent with genetic control of resistance by a single dominant gene. Preliminary molecular marker analysis suggests that one dominant gene and possibly one QTL control clubroot resistance introgressed from ECD 02.

Using knowledge on plant hormone metabolism by *Plasmodiophora brassicae* - a possibility to control the clubroot pathogen? S. JÜLKE, D. SEIDLER, R. MENCIA, E. WELCHEN, J. LUDWIG-MÜLLER *Institut für Botanik, Technische Universität Dresden, 01062 Dresden, Germany;(R.M., E.W.) Instituto de Agrobiotecnología del Litoral (CONICET-UNL), Cátedra de Biología Celular y Molecular, Facultad de Bioquímica y Ciencias Biológicas, Universidad Nacional del Litoral, 3000 Santa Fe, Argentina*

Clubroot disease symptoms in the Brassicaceae, caused by the soilborne obligate biotrophic pathogen *Plasmodiophora brassicae,* are determined by the modulation of plant hormones such as auxins and cytokinins, inducing hypertrophy and metabolic sinks in the root galls. Alterations in defense hormones can also occur. *Arabidopsis thaliana* is used as a model host to understand the molecular biology underlying these processes. The genome sequence of *P. brassicae* has opened up novel approaches to study this pathogen. We could identify several genes encoding putative plant hormone metabolizing enzymes, such as a SABATH-type methyltransferase, but also a GH3-family protein involved in the conjugation and thereby inactivation of indole-3-acetic and jasmonic acids. Further, a functional cytokinin oxidase was found. We have studied why the treatment of host plants with the defense compound salicylic acid (SA) did not result in increased tolerance and found that the methyltransferase is able to methylate mainly SA and to a lesser extent benzoic and anthranilic acids. Overexpression of this gene, *PbBSMT*, in Arabidopsis resulted in plants

more susceptible to the clubroot pathogen. Selected mutants with higher constitutive levels of SA, however, were more tolerant to *P. brassicae*. The possible roles for the other plant hormone metabolizing enzymes will be discussed.

Can *Brassica rapa* contribute a new clubroot resistance gene for use in the breeding of *Brassica napus* canola for Canada? KAWALPREET KAUR AND HABIBUR RAHMAN
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Clubroot disease of crucifers, caused by *Plasmodiophora brassicae*, is a threat to Brassica crop production. Various pathotypes of this pathogen have been reported in different countries and clubroot causes about 10-15% yield loss worldwide. This disease has been reported in canola fields in North America since 2003; therefore, the development of clubroot resistant canola cultivars has been a priority for canola breeders. Different *Brassica* accessions have been reported to carry resistance to different pathotypes of *P. brassicae*. The objective of this research was to introgress resistance to pathotype 3 from *B. rapa* into *B. napus* canola. For this, an interspecific cross between a spring *B. napus* canola and *B. rapa* var. *rapifera* was made and conventional breeding was followed. The self-pollinated populations were grown in a greenhouse and selection for resistance to *P. brasssicae* pathotype 3 was done, from where several resistant families were developed. Genotyping of this population will be conducted with simple sequence repeat (SSR) markers, including the published markers reported to be associated with resistance, to identify the markers linked to the clubroot resistance gene for use in marker-assisted selection. The materials developed in this study can be used in breeding programs to develop clubroot resistant *B. napus* canola cultivars.

Development of isolate-specific molecular markers for detecting Korean *Plasmodiophora brassicae*; the causal agent of clubroot H.-T. KIM, J.-Y. JEONG, J.-I. PARK AND I.-S. NOU
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Clubroot, caused by an obligate parasite *Plasmodiophora brassicae*, is one of the most economically important diseases of the *Brassicaceae* family. In Korea at least five races of the pathogen, namely races 1, 2, 4, 5 and 9, have been detected and fall under four pathotype groups. This study was planned to develop isolate-specific markers by exploiting genomic sequence variations. A total of 119 markers were developed based on unique variation in genomic sequences of each of the races. Only 12 markers were able to detect *P. brassicae* strains under each isolate/race/pathotype. Ycheon9 and Ycheon10 markers were specific to the Yeoncheon isolate (race 2, pathotype 3); Ycheon14 markers were specific to race 2-isolates Yeoncheon and Hoengseong; ZJ1-3, ZJ1-4 and ZJ1-5 markers were specific to Haenam2 (race 4) isolate; ZJ1-35, ZJ1-40, ZJ1-41 and ZJ1-49 markers were specific to Hoengseong isolate; and ZJ1-56 and ZJ1-64 markers were specific to the Pyeongchang isolate (race 4, pathotype 3). PCR-based SCAR markers developed in this study were able to detect five Korean isolates of *P. brassicae.* These markers can be utilized to identify Korean *P. brassicae* isolates from different regions. Additional effort is required to develop isolate-specific markers for the remaining Korean isolates.

Clubroot resistance genes in *Brassica rapa*: utility for differentiation of *Plasmodiophora brassicae* pathotypes and CR breeding in *Brassica* crops XIAONAN LI, WENXING PANG, ZHONGXIANG ZHAN, YUE LIANG, CHHUNYU ZHANG, ZHONGYUN PIAO *College of Horticulture, Shenyang Agricultural University, Shenyang, China, 110866; (W.X.P., Z.X.Z., Z.Y.P) College of Horticulture, Shenyang Agricultural University, Shenyang, China, 110866; (Y.L.) College of Plant Protection, Shenyang Agricultural University, Shenyang, China, 110866; (C.Y.Z.) College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China, 430070*

Clubroot disease, one of the most devastating diseases of *Brassica* crops, is increasingly prevalent in *Brassica* vegetable and oil crops in China. *Plasmodiophora brassicae,* the causal agent of clubroot, undergoes rapid variation, leading to the fast erosion of clubroot resistance (CR). Therefore, accurate classification and identification of *P. brassicae* pathotypes is fundamental to guiding the breeding of CR cultivars, the most economic and efficient approach of clubroot management. So far, more than 10 CR loci showing pathotype-specific resistance

have been identified in *B. rapa*. Mining of CR germplasm provides an opportunity to differentiate *P. brassicae* pathotypes, in addition to the Williams' differential set and the European Clubroot Differential set. Based on Williams' system, four pathotypes have been found in China. However, 12 pathotypes were differentiated with a newly developed Sinitic Clubroot Differentiation set consisting of different CR genotypes of *B. rapa* with known or unknown *CR* genes. These CR genotypes were successfully used to transfer *CR* genes into Chinese cabbage (*B. rapa*), Zhacai (*B. juncea*) and rapeseed (*B. napus*), based on which new cultivars were released. Understanding the molecular mechanisms of clubroot resistance, the interaction between *CR* genes and *P. brassicae* pathotypes, and the genetic interaction between *CR* genes will accelerate the breeding process for durable clubroot resistance.

Verification of two candidate genes resistance to *Plasmodiophora brassicae* in broccoli Z. S. LI, X. L. ZHANG, Y. M. LIU, Z.Y. FANG, L. M. YANG, M. ZHUANG, Y. Y. ZHANG AND H. H. LV *Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China, +86 100081; Key Laboratory of Biology and Genetic Improvement of Horticultural Crops, Ministry of Agriculture Beijing, China, +86 100081*

Clubroot is one of the most devastating diseases of the *Brassicaceae* family. In this study, two candidate resistance genes for *Plasmodiophora brassicae* in broccoli were detected based on a transcriptome analysis method. Two genotypes of broccoli, one clubroot-resistant wild cabbage B863 (*Brassica macrocarpa* Guss.) and another clubroot-susceptible broccoli B196 (*Brassica oleracea* var. *italica*), were inoculated with *Plasmodiophora brassicae* and sequenced in young plants. The results revealed that two differentially expressed genes *Bol R1* and *Bol R2* might be related to the defense response against *Plasmodiophora brassicae*. Both genes were located on scaffold 000004 on chromosome C09. Bioinformatics and genome annotation of cabbage suggests that *Bol R1* belongs to the NBS gene family while *Bol R2* is not annotated. The candidate genes *Bol R1* and *Bol R2* were both verified by agrobacterium-mediated genetic transformation in *Arabidopsis thaliana* and broccoli, respectively, and positive plants were obtained from T0 generation of *A. thaliana*. The result of this analysis will provide new insights into clubroot resistance in *Brassica* plants.

Genetic analysis of clubroot resistance using multiple populations in *Brassica rapa* Y.P. LIM, S.R. CHOI AND S. PARK *Department of Horticulture, Chungnam National University, Daejeon 305-764, Korea; (S.P.) Vegetable Research Division, National Institute of Horticultural & Herbal Science, Suwon 441-440, Korea*

Clubroot disease is one of the most serious soilborne contagious diseases of *Brassica* crops and is caused by *Plasmodiophora brassicae*. Traditional QTL mapping is used with a bi-parental mapping population and can identify a significant locus. In comparison, Genome-Wide Association Study (GWAS) is an approach that involves scanning genetic loci associated with traits across natural populations and may screen out all candidate loci contained in populations. In this study, we used a joint QTL and association-mapping approach with 232 inbred lines and 3 years of replications, resulting in the identification of associated SNP markers. Most of these were located on chromosomes A2 and A3, as already known, while several new candidate loci were identified on A6 and A9. This study may serve as the basis for further studies to identify clubroot resistance or defense-associated genes, and in marker-assisted selection for clubroot resistance breeding.

Fine mapping of *BraCrr5,* a novel gene conferring resistance to clubroot disease (*Plasmodiophora brassicae* Woronin) in *Brassica rapa* **L. L.X. MAO, Y.X. YUAN, S.J. YANG, X.C. WEI, Y.Y. ZHAO, Z.Y. WANG, X.W. ZHANG AND B.M. TIAN** *(L.X.M., X.W.Z., B.M.T.) College of Life Science, Zhengzhou University, Zhengzhou, Henan, P. R. China; (L.X.M., Y.X.Y.,S.J.Y.,X.C.W.,Y.Y.Z.,Z.Y.W., X.W.Z.) Institute of Horticulture, Henan Academy of Agricultural Sciences,Zhengzhou City, Henan, P. R. China.*

Clubroot, caused by *Plasmodiophora brassicae* Woronin, is an important disease of Chinese cabbage (*Brassica rapa* L. ssp. *pekinesis*) in China and worldwide. In this study, a novel CR resistance gene *BraCrr5* from ECD01-1 (a European Clubroot Differential inbred line) was identified and preliminary mapped to an interval of 0.5Mb between 0.45 and 1.95 Mb on chromosome A08 via Bulked Segregant DNA Sequencing (BSA-seq). Three Leucine-rich repeat receptor-like protein kinase (LRR-RLK) genes (*BraA08000045*, *BraA08000049* and

BraA08000154) were identified in the target region. A total of 3379 single-nucleotide polymorphisms (SNPs) were detected between one pooled resistant (R) and one pooled susceptible (S) sample in the region. One-hundred thirty three polymorphic SNP sites were nonsynonymous among them. A segregating population consisting of 520 plants was analyzed with 12 SNP sites in the region using the Kompetitive Allele Specific PCR (KASP) method, further narrowing the candidate region to the interval between 0.4 and 1.1 Mb. Five robust SNP markers associated with *BraCrr5* were developed. These markers could provide an effective and robust basis for introgression of *BraCrr5* into Chinese cabbage by marker assisted selection.

Development of a highly clubroot-resistant F_1 cultivar Chinese cabbage (*Brassica rapa* L.) carrying three resistance genes, *Crr1, Crr2* and *CRb* S. MATSUMOTO *Institute of Vegetable and Floriculture Science, NARO, 360 Kusawa, Ano, Tsu, Mie, 514-2392, Japan*

Clubroot-resistant (CR) Chinese cabbage (*Brassica rapa* L.) cultivars play an important role in controlling the disease. However, the breakdown of resistance in CR cultivars caused by genetic variability in the clubroot pathogen (*Plasmodiophora brassicae* Wor.) has been observed. Four pathotypes (group 1 to group 4) of *P. brassicae* have been identified in Japanese field isolates using two commercial CR F_1 cultivars of Chinese cabbage as differential hosts. 'Parental line No.9' (PL9) carries the CR loci *Crr1* (*Crr1a* and *Crr1b*) and *Crr2*, which were introduced from the genetic resource 'G004' derived from the European fodder turnip 'Siloga', and shows strong resistance to *P. brassicae* isolates from the pathotype group 1, which is pathogenic to most of Japanese Chinese cabbage F_1 cultivars. It also shows strong resistance to groups 2 and 4, but not to group 3. The parents of the F_1 hybrid 'Akiriso', T-line and V-line, were crossed with PL9. A T-line harboring another CR gene, *CRb* (CRb^{Kato}), confers resistance to pathotype group 3. The progenies were selected by DNA makers linked to *Crr1* and *Crr2*, and backcrossed with each parent. Since CRb^{Kato} is a dominant resistance gene, the F_1 hybrid between the new parental lines was highly resistant to all isolates from pathotype groups 1 to 4. Clubroot resistance breeding with DNA markers is also used for other vegetables and rapeseed (*Brassica rapa* and *Brassica napus*).

Management of clubroot: An overview of the challenges M.R. MCDONALD AND B.D. GOSSEN *Department of Plant Agriculture, University of Guelph, 50 Stone Road East, Guelph, ON N1G 2W1, Canada;*, (BDG) *Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada (AAFC), 107 Science Place, Saskatoon, SK S7N 0X2, Canada*

Management of clubroot (*Plasmodiophora brassicae* Wor.) is a challenge worldwide, wherever brassica crops are grown. Major gene resistance has been an effective short-term strategy, but is not sustainable because of the high risk of erosion of resistance. To reduce this risk, populations of resting spores in soil must be monitored and kept low. However, spore populations in soil are difficult to quantify. Molecular approaches have been developed to quantify total or viable resting spores in soil, but these cannot reduce the high levels of variability that are present both horizontally and vertically within the soil profile. For example, resting spore concentration ranged from 10^3 to 10^6 within a 0.4 ha spot in a heavily infested field. Also, spore levels in heavily infested fields can be so high ($> 10^7$) that a reduction of 99% would leave enough spores to cause severe clubroot, which makes assessment of management strategies more difficult. Management strategies need be used in combination to be effective. These include rotation and / or stacking of major resistance genes and quantitative resistance genes, in combination with crop rotation, seeding into cool soil (5-14 °C), soil amendments with lime, boron or calcium cyanamide, solarization, fumigation, and use of non-host crops to stimulate the germination of resting spores. None of these approaches are effective on their own, so effective combinations must be identified that are economical and sustainable alternatives for producers.

Identification of a novel clubroot resistance QTL in Chinese cabbage (*Brassica rapa* L.) JONG-IN PARK, RAWNAK LAILA, ARIF HASAN KHAN ROBIN, HOY-TAEK KIM AND ILL-SUP NOU *Department of Horticulture, Sunchon National University, Suncheon 57922, Republic of Korea*

Plasmodiophora brassicae Wor. is a soil-borne plant pathogen that causes clubroot disease, resulting in crop yield losses in cultivated Brassica species. Here, we investigated whether a quantitative trait locus (QTL) in *Brassica rapa* L. might confer resistance to a Korean pathotype or isolate, Seosan. We identified and mapped a novel clubroot resistance QTL using a mapping

population that included susceptible Chinese cabbage and resistant turnip lines. We crossed resistant and susceptible parental lines and analyzed the segregation pattern in 348 F2 plants. A 3:1 ratio was observed for resistant: susceptible genotypes, which is in accordance with Mendelian segregation. Further, 45 resistant and 45 susceptible F2 plants along with their parental lines were used for double digest restriction site-associated DNA sequencing (ddRAD-seq), which identified a new locus, CRs, on chromosome A08, which is different from the clubroot resistance (CR) locus Crr1. The newly identified locus is novel since the Akimeki cultivar bearing Crr1 was susceptible to the Seosan isolate. These results could be exploited to develop molecular markers that can be helpful to develop Seosan resistant Chinese cabbage cultivars.

The effect and durability of incomplete resistance, based on two clubroot resistance genes, against a new pathotype of *Plasmodiophora brassicae* G. PENG, T. SONG, N. TONU, K. HORNADAY, J. LEE, J. BUSH, R. WEN AND F. YU *Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, Saskatchewan S7N 0X2, Canada*

Single-gene resistance against clubroot (*Plasmodiophora brassicae* Woronin) is often short lived. For example, in Canada almost all monogenic resistant canola (*Brassica napus* L) cultivars became susceptible to new pathotypes of *P. brassicae* only 3-4 years after introduction. The objectives of this study were to examine root infection in new canola hybrids carrying two clubroot resistance (CR) genes (A3, A8) that showed incomplete (moderate) resistance to the pathotype X of *P. brassicae*. The durability of resistance was also assessed by inoculating two of double CR-gene hybrids with the inoculum of a field pathotype X population from the prior planting cycle over five generational cycles (6 weeks per cycle) under controlled environment. Root infection was examined using fluorescent microscopy. At 10 and 35 days post inoculation (dpi), plasmodia, zoosporangia and/or resting spores were seen in both resistant and susceptible roots, but fewer were found in resistant roots, especially in cortical tissues at 35 dpi. Enhanced lignin autofluorescence was observed in the cell wall of resistant roots. In repeated resistance durability tests, harvested galls were let mature in damp soil for 3 weeks before being mixed thoroughly in recycled growth media. Resting spores in the media were quantified using qPCR

just before each planting. After being exposed to the same pathotype population for five cycles, the two double CR-gene hybrids maintained the moderate level of resistance. The inoculum concentration increased slightly in the media of susceptible canola lines (controls), but decreased slightly in the media of resistant hybrids during the 5 generational cycles.

Genome-wide transcriptomic analysis used to identify putative effector proteins in *Plasmodiophora brassicae* E. PÉREZ-LÓPEZ, J. TU, M. WALDNER, A. J. KUSALIK, M. HOSSAIN, C. D. TODD AND P. C. BONHAM-SMITH. *Department of Biology, University of Saskatchewan (U of S), Saskatoon SK S7N 5E2, Canada; (M.W., A.J.K.) Department of Computer Science, U of S, Saskatoon SK S7N 5C9, Canada.*

Plasmodiophora brassicae (Wor.) is an obligate plant pathogen, which makes study of its biology and mechanism of infection difficult. Identification of effector proteins could be an important key to understanding the interaction between this pathogen and the genes involved in host resistance. *Arabidopsis thaliana* ecotype Columbia (Col.0, susceptible to clubroot), was used as a model plant host. Each 13-day-old seedling was inoculated at the base of the stem with 400 µL of 5×10^7 resting spores/mL from clubbed roots of canola plants. Root tissue from inoculated and control plants were collected at 17, 20 and 24 days post-inoculation (dpi) for RNA-seq analysis, with 24 plants per biological replicate, 2 biological replicates, and 3 technical replicates. After RNA extraction and library construction, library sequencing was conducted from both ends on an Ilumina HiSeq 2500. More differentially expressed genes (DEGs) were identified at 24 dpi relative to the two earlier time points. RNAseq data and a bioinformatics pipeline were used to identify 32 small-secreted proteins of *P. brassicae* that were highly expressed in *Arabidopsis*. BLAST2GO, 3D structure, and phylogenetic analysis indicated that at least a quarter of these proteins were involved in cell division and plant defense manipulation. Further studies will be performed to identify the subcellular localization of the proteins and to assess their role in infection and subsequent symptom development.

Gene similarity of *Plasmodiophora brassicae* collections from Canada A. SEDAGHATKISH, B. D. GOSSEN AND M. R. MCDONALD *Department of Plant Agriculture, University of*

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Clubroot, caused by *Plasmodiophora brassicae* Wor., is generally managed using resistant cultivars, but new, virulent pathotypes are increasing rapidly on canola (*Brassica napus* L.) in Canada. Information on genetic similarity among pathogen populations could inform the development of sustainable management approaches. The objective of this study was to develop whole-genome sequences of pathogen collections from across Canada, assess their genetic similarity, and compare with collections from the USA, China and Europe. In total, 52 single-spore and field collections from clubbed roots were increased on the highly susceptible 'Mei Qing Choi' (*Brassica rapa var. chinensis*). DNA was extracted from clubs and *P. brassicae*-induced callus cultures. Sequencing reads were mapped to the published genome of *P. brassicae* isolate e3 (from Europe) to remove plant DNA sequences. A phylogenetic tree prepared using R software clustered the collections into five clades. The collections from Canada were separated into four clades: one from the Prairie provinces, one of new pathotypes from Alberta, and two clades from eastern / central Canada plus British Colombia. The collections from China formed the fifth clade. There was some overlap among clades. The similarity between samples collected from a site before and after a change of pathotype was generally low. Collections from the USA grouped with eastern / central Canada. This indicated that collections across much of North America differed from the initial collections in Alberta, which in turn differed from the new pathotypes.

Production of single-spore isolates of *Plasmodiophora brassicae* using micromanipulation of resting spores A. SEDAGHATKISH, B. D. GOSSEN, J. SINGH AND M. R. MCDONALD *Department of Plant Agriculture, University of Guelph, 50 Stone Rd E, Guelph, ON N1G 2W1, Canada; (B. D. G.) Agriculture and Agri-Food Canada, 107 Science Place, Saskatoon, SK S7N 0X2, Canada; (J. S.) Veterinary Biomedical Sciences, University of Saskatchewan, 52 Campus Drive, Saskatoon SK S7N 5B4 Canada*

Clubroot, caused by *Plasmodiophora brassicae* Wor., is an important disease of Brassicaceae crops. The molecular basis of clubroot pathogenicity and resistance are poorly understood.

Extraction of high quality DNA of single spores for genomic and genetic studies is a challenge because *P. brassicae* often occurs as a mixture of pathotypes, is associated with other soil microbes, and cannot be cultured in the absence of host tissue. The objective of this study was to isolate single spores for inoculation and high quality DNA for downstream applications. A technique was developed to isolate and culture single resting spores of *P. brassicae* using micromanipulation. Spores were extracted from clubbed roots, were isolated using a micromanipulator and inoculated individually onto the root of three-day-old seedling of the highly susceptible 'Mei Qing Choi' (*Brassica rapa* var. *chinensis*) grown in sterile Hoagland's solution media. Clubroot formation was visible in 8% of inoculated plants at 6 weeks after inoculation. This is a high rate of success compared with other published methods for inoculation with single spores. The approach was also effective for inoculation of root tissue sections that had been plated onto solid MS media. This method is fast and efficient and results in clean single-spore isolates of *P. brassicae* for molecular and genomic studies.

The changing face of clubroot (*Plasmodiophora brassicae*) in Canada S.E. STRELKOV, S.F. HWANG, M.D. HOLTZ, V.P. MANOLII AND M.W. HARDING *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada; (S.F.H.) Crop Diversification Centre North, Alberta Agriculture and Forestry, Edmonton, AB, T5Y 6H3, Canada; (M.D.H.) Field Crop Development Centre, Alberta Agriculture and Forestry, 5030-50 Street, Lacombe, AB T4L 1W8, Canada; (M.W.H.) Crop Diversification Centre South, Alberta Agriculture and Forestry, Brooks, AB, T1R 1E6, Canada.*

Clubroot, caused by *Plasmodiophora brassicae* Wor., has become an important disease of canola (*Brassica napus* L.) in western Canada. Despite efforts to slow its spread, the number of confirmed *P. brassicae* field infestations has increased from just 12 in 2003 to more than 2,700 by 2017. Management of clubroot relies mainly on the planting of clubroot resistant (CR) canola cultivars. Unfortunately, populations of *P. brassicae* capable of overcoming the resistance in most cultivars have been identified with increasing frequency since 2013. At present, the erosion or loss of resistance has been documented in 104 fields. Characterization of pathogen field isolates on the hosts of the Canadian Clubroot Differential (CCD) Set revealed the occurrence of 17 distinct pathotypes of *P. brassicae* in Canada, most of which are virulent on CR canola.

These include pathotype 5X, which represents the first field isolates found to overcome resistance, and pathotype 3A, which is predominant among all collections examined from CR canola. An evaluation of genetic diversity within *P. brassicae* by restriction site-associated DNA sequencing showed that pathotype 5X is distinct from older pathotypes that do not cause clubroot on resistant canola. Testing of root galls using quantitative PCR assays developed to distinguish *P. brassicae* populations that are virulent or avirulent on CR canola indicated that virulent strains of the pathogen were present at a low frequency prior to the introduction of clubroot resistance. Collectively, these results indicate that the canola crop is still at risk from *P. brassicae* in Canada.

Detection service for clubroot resting spores by the LAMP method in Japan K. WAKAYAMA, T. USUI, M. OKADA AND Y. KAWAHARA *Plant Hospital of Plant Science, Vegetalia, Inc. Tokyo University Food Science 304, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8654 Japan*

In Japan, *Plasmodiophora brassicae*, the cause of clubroot, is a serious pathogen of cabbage, Chinese cabbage, broccoli and other crops in the Brassica family. If this pathogen becomes established in the soil throughout a field, serious yield loss can occur. The use of pesticides for the management of clubroot costs more than 3 billion yen/year in Japan. The aim of this work was to implement a fast and reliable screening method to detect *P. brassicae*. By quantifying clubroot in the soil, we eliminate unnecessary control and help to identify what kind of control measures might be appropriate. If necessary, infestation maps can be generated, helping to prevent spread of the pathogen before it occurs. The loop-mediated isothermal DNA amplification (LAMP) technique was selected for detection of *P. brassicae*, since LAMP is easy, fast and accurate. By applying the LAMP protocol, detection and quantification of *P. brassicae* also can be conducted at a reasonable cost. The results of sample analysis can be obtained within about 1 week. The detection limit with the LAMP technique is 1,000 spores/g soil, which is similar to that reported with quantitative PCR-based assays. The technique is simple, so it is a good alternative to other methods when it comes to practical application and the assessment of numerous samples.

Integrated management of clubroot - crucial for a sustainable oilseed rape production A.C. WALLENHAMMAR, Z. OMER AND A. JONSSON *Rural Economy and Agricultural Society|HS Konsult AB. P.O. Box 271, SE- 701 45 Örebro, Sweden; (Z.O.) Rural Economy and Agricultural Society|HS Konsult AB. P.O. Box 412, SE- 750 12 Uppsala, Sweden; (A.J.) RISE Agrifood and Bioscience, P.O. Box 63, SE-532 21 Skara, Sweden*

Clubroot is a serious threat to oilseed rape (OSR) production in Sweden and genetic resistance is the most important factor in a cropping strategy. A new project aiming at developing a concept for integrated production of winter OSR supported by DNA technology started in 2017. The objective is to provide an improved decision support and guidelines for growing winter OSR in fields where *Plasmodiophora brassicae* DNA occurs. *Plasmodiophora brassicae* infestation levels and yield of clubroot resistant (CR) and susceptible cultivars were investigated at four field sites established in 2017 in south and central Sweden. Three CR resistant cultivars of winter OSR were selected together with a mixture of susceptible cultivars. The selected fields showed *P. brassicae* at levels ranging from 5,000 to 2.5 million target copies g^{-1} soil at pre-sampling in July. Soil samples were then collected plot-wise immediately prior to seeding the trials. Quantification of *P. brassicae* by qPCR was performed and bioassays were carried out to ensure optimal infection of the tested cultivars. Plants were sampled plot-wise in late autumn and roots were assessed for disease symptoms. Preliminary results will be discussed as soil DNA-analyses will be correlated with disease severity and yield.

Effects of root exudates on *Plasmodiophora brassicae* resting spore germination Y. WANG, B. KOOPMANN, P. KARLOVSKY AND A. VON TIEDEMANN *Department of Crop Sciences, Georg-August University Göttingen, Grisebachstrasse 6, 37077, Göttingen, Germany*

Clubroot, caused by the obligate parasite *Plasmodiophora brassicae* Woronin, is one of the most devastating soil-borne diseases of cruciferous crops throughout the world. Germination of dormant *P. brassicae* resting spores is essential for the development of clubroot. Root exudates of several host and non-host plants can stimulate resting spore germination in laboratory experiments. A hydrophobic root exudates trapping system based on a hydroponic sand substrate

system was established for continuous trapping of exudates from undisturbed living roots. The system provided sterile growth conditions for the experimental plants in order to exclude any modulations of root exudates by microbes. Hoagland's solution was continuously circulated through the root system and a XAD8 resin column, followed by a XAD4 resin column. Extracellular hydrophobic metabolites were selectively adsorbed by the resin, while inorganic nutrients were recycled to sustain plant growth. Metabolite profiling by HPLC-MS was conducted on root exudates of the host plants (oilseed rape) and non-host plants (tomato and ryegrass). Candidate substances were selected and their effect on the germination of resting spores will be tested in bioassays. The objective of this study is to identify the substances that trigger the germination of *P. brassicae* resting spores.

Infection of canola with *Plasmodiophora brassicae* increases resistance to herbivory by Bertha armyworm, *Mamestra configurata* C.D.S. WEERADDANA, V.P. MANOLII, S.E. STRELKOV, A.P. DE LA MATA, J.J. HARYNUK AND M.L. EVENDEN *Department of Biological Sciences, University of Alberta, Edmonton, Canada; (V.P.M., S.E.S.) Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada; (A.P.D.L.M., J.J.H.) Department of Chemistry, University of Alberta, Edmonton, Canada*

Canola (*Brassica napus* L.) is an economically important crop grown in Canada. On the Canadian Prairies, clubroot disease, caused by the soil borne protist *Plasmodiophora brassicae* Woronin, has emerged as an important economic pest that impacts canola production. Bertha armyworm (BAW), *Mamestra configurata* Walker (Lepidoptera: Noctuidae), is a generalist herbivore and significant insect pest of canola in Canada. Both *P. brassicae* infection and BAW infestation occur in the agroecosystems of Alberta canola fields, and thus it is important to study the potential interaction between *P. brassicae*-infected plants and BAW to properly manage both threats. The effect of *P. brassicae* inoculation on subsequent BAW oviposition and offspring performance was tested using *P. brassicae* susceptible and resistant canola. Fewer eggs were laid on susceptible canola inoculated with *P. brassicae* when compared with non-inoculated susceptible canola. A similar number of eggs, however, was laid on inoculated and non-inoculated resistant canola. Inoculation with *P. brassicae* influenced larval development as pupae weighed more when reared on non-inoculated as compared with inoculated susceptible

canola. Higher levels of salicylic acid were found in *P. brassicae*-inoculated susceptible canola as compared with non-inoculated canola. Analysis of the volatile organic compounds (VOCs) released from the variously treated plants showed that *P. brassicae*-inoculated plants emit a different VOC profile than non-inoculated plants.

Genetic diversity of rutabaga accessions as sources of clubroot resistance ZHIYU YU, RUDOLPH FREDUA-AGYEMAN, SHEAU-FANG HWANG AND STEPHEN E. STRELKOV *Department of Agricultural, Food and Nutritional Sciences, University of Alberta, Edmonton, AB, T6G 2P5; (R.D.F., S.F.H.) Alberta Agriculture and Forestry, 17507 Fort Road NW, Edmonton, AB, T5Y 6H3, Canada*

Rutabaga or swede (*Brassica napus* L. spp. *napobrassica*) is a root crop derived from the hybridization between turnip (*B. rapa* L.) and cabbage or kale (*B. oleracea* L.). It is cultivated as a minor crop in Europe and North America for use as a table vegetable and as fodder for animals. Rutabagas vary in root and leaf shape and colour, foliage growth habits, quality parameters and maturity date. They also have been used in breeding programs as sources of clubroot resistance. Despite these differences, no in-depth molecular research has been conducted on the genetic diversity of rutabaga accessions. In this study, 134 rutabaga accessions from Scandinavia (Sweden, Norway, Denmark, Finland and Iceland) were genotyped with a 15K Brassica SNP array from TraitGenetics. After excluding markers that did not amplify genomic DNA and also those with >20% missing data, 118 accessions were genotyped with 6000 SNP markers. Both UPGMA and NJ methods clustered the accessions into two major groups, with each branching into three sub-groups. Rutabaga accessions from Norway, Sweden and Finland were the most diverse and were distributed across the two major groups. In contrast, accessions from Denmark and Iceland clustered into each of the two major groups. Clubroot screening will establish if resistance is correlated with geographic distribution.

Suppression of *Plasmodiophora brassicae* in infested oilseed rape farms by soil amendment with calcium cyanamide and burnt lime N. ZAMANI-NOOR *Julius Kühn-Institut, Institute*

for Plant Protection in Field Crops and Grassland, Messeweg 11-12, D-38104 Braunschweig, Germany

Clubroot, caused by *Plasmodiophora brassicae*, is an important disease of oilseed rape (OSR), causing serious losses in Germany. The detection of 124 new *P. brassicae*-infested fields during 2013-2017 across several federal states in Germany suggests that clubroot disease may be more widespread in oilseed rape than previously thought. In the present study, the application of calcium cyanamide and burnt lime in combination with cultivar resistance were evaluated for their potential in suppression of clubroot. Multifactorial field trials with natural infection were conducted at three locations in Germany from 2014 to 2016. The plots consisted of two OSR cultivars differing in their levels of resistance to clubroot and subplots of two soil amendments which were applied at different time points. Calcium cyanamide (300 kg/ha; 50% calcium oxide) and burnt lime (1500 kg/ha) were distributed evenly on the soil surface one day prior to the sowing or when the OSR had reached the growth stage 11-12. Soil moisture, temperature and pH at two depths were measured at regular intervals over the growing season. Clubroot incidence and severity were assessed visually. The results showed that the incidence and severity of clubroot varied across locations and years. The most severe disease was observed in all locations in 2014 in which the clubroot-resistant OSR also showed strong infection in one field. In the other two fields, the resistant cultivar provided up to 90% disease control. In general, a slight increase in soil pH, about 0.2-0.5 units higher than the natural soil suspension pH, was observed after application of calcium cyanamide or burnt lime. However, some days later the soil pH decreased again and was equal to that in control plots. Changing the time of fertilizer application had a significant effect on the final disease severity. Relative to untreated controls, clubroot incidence and severity were decreased by application of fertilizers at later growth stages. In comparison with calcium cyanamide, burnt lime application had a smaller effect. Nearly 30% yield losses were recorded in the susceptible cultivar in non-treated plots in compared with the treated ones.

Epidemiology of clubroot disease and pathogenic variation among isolates of *Plasmodiophora brassicae* from oilseed rape growing in Europe N. ZAMANI-NOOR; E. DIEDERICHSEN, A.-C. WALLENHAMMAR; G. CORDSEN-NIELSEN; G. ORGEUR; V.

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Clubroot, caused by *Plasmodiophora brassicae*, is one of the most destructive diseases in oilseed rape (OSR) cultivation and has become increasingly important in central Europe. The disease has been monitored by collaborators through field surveys in the Czech Republic, Denmark, France, England and Scotland, Germany, Poland and Sweden. Infected plants and soil samples were collected randomly from clubroot-infested fields in different countries. Location, soil type, soil pH, plant genotype and rotation regime were recorded for each field. The presence of *P. brassicae*-resting spores in the soil was assessed by standard bioassays. Pathotype classification of the *P. brassicae*-populations was conducted on two or three known differential sets. Additionally, the degree of virulence of the collected isolates was analyzed on the clubroot-resistant OSR cv. Mendel. Clubroot monitoring revealed that the disease occurred in different regions in all stated countries with different intensities. It was a particularly major issue when OSR was grown in a 2 or 3-year rotation, which could lead to a rapid increase in clubroot severity. A slight significant negative correlation was found between soil pH and the disease incidence in infested fields. Variation in pathotype distribution was observed in different countries. In Czech Republic and Poland, there were nine pathotypes according to the evaluation system of Williams, four pathotypes based on Somé et al. and 15 with the European Clubroot Differential (ECD) set. In Germany, five pathotypes were found according to Somé et al. and 20 according to the ECD set. In France, six pathotypes were classified according to the set of Somé et al. Although the population of *P. brassicae* appears to be very diverse in the UK, three pathotypes were found to be dominant when tested on the ECD set. In Sweden, pathotypes were evaluated earlier according to Williams and four pathotypes were found. In 2010, a range of isolates originating from different Brassica species were evaluated on the ECD set and different

virulence patterns were observed. From all populations tested for virulence on cv. Mendel, several isolates were found to be moderately or highly virulent. These virulent populations were not restricted to a small geographical area in different countries.

Development of clubroot resistant interspecific hybrids of *Brassica oleracea* × *Brassica rapa* ssp. *rapa* S. ZHANG, S. SHEN, Q. LI, X. REN, H. SONG AND J.SI *Key Laboratory of Horticulture Science for Southern Mountains Regions, Ministry of Education, Chongqing Key Laboratory of Olericulture, College of Horticulture and Landscape Architecture, Southwest University，Chongqing 400715, China*

To develop new clubroot (*Plasmodiophora brassicae*) resistant Brassica germplasm resources, four interspecific crosses were made using three *B. oleracea* lines D_3, E_4 and 545 as female and two *Brassica rapa* ssp. *rapa* lines WJ-1 and WJ-2 as male. Interspecific hybrid plants were obtained through the application of the embryo rescue technique. While comparing compatibility of these crosses, the formation of a silique per pollinated flower was about 90% higher in E_4 × WJ-1 as compared with the other crosses; however, survival of immature embryos per pollinated flower was higher (3.09%) in D_3 × WJ-1. The number of interspecific hybrid plants obtained from the four crosses D_3 × WJ-1, E_4 × WJ-1, E_4 × WJ-2, 545 × WJ-2 were 5, 0, 0, and 0, respectively. These plants were identified to be true hybrids based on morphological and cytological observation, and had a somatic chromosome number $2n = 19$ and intermediate morphology of the two parents. Pollen fertility of the five plants derived from D_3 × WJ-1 was almost zero; however, they showed resistance to *P. brassicae* race 4. Results from this study demonstrated that clubroot resistant plants can be developed through interspecific hybridization between *B. oleracra* and *B. rapa* ssp. *rapa*.

Developing spring type *Brassica napus* lines containing single clubroot resistance genes Y. ZHANG, J. WANG, M. KEHLER, S.E. STRELKOV, B.D. GOSSEN, G. PENG AND F. YU *Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, 107 Science*

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Clubroot, caused by *Plasmodiophora brassicae* Woronin, poses a serious threat to canola (*Brassica napus* L.) production in Canada. Pathotypes are characterized based on differential reactions on *Brassica* cultivars, but most of the differential cultivars are vegetable or fodder types. Ideally, a set of near-isogenic spring canola lines containing single clubroot resistance genes could be used to differentiate races of *P. brassicae*. Genetic mapping has identified more than 10 genes for clubroot resistance that occur in *Brassica* species. The objective of this study was to develop a set of near-isogenic lines of *B. napus* with single genes, suitable for identification of races of *P. brassicae*. Resistant lines of *B. rapa* carrying *Rcr1*, *Rcr2*, *Crr1* to *Crr4*, *CRa*, *CRb*, *CRc* and *CRk*, and the European *B. napus* cultivar 'Mendel' carrying a clubroot resistance gene, were crossed with the susceptible spring type canola line DH16516. The resulting F_1 plants were crossed into the susceptible background to back cross generations 3 to 4 (BC_3 to BC_4). SNP markers linked to most of the clubroot resistance genes were validated. Marker assisted selection was performed at each generation. Plants were tested with pathotypes 3H and 5X from Canada. Homozygous lines containing *Rcr1* or *Rcr2* in BC_4 were obtained. Plants carrying the other CR genes in BC_3 were developed. Microspore culture is being used to develop homozygous lines carrying the other CR genes.

QTL-seq analysis identifies two clubroot (*Plasmodiophora brassicae*) resistance QTL in turnip (*Brassica rapa* ssp. *rapifera*). H. ZHANG, S. J. ZHANG, F. CHENG, F. LI, S. F. ZHANG AND R. S. SUN *Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, 100081, China*

European fodder turnips (*Brasscia rapa* ssp. *rapifera*) were identified as sources of clubroot resistance (CR) and have been widely used in Brassica resistance breeding. In this study, an F2 mapping population derived from a cross between a resistant turnip and a susceptible Chinese cabbage was used to determine the inheritance and location(s) of the resistance gene(s). The parents were very resistant or susceptible to three field isolates of *Plasmodiophora brassicae* from Yunan (Pby), Henan (Pbh) and Liaoning (Pbl) provinces in China. After inoculation with

Pbh (pathotype 4), the 206 F2 individuals showed a 9:7 segregation ratio for resistance, indicating that clubroot resistance is controlled by multiple genes with a complementary effect in this population. Next generation sequencing-based QTL–seq was used to locate resistance genes. Each of 27 very resistant (R)/susceptible (S) individuals was selected to construct R/S bulks, respectively. SNP index and Δ(SNP-index) graphs were identified base on bioinformatics information from the *B. rapa* genome. Two regions on chromosome A03 (upstream of Crr3) and A08 (downstream of Crr1) showed significant differences, respectively. There are four and five genes in the A03 and A08 candidate regions, respectively.

Isolation and evaluation of potential biocontrol agents against clubroot (*Plasmodiophora brassicae*) of *Brassica napus* L. ZHENG, Y.W. HE, M.L. ZHU, D.H. JIANG AND J.B. HUANG *Key Lab of Plant Pathology of Hubei Province, Huazhong Agricultural University, Wuhan, 430070, China*

The soilborne pathogen *Plasmodiophora brassicae* causes clubroot, one of the most damaging diseases of the family Brassicaceae in China. Microorganisms and their metabolites have attracted attention as potential biocontrol agents to reduce fungicide use. In this study, a total 667 microbial strains were isolated from the rhizosphere soil of rapeseed collected from severely diseased crops in Dangyang City, Hubei Province, China. These included 323 strains of bacteria, 253 strains of fungi, and 91 strains of actinomycetes. As previously reported, strains of *Fusarium oxysporum* and *Magnaporthe oryzae* could be used as indicators for *P. brassicae* in the first round screening of biocontrol agents. Fifty-four strains with an inhibition zone of > 3 mm by dual culture test were obtained. Among these, two potential biocontrol strains F85 and T113, which were identified as *Bacillus* sp., were found to have a control efficiency of > 60% in pot experiments. Strains F85 and T113 significantly inhibited root hair infection, and reduced the differentiation of primary plasmodia of *P. brassicae* as well as the formation of secondary zoosporangia. These two *Bacillus* strains F85 and T113 could be tested further in naturally diseased fields.

What we should do before clubroot disease of *Brassica Napus* L. happened in Jiangsu province of China. Q. PENG, S. CHEN, W. ZHANG, J.Q. GAO, F. CHEN, M.L. HU, S.X. FU, W.H. LONG, X.D. WANG, X.Y. ZHOU, H.M. PU, J.F. ZHANG *Key Laboratory of Cotton and Rapeseed, Ministry of Agriculture, Institute of Industrial Crops, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, People's Republic of China*

In recent years, the clubroot disease of *Brassica napus* happened in Yangtze River basin of China is increasingly severe. Especially in Sichuan, Yunnan, Hubei and Anhui province, the damaged area has expanded to 70,000 hectares. So, it's time for us do something to cut our losses before the disease spread to Jiangsu province. In this research, we evaluated 30 different Jiangsu cultivars of rapeseed's disease-resistance to the pathotype 4 in the infected field. Then, the molecular marker A08-300, was also tested in 30 different cultivars by PCR amplification, which closely-linked to the pathotype 4 resistance loci PbBa8.1. Unfortunately, all the Jiangsu cultivars of *Brassica napus* were highly susceptible to race 4 of clubroot, the disease indexes were greater than 59.26. The molecular marker resistant to race 4 was not detected in any Jiangsu cultivar. This means that the consequence will be serious once clubroot disease spreads to Jiangsu province's rapeseed. Breeding of resistant cultivars, therefore, is a promising alternative. We use our parental plants crossing with resistant cultivars and increased the disease-resistance of the cross-generation. After multiple generations of breeding, the disease-resistant varieties are expected to replace the old ones.