AN ABSTRACT OF THE THESIS OF

Shengwei Hu for the degree of Master of Science in Crop Science presented on November 19, 2021.

Title: Comparative Genomics of Different Races of Columbia Root-knot Nematode and Development of Molecular Markers Linked to Corky Ringspot Resistance in Potato

Abstract approved:

_____

Sathuvalli Rajakalyan, Vidyasagar

On a global scale, potato (*Solanum tuberosum* L.) plays an important role in tackling the threat of food insecurity due to its high yield and broad global acceptance. However, pathogens threaten potato production, causing direct yield loss and rendering potatoes tubers unmarketable. Breeding new cultivars that carry multiple resistances is an efficient way for sustainable potato production.

Columbia root-knot nematode (CRKN, *Meloidogyne chitwoodi*) parasitizes potato plants and causes small brown dots in the tuber flesh that dramatically reduce the market value of the crop. In the Pacific Northwest (PNW) two races of *M. chitwoodi* exist, Race 1 and Race 2; a pathotype of Race 1, Race 1_{Roza} also occurs. The races of *M. chitwoodi* are primarily identified based on a differential host test. In order to understand the phylogeny of *M. chitwoodi* and develop molecular markers to identify the different races, we sequenced the genomes of *M. chitwoodi* Race 1, Race 2 and Race 1_{Roza} using Illumina and PacBio sequencing. Each genome was assembled and annotated. Comparisons of syntenies and orthologs elucidate the complex

evolutionary history of this species and facilitate molecular marker development and analysis of host plant resistance to these root-knot nematodes.

Based on the genome comparisons of *M. chitwoodi* Race 1, Race 2 and Race $1_{Roza,}$ we developed 36 pairs of PCR primers for SSR markers and 17 pairs of PCR primers for INDEL markers. Four of those molecular markers, HSINDEL8, HSINDEL5, HSINDEL9 and HSINDEL10, can successfully differentiate the three pathotypes of *M. chitwoodi* used in this study on agarose gel electrophoresis. These markers have application in plant disease diagnostics.

Corky ringspot (CRS) disease caused by tobacco rattle virus (TRV) and vectored by stubby root nematodes, can render 6-55% of potatoes in an infested field unmarketable. Previous studies identified 22 SNP markers that are significantly associated with CRS resistance from 'Castle Russet' using a progeny of 48 seedlings. In this study we developed 44 pairs of PCR primers around previously identified significant SNPs. SNP marker PotVar0108448 on chromosome 9 shows polymorphisms on agarose gel electrophoresis and explains the highest percentage of phenotypic variance. Based on the initial marker screening, we developed 36 pairs of SSR primers, 72 pairs of primers for short INDELs and 36 pairs of primers for long INDELs on the upstream and downstream of SNP marker PotVar0108448. We screened them on 48 seedlings of progeny POR15V001 and 170 seedlings of progeny POR16V001. Markers INDEL20, INDEL490-7, Potvar008448 are linked to CRS resistance from 'Castle Russet'. Of these, marker INDEL490-7 was robust and able to identify resistance from diverse germplasm. It has the potential for use in marker-assisted selection (MAS).

Comparative Genomics of Different Races of Columbia Root-knot Nematode and Development of Molecular Markers Linked to Corky Ringspot Resistance in Potato

by
Shengwei Hu

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented November 19, 2021
Commencement June 2022

Master of Science thesis of Shengwei Hu presented on November 19, 2021

APPROVED:

Major Professor, representing Crop Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Shengwei Hu, Author

ACKNOWLEDGEMENTS

I would like to thank my major advisor, Dr. Sathuvalli, for contributing countless hours towards my education, having me in his group and providing me with invaluable advice and support along the way. I would also like to thank my co-advisor Dr. Vining, for helping me learn bioinformatics which I was not familiar with before and guide me on my thesis. I would also like to thank all my committee members, Dr. Mehlenbacher, Dr. Ingham, Dr, Lukas and all the other researchers who lent time, advice, and expertise, allowing these projects to be at the level they are. I would also like to thank my family and friends, who supported and encouraged me throughout my education.

CONTRIBUTION OF AUTHORS

Chapter2. Dr. Kelly Vining assisted with the experimental design. Dr. Sapinder Bali assisted with the DNA extraction for Illumina Sequencing. Dr. Cynthia Gleason provided the DNA for PacBio sequencing. Dr. Vidyasagar Sathuvalli secured the funding, assisted with the experimental design, interpretation of results, and writing the manuscript.

Chapter3. Mr. Rich Quick and Ms. Launa Cimrhakl provided the DNA of *M. chitwoodi* Race 1, Race 2 and Race 1_{Roza} for marker screening. Dr. Sam Chavoshi provided the nematode sample for marker validation. Dr. Sapinder Bali assisted with designing SSR marker primers. Dr. Kelly Vining assisted with the experimental design. Dr. Vidyasagar Sathuvalli secured the funding, assisted with the experimental design, interpretation of results, and writing the manuscript.

Chapter4. Mr. Moises Aguilar helped maintain the germplasm to evaluate. Ms. Launa Cimrhakl, Mr. Rich Quick and Dr. Max Feldman conducted the phenotypic evaluation for corky ringspot resistance. Mr. Solomon Yilma assisted with germplasm maintenance and marker screening. Dr. Vidyasagar Sathuvalli secured funding and assisted in all stages of this experiment and writing the manuscript.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

Figure Page

LIST OF FIGURES(Continued)

LIST OF TABLES

Table Page

LIST OF TABLES(Continued)

1. Introduction

1.1 The importance of potatoes in the global food supply

On a global scale, potato (*Solanum tuberosum* L.) plays an important role against the threat of food insecurity. Potato is a high yielding crop per unit area and feeds a large population worldwide. In 2019, total U.S. potato production was 21.5 million metric tons. Of these, 14.4 million metric tons were processed, with 8.8 million metric tons frozen as French fries and an additional 3.0 million metric tons processed as chips and shoestrings (National Agricultural Statistics Service 2020). Washington and Oregon ranked second and fourth respectively in U.S. potato production in 2019. Oregon producers planted 43,000 acres of potatoes in 2019 with an average yield of 81.6 metric tons per hectare, for a total of 1.3 million metric tons. In the same year, Washington producers planted 165,000 acres with an average yield of 88.4 metric tons per hectare, for a total of 5.3 million metric tons (Nadeem et al., 2018; National Agricultural Statistics Service 2020). The Columbia Basin, with its favorable climate, soil, and ample supply of irrigation water, is the largest potato growing region in Washington and Oregon.

Potato provides more vitamins and minerals per serving than other main food crops and contributes to a healthy diet (Brown 2008). Potato is a less expensive vitamin C source than other high vitamin C vegetables (Drewnowski and Rehm 2013). Potato is also a source of important B vitamins, including B1, B6 and B9 (Alfthan et al. 2003; Brevik et al. 2005, Goyer and Sweek 2011). Potato is also a good source of

potassium, iron, and magnesium; one medium sized red potato (173g) meets 10- 36% of the daily recommended dietary allowance of Vitamin C (Navarre et al., 2019). The secondary plant metabolites, phenylpropanoids and anthocyanins, are known for their health-promoting effects (Navarre et al. 2019) and make potato a healthy food choice. The four groups of domesticated potato species are *S. tuberosum* tetraploid group Andigena, which is adapted to the tropics, *S. tuberosum* tetraploid group Tuberosum, which is adapted to temperate zones and *S. tuberosum* diploid groups Phureja and Stenotomum (Spooner and Hetterscheid 2006; Barrell et al. 2013). Other *Solanum* species related to *S. tuberosum* have ploidy levels ranging from diploid to hexaploid (van den Berg and Jacobs 2007; Barrell et al. 2013). With heterozygosity often > 0.8, *S. tuberosum* is a highly heterozygous species (Provan et al. 1996), with inbreeding depression (Simko et al. 2006; Bradshaw 2007).

Pests and diseases threaten potato production. Soilborne pathogens include fungi, bacteria, protists and nematodes that cause root diseases and reduce marketable tuber yield. In the US, potato virus Y (PVY) and *Phytophthora infestans* (late blight) are two common pathogens. In the Columbia Basin, in addition to PVY, the following pathogens significantly impact potato yield: corky ringspot (CRS) disease caused by tobacco rattle virus (TRV), Columbia root-knot nematode (CRKN, *Meloidogyne chitwoodi*), verticillium wilt (VW; *Verticillium dahliae*), and potato mop-top virus vectored by powdery scab *Spongospora subterranea* f.sp. *subterranea.* The two pathogens of prime importance are CRKN and TRV.

1.2 Molecular markers and genomic resources in potato

Genetic markers are important tools in plant breeding. Potato breeders have used molecular markers to identify cultivars (Gebhardt et al., 1989a), analyze recombination between genomes (Williams et al. 1993), study phylogenetics (Kardolus et al.1998), and for marker-assisted selection (MAS) (Hämäläinen et al. 1997). In marker-assisted breeding, the first step is to identify one or more markers closely linked to the gene or trait of interest.

Markers are genes or DNA sequences with known chromosome locations that are closely linked to the target genes (Nadeem et al. 2018). Genetic markers can be grouped into two categories: classical morphological, cytological and biochemical markers and DNA/molecular markers including restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSRs), single nucleotide polymorphisms (SNP) and diversity array technology (DArT) (Jiang, 2013).

Simple sequence repeats (SSRs) are tandem repeat motifs of one to six nucleotides (Weber, 1990). These SSR markers are PCR based and are co-dominant.

Development of high throughput DNA sequencing techniques has reduced the cost of genome sequencing. Identified specific DNA sequences can also be used as molecular markers. Single nucleotide polymorphism (SNP) markers are single base-pair changes in a genome sequence. These SNPs can be transitions, transversions, insertions, or deletions of single nucleotides. Single-nucleotide changes provide many markers that are widely used in genetic mapping studies.

Many types of markers have been mapped and used in MAS in potato (Hirsch et al. 2014). Most of these markers are associated with disease resistance including late

blight (*Phytophthora infestans*), potato virus X, PVY and various nematodes (Ramakrishnan et al., 2015). Marker development for traits controlled by multiple genes, such as stress tolerance or cold tolerance, lags far behind that for disease resistance genes (Wang et al. 2003; Watanabe et al. 2011). New techniques allow researchers to map quantitative trait loci (QTLs) in large potato populations. The Solanaceae Coordinated Agricultural Project has identified an Infinium 8303 SNP array from the transcriptome of one diploid and six tetraploid cultivars (Hamilton et al. 2011; Douches et al., 2014). This SNP array has been used for genome-wide associations and fingerprinting studies (Lindqvist-Kreuze et al. 2014; Schreiber et al.2014; Endelman and Jansky 2014; Bali et al., 2017).

MAS in potato breeding has been widely used to screen seedlings for their resistance to PVY. There are two types of PVY resistance based on plant response to the virus: extreme resistance (ER) and hypersensitive response (HR). In the case of ER, potato plants inhibit the multiplication and cell-to-cell movement of the virus (Solomon-Blackburn and Barker 2001; Valkonen 2015). The plants show no symptoms or very limited necrosis (Valkonen et al. 1996), and the virus titer is below the limit of detection. In the case of HR, systemic virus movement is prevented by the rapid death of infected cells. Plants show limited infection on tissue surrounding the initially infected cells (Valkonen 2015). Table 1.1 shows various markers being employed in breeding programs for PVY resistance.

Table 1.1. Molecular markers for PVY resistance selection in potato

Resistance type	Gene/Source	Chr.	Marker name	Marker type	Reference
Hypersensitive response	*Ny_{tbr}*	IV	TG506	RFLP	(Celebi-Toprak et al. 2002)

	Ny-1	IX	$SC895_{1139}$	PCR based	(Szajko et al. 2008)
Extreme resistance	Ry_{adg}	XI	TG508	RFLP	(Hämäläinen et al. 1997)
	Ry_{adg}	XI	ADG1, ADG2	PCR based	(Hämäläinen et al. 1998)
	Ry_{adg}	XI	RYSC3	PCR based	(Kasai et al. 2000)
	Ry_{sto}	XII	$SCAR_{YSTO4}$	PCR based	(Cernák et al. 2008)
	Ry_{sto}	XII	YES3-3A, YES3-3B	ESTS	(Song et al. 2005; Song and Schwarzfischer 2008)
	Ry_{chc}	IX	CT220	RFLP	(Sato et al. 2006)
	Ry_{chc}	IX	RY186	PCR based	(Mori et al. 2011)
	$Ry\text{-}f_{sto}$	XII	$GP122_{564}$, $GP122_{718}$	CAPS	(Witek et al. 2006; Flis et al. 2005)

The first potato genome was sequenced in 2011, a doubled monoploid Phureja clone. The genome was assembled into 12 chromosome pseudomolecules and 39,031 protein-coding genes were annotated (The Potato Genome Sequencing Consortium 2011). The wild species *S. commersonii* genome was sequenced and assembled in 2015 using the potato genome sequence as reference (Aversano et al. 2015). In 2018, the genome of another wild species, *S. chacoense,* was sequenced using a diploid inbred clone (Leisner et al. 2018). In addition, Oregon State University's potato breeding program has sequenced *S. bulbocastum* clone SB2 that carries resistance to CRKN. As the cost of sequencing per mega base has fallen from over USD $1500 to under $0.05, we can expect more genome resources of potato to be released in the future (Wetterstrand 2014).

1.3 Columbia root-knot nematode

Plant parasitic root-knot nematodes are members of the genus *Meloidogyne*. Worldwide, six *Meloidogyne* species are potato pathogens: *M. incognita*, *M. javanica, M. arenaria, M. chitwoodi, M. fallax* and *M. hapla* (Brodie et al., 1993; Onkendi et al., 2014). Columbia root-knot nematode (CRKN), *Meloidogyne chitwoodi*, parasitizes a wide range of plants in the Pacific Northwest. It has four juvenile stages (J1- J4) and an adult stage. The second-stage juveniles (J2) of CRKN invade the root elongation region. In potato, CRKN also attacks tubers and causes small brown dots in the flesh, dramatically reducing the market value of the crop. *M. chitwoodi* is most abundant in the Columbia Basin potato growing region of Oregon and Washington, but is also found in California, Idaho, Colorado, New Mexico, and Texas (Powers et al. 2005). *M. chitwoodi* is difficult to control because of its wide host range and the minimal damage it causes to most of the alternate host species. The *M. chitwoodi* population supported by these alternate hosts can increase in soil sharply by the end of the warm growing season and inflict serious economic losses on the subsequent potato crop. Furthermore, the low level of damage to several host species makes it difficult to identify the nonhost and remove the potential threat from the rotation. Fumigation before planting is an effective method to control the nematodes but it is costly and leads to negative environmental impacts (Brown et al. 2009).

In the Pacific Northwest, an *M. chitwoodi* population designated Race 1 has been confirmed as unable to reproduce on alfalfa. A population that can reproduce on 'Thor' alfalfa has been designated as Race 2 (Santo et al., 1980, Santo et al., 1985). Races 1 and 2 infect unique sets of host plants. A key difference between these races

is that Race 2 can reproduce on 'Thor' alfalfa, while Race 1 cannot. Race 1 can reproduce on carrot while Race 2 cannot (Mojtahedi et al. 1994).

Two genes in potato (*RMc1 (blb)* and *RMctuber (blb)*) that confer resistance to *M. chitwoodi* are being employed in cultivar development efforts. Both genes were introgressed from *S. bulbocastanum* clone SB22 (PI 275187), a diploid wild potato. *RMc1 (blb)* confers resistance to Race 1 of *M. chitwoodi*, apart from Race 1 isolate Roza. Race 1_{Roza} was identified in experimental plots that repeatedly had been planted with clones carrying *RMc1 (blb)* (Mojtahedi et al. 2007). *RMctuber(blb)* confers tuber resistance to both Race 1 and Race 2 of *M. chitwoodi*.

Currently, Races 1 and 2 can only be differentiated via tedious differential host testing. They cannot yet be differentiated morphologically, and no molecular markers are available to differentiate them. The key to *M. chitwoodi* control is accurate race identification. The economic impact of *M. chitwoodi* in Columbia Basin potato production increases the importance of molecular marker development that can differentiate these races.

A genomic study of *M. chitwoodi* will increase an understanding of its biology and epidemiology. High throughput sequencing technology will reduce the cost of analyses. PacBio sequencing, which is also called single-molecule real-time (SMRT) sequencing, sequence the closed, single-stranded circular DNA on the chip called SMRT cell. Each SMRT cell can generate 0.5–1 Gb of sequence in a run with average read lengths over 10 kb (Rhoads & Au, 2015). Long reads of SMRT sequencing will improve the contiguity and completeness of genomic assemblies. Some important root-knot nematodes have been sequenced in the past.

The *Meloidogyne hapla* genome was sequenced and published in 2008 (Opperman et al. 2008) and assembled into 1523 scaffolds with assembly size of 54 Mbps. Genomes of five other species (*M. incognita, M. javanica, M. arenaria, M. enterolobii*, and *M. floridensis*) were sequenced and assembled for comparative and evolutionary analysis (Szitenberg et al. 2017). Molecular makers based on loop-mediated isothermal amplification (LAMP) were developed to detect root-knot nematodes *M. chitwoodi* and *M. fallax* (Zhang et al. 2018). Bioinformatic tools and improved sequencing technologies will permit us to generate more complete and contiguous genome sequences for comparisons and molecular marker design. Currently there is no commercial potato variety resistant to *M. chitwoodi*. Tuber and root resistance to *M. chitwoodi* Race 1 and Race 2 have been introgressed into elite potato germplasm, although no resistant cultivars have been released. Graebner et al. (2018) identified four clones, PI239424hou-2mc, PI239424hou-6mc, PI283107hou-5mc, and PI283107hou-9mc from *S. hougasii* (6X) that are significantly resistant to Race 1, Race 2 and Race 1_{Roza} of *M. chitwoodi*. These new sources are being introgressed into elite potatoes.

1.4 Corky ringspot

Corky ringspot (CRS) is a disease of economic importance in the Columbia Basin caused by Tobacco rattle virus (TRV) and vectored by stubby root nematodes (*Trichodorus* sp. and *Paratrichodorus sp.*). In potato, CRS is characterized by necrotic rings in the tuber flesh, which can render 6-55% of potatoes in an infested

field unmarketable (Hafez and Sundararaj 2009). Typically, the most effective control of damage caused by TRV is to control the vector with nematicides. Before 1990, fumigants and aldicarb were used to control nematodes. Corky ringspot disease has increased in the Columbia Basin since 1989, when aldicarb use on potato ended (Weingartner and Shumaker 1990). Introgression of resistance into potato cultivars is the most efficient and environmentally sustainable method to control CRS.

In the United States, stubby-root nematode has been reported in most of the states. Stubby-root nematodes are ectoparasitic nematodes, which live in the soil and feed on root tips. Unlike the plant-parasitic nematodes, stubby root nematodes must puncture plant cells with a dagger-like onchiostyle. They then inject saliva into the punctured cell and ingest the cell contents (Crow, 2019). This feeding behavior makes the stubby-root nematode a natural vector to spread TRV*, Pea early-browning virus* (PEBV) and *Pepper ringspot virus* (PepRSV). Due to TRV's damage to potato's marketable yield, US seed certification programs have classified it as a zero-tolerance disease (Brown and Mojtahedi 2005).

Tobacco rattle virus is a member of the genus *Tobravirus* and has two parts of single-stranded, positive sense RNA. Genomic RNA1 encodes the replicase protein, cell-to-cell movement protein and silencing suppressor protein, while RNA2 encodes the coat protein and nematode-transmission factor (Donaire et al. 2008).

'Castle Russet', a recently released variety from the Northwest potato variety development program, has improved agronomic performance and resistance to PVY and CRS. Based on single marker association analysis, resistance to CRS from 'Castle Russet' was mapped to a major peak on 2 Mbp region on chromosome 9 and

two minor peaks on chromosomes 1 and 10 (Graebner, 2018). Twenty-two SNP markers were identified as significantly associated with CRS severity. Development of efficient molecular markers linked to CRS resistance from 'Castle Russet' can aid in MAS.

1.5 References

Alfthan, G., Laurinen, M. S., Valsta, L. M., Pastinen, T., & Aro, A. (2003). Folate intake, plasma folate and homocysteine status in a random Finnish population. *European Journal of Clinical Nutrition*, *57*(1), 81–88. https://doi.org/10.1038/sj.ejcn.1601507

Aversano R, Contaldi F, Ercolano MR, Grosso V, Iorizzo M, Tatino F, Delledonne M. 2015. The *Solanum commersonii* genome sequence provides insights into adaptation to stress conditions and genome evolution of wild potato relatives. *Plant Cell, 27*, 954–968. https://doi.org/10.1105/ tpc.114.135954

Bali, S., Sathuvalli, V., Brown, C., Novy, R., Ewing, L., Debons, J., Douches, D., Coombs, J., Navarre, D., & Whitworth, J. (2017). Genetic fingerprinting of potato varieties from the northwest potato variety development program. *American Journal of Potato Research*, *94*(1), 54–63.

Bradshaw, J. E. (2007). Potato-breeding strategy. *Potato Biology and Biotechnology* (pp. 157–177). https://doi.org/10.1016/b978-044451018-1/50050-6

Brevik, A., Vollset, S. E., Tell, G. S., Refsum, H., Ueland, P. M., Loeken, E. B., Drevon, C. A., & Andersen, L. F. (2005). Plasma concentration of folate as a biomarker for the intake of fruit and vegetables: The Hordaland Homocysteine Study. *The American Journal of Clinical Nutrition*, *81*(2), 434–439.

Brodie, B. B., Evans, K., & Franco, J. (1993a). Nematode parasites of potatoes. *Plant Parasitic Nematodes in Temperate Agriculture.*, 87–132.

Brown, C. R. (2008). Breeding for phytonutrient enhancement of potato. *American Journal of Potato Research*, *85*(4), 298–307.

Brown, C. R., & Mojtahedi, H. (2005). Breeding for resistance to *Meloidogyne species* and *trichodorid*-vectored virus. *Genetic Improvement of Solanaceous Crops*, *1*, 267–292.

Brown, C. R., Mojtahedi, H., Zhang, L.-H., & Riga, E. (2009). Independent resistant reactions expressed in root and tuber of potato breeding lines with introgressed resistance to *Meloidogyne chitwoodi*. *Phytopathology*, *99*(9), 1085–1089.

Celebi-Toprak, F., Slack, S. A., & Jahn, M. M. (2002). A new gene, Ny_{tbr}, for hypersensitivity to Potato virus Y from Solanum tuberosum maps to chromosome IV. *Theoretical and Applied Genetics*, *104*(4), 669–674.

Cernák, I., Decsi, K., Nagy, S., Wolf, I., Polgár, Z., Gulyás, G., Hirata, Y., & Taller, J. (2008). Development of a locus-specific marker and localization of the Ry_{sto} gene based on linkage to a catalase gene on chromosome XII in the tetraploid potato genome. *Breeding Science*, *58*(3), 309–314.

Crow, W. T. (2019). Stubby-root nematode, *Trichodorus obtusus cobb* (*Nematoda*: *Adenophorea*: *Triplonchida*: *Diphtherophorina*: *Trichodoridea*: *Trichodoridae*). *EDIS*, *2005*(5). https://doi.org/10.32473/edis-in617-2004

Donaire, L., Barajas, D., Martínez-García, B., Martínez-Priego, L., Pagán, I., & Llave, C. (2008). Structural and genetic requirements for the biogenesis of tobacco rattle virus-derived small interfering RNAs. *Journal of Virology*, *82*(11), 5167–5177.

Douches, D., Hirsch, C. N., Manrique-Carpintero, N. C., Massa, A. N., Coombs, J., Hardigan, M., Bisognin, D., De Jong, W., & Buell, C. R. (2014). The contribution of the Solanaceae coordinated agricultural project to potato breeding. *Potato Research, 57*(3), 215–224.

Drewnowski, A., & Rehm, C. D. (2013). Vegetable cost metrics show that potatoes and beans provide most nutrients per penny. *PloS One*, *8*(5), e63277.

Endelman, J., & Jansky, S. H. (2015). Genotyping-by-sequencing of a diploid potato F2 population. *Plant and Animal Genome XXIII, San Diego, CA*.

Gebhardt, C., Blomendahl, C., Schachtschabel, U., Debener, T., Salamini, F., & Ritter, E. (1989). Identification of 2n breeding lines and 4n varieties of potato (*Solanum tuberosum, ssp. Tuberosum*) with RFLP-fingerprints. *Theoretical and Applied Genetics*, *78*(1), 16–22.

Goyer, A., & Sweek, K. (2011). Genetic diversity of thiamin and folate in primitive cultivated and wild potato (Solanum) species. *Journal of Agricultural and Food Chemistry*, *59*(24), 13072–13080.

Graebner, R. C. (2018). *Breeding qualitative and quantitative traits for potatoes in the Columbia Basin*. Oregon State University.

Hafez, S. L., & Sundararaj, P. (2009). Management of corky ringspot disease of potatoes in the Pacific Northwest. *University of Idaho. Extension*, 1–6.

Hämäläinen J. H., KN Watanabe, JPT Valkonen, A Arihara, RL Plaisted, E Pehu, L Miller, & SA Slack. (1997). Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. *Theoretical and Applied Genetics*, *94*, 192-197.

Hämäläinen, J. H., Sorri, V. A., Watanabe, K. N., Gebhardt, C., & Valkonen, J. P. T. (1998). Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato. *Theoretical and Applied Genetics*, *96*(8), 1036–1043.

Hamilton, J. P., Hansey, C. N., Whitty, B. R., Stoffel, K., Massa, A. N., Van Deynze, A., De Jong, W. S., Douches, D. S., & Buell, C. R. (2011). Single nucleotide polymorphism discovery in elite North American potato germplasm. *BMC Genomics*, *12*(1), 1–12.

Hirsch, C. D., Hamilton, J. P., Childs, K. L., Cepela, J., Crisovan, E., Vaillancourt, B., Hirsch, C. N., Habermann, M., Neal, B., & Buell, C. R. (2014). Spud DB: A resource for mining sequences, genotypes, and phenotypes to accelerate potato breeding. *The Plant Genome*, *7*(1), plantgenome2013-12.

Jiang, G.-L. (2013). Molecular markers and marker-assisted breeding in plants. *Plant Breeding from Laboratories to Fields*, 45–83.

Kardolus, J. P., van Eck, H. J., & van den Berg, R. G. (1998). The potential of AFLPs in biosystematics: A first application in Solanum taxonomy (Solanaceae). *Plant Systematics and Evolution*, *210*(1), 87–103.

Kasai, K., Morikawa, Y., Sorri, V. A., Valkonen, J. P. T., Gebhardt, C., & Watanabe, K. N. (2000). Development of SCAR markers to the PVY resistance gene Ry_{adg} based on a common feature of plant disease resistance genes. *Genome*, *43*(1), 1–8.

Leisner, C. P., Hamilton, J. P., Crisovan, E., Manrique-Carpintero, N. C., Marand, A. P., Newton, L., Pham, G. M., Jiang, J., Douches, D. S., & Jansky, S. H. (2018). Genome sequence of M6, a diploid inbred clone of the high-glycoalkaloid-producing tuber-bearing potato species *Solanum chacoense*, reveals residual heterozygosity. *The Plant Journal*, *94*(3), 562–570.

Lindqvist-Kreuze, H., Gastelo, M., Perez, W., Forbes, G. A., de Koeyer, D., & Bonierbale, M. (2014). Phenotypic stability and genome-wide association study of late blight resistance in potato genotypes adapted to the tropical highlands. *Phytopathology*, *104*(6), 624–633.

Mojtahedi, H., Brown, C. R., Riga, E., & Zhang, L. H. (2007). A new pathotype of *Meloidogyne chitwoodi* Race 1 from Washington State. *Plant Disease*, *91*(8), 1051–1051.

Mojtahedi, H., Santo, G. S., Brown, C. R., Ferris, H., & Williamson, V. (1994). A new host race of *Meloidogyne chitwoodi* from California. *Plant Disease*, *78*(10).

Mori, K., Sakamoto, Y., Mukojima, N., Tamiya, S., Nakao, T., Ishii, T., & Hosaka, K. (2011). Development of a multiplex PCR method for simultaneous detection of diagnostic DNA markers of five disease and pest resistance genes in potato. *Euphytica*, *180*(3), 347–355.

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., & Labhane, N. (2018). DNA molecular

markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, *32*(2), 261–285.

National Agricultural Statistics Service. 2020, September 17. Potato Annual Summary. Retrieved from https://downloads.usda.library.cornell.edu/usda-esmis/files/fx719m44h/ks65j252d/wm118c91g/pots0920.pdf

Navarre, D. A., Brown, C. R., & Sathuvalli, V. R. (2019). Potato vitamins, minerals and phytonutrients from a plant biology perspective. *American Journal of Potato Research*, *96*(2), 111–126.

Opperman, C. H., Bird, D. M., Williamson, V. M., Rokhsar, D. S., Burke, M., Cohn, J., Cromer, J., Diener, S., Gajan, J., & Graham, S. (2008). Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences*, *105*(39), 14802–14807.

Powers, T. O., Mullin, P. G., Harris, T. S., Sutton, L. A., & Higgins, R. S. (2005). Incorporating molecular identification of *Meloidogyne spp*. Into a large-scale regional nematode survey. *Journal of Nematology*, *37*(2), 226.

Provan, J., Powell, W., & Waugh, R. (1996). Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). *Theoretical and Applied Genetics*, *92*(8), 1078–1084.

Ramakrishnan, A. P., Ritland, C. E., Blas Sevillano, R. H., & Riseman, A. (2015). Review of Potato Molecular Markers to Enhance Trait Selection. *American Journal of Potato Research*, *92*(4), 455–472. https://doi.org/10.1007/s12230-015-9455-7

Rhoads, A., & Au, K. F. (2015). PacBio sequencing and its applications. *Genomics, Proteomics & Bioinformatics*, *13*(5), 278–289.

Santo, G. S., O'bannon, J. H., Finley, A. M., & Golden, A. M. (1980). Occurrence and host range of a new root-knot nematode (*Meloidogyne chitwoodi*) in the Pacific Northwest. *Plant Disease*, *64*(10), 951–952.

Santo, G. S., and J. N. Pinkerton. 1985. A second race of *Meloidogyne chitwoodi* discovered in Washington. *Plant Disease*, *69*, 631.

Sato, M., Nishikawa, K., Komura, K., & Hosaka, K. (2006). Potato virus Y resistance gene, Ry chc, mapped to the distal end of potato chromosome 9. *Euphytica*, *149*(3), 367–372.

Schreiber, L., Nader-Nieto, A. C., Schönhals, E. M., Walkemeier, B., & Gebhardt, C. (2014). SNPs in genes functional in starch-sugar interconversion associate with natural variation of tuber starch and sugar content of potato (*Solanum tuberosum L.*). *G3: Genes, Genomes, Genetics*, *4*(10), 1797–1811.

Simko, I., Haynes, K. G., & Jones, R. W. (2006). Assessment of linkage disequilibrium in potato genome with single nucleotide polymorphism markers. *Genetics*, *173*(4), 2237–2245.

Solomon-Blackburn, R. M., & Barker, H. (2001). Breeding virus resistant potatoes (Solanum tuberosum): A review of traditional and molecular approaches. *Heredity*, *86*(1), 17–35.

Song, Y.-S., Hepting, L., Schweizer, G., Hartl, L., Wenzel, G., & Schwarzfischer, A. (2005). Mapping of extreme resistance to PVY (Ry_{sto}) on chromosome XII using

anther-culture-derived primary dihaploid potato lines. *Theoretical and Applied Genetics*, *111*(5), 879–887.

Song, Y.-S., & Schwarzfischer, A. (2008). Development of STS markers for selection of extreme resistance (Ry sto) to PVY and maternal pedigree analysis of extremely resistant cultivars. *American Journal of Potato Research*, *85*(2), 159–170.

Spooner, D. M., & Hetterscheid, W. L. (2006). 13. Origins, Evolution, and Group Classifi cation of Cultivated Potatoes. In *Darwin's Harvest* (pp. 285–307). Columbia University Press.

Szajko, K., Chrzanowska, M., Witek, K., Strzelczyk-Żyta, D., Zagórska, H., Gebhardt, C., Hennig, J., & Marczewski, W. (2008). The novel gene Ny-1 on potato chromosome IX confers hypersensitive resistance to Potato virus Y and is an alternative to *Ry* genes in potato breeding for PVY resistance. *Theoretical and Applied Genetics*, *116*(2), 297–303.

Szitenberg, A., Salazar-Jaramillo, L., Blok, V. C., Laetsch, D. R., Joseph, S., Williamson, V. M., Blaxter, M. L., & Lunt, D. H. (2017). Comparative genomics of apomictic root-knot nematodes: Hybridization, ploidy, and dynamic genome change. *Genome Biology and Evolution*, *9*(10), 2844–2861.

The Potato Genome Sequencing Consortium. 2011. Genome sequence and analysis of the tuber crop potato. *Nature*, *475*, 189–195.

USDA - National Agricultural Statistics Service—Washington—Potatoe Reports. (n.d.). Retrieved July 6, 2021, from https://www.nass.usda.gov/Statistics_by_State/Washington/Publications/Potatoes/index.php

Valkonen, J. P. (2015). Elucidation of virus-host interactions to enhance resistance breeding for control of virus diseases in potato. *Breeding Science*, *65*(1), 69–76.

Valkonen, J. P. T., Jones, R. A. C., Slack, S. A., & Watanabe, K. N. (1996). Resistance specificities to viruses in potato: Standardization of nomenclature. *Plant Breeding*, *115*(6), 433–438.

van den Berg, R. G., & Jacobs, M. M. J. (2007). Chapter 4—Molecular Taxonomy. In D. Vreugdenhil, J. Bradshaw, C. Gebhardt, F. Govers, D. K. L. Mackerron, M. A. Taylor, & H. A. Ross (Eds.), *Potato Biology and Biotechnology* (pp. 55–76). Elsevier Science B.V. https://doi.org/10.1016/B978-044451018-1/50046-4

Wang, W., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, *218*(1), 1–14.

Watanabe, K. N., Kikuchi, A., Shimazaki, T., & Asahina, M. (2011). Salt and drought stress tolerances in transgenic potatoes and wild species. *Potato Research*, *54*(4), 319–324.

Weber, J. L. (1990). Informativeness of human (dC-dA) n·(dG-dT) n polymorphisms. *Genomics*, *7*(4), 524–530.

Weingartner, D. P., & Shumaker, J. R. (1990). Control of nematodes and soil-borne diseases in Florida potatoes with aldicarb and 1, 3-D. *Journal of Nematology*, *22*(4S), 775.

Wetterstrand, K. (2020). *The Cost of Sequencing a Human Genome*. Genome.Gov. https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost

Williams, C. E., Wielgus, S. M., Haberlach, G. T., Guenther, C., Kim-Lee, H., & Helgeson, J. P. (1993). RFLP analysis of chromosomal segregation in progeny from an interspecific hexaploid somatic hybrid between *Solanum brevidens* and *Solanum tuberosum*. *Genetics*, *135*(4), 1167–1173.

Witek, K., Strzelczyk-Żyta, D., Hennig, J., & Marczewski, W. (2006). A multiplex PCR approach to simultaneously genotype potato towards the resistance alleles Ry-f sto and Ns. *Molecular Breeding*, *18*(3), 273–275.

Zhang, L., & Gleason, C. (2019). Loop-mediated isothermal amplification for the diagnostic detection of *Meloidogyne chitwoodi* and *M. fallax. Plant Disease*, *103*(1), 12–18.

2. Gene annotation and comparison analyses of *Meloidogyne chitwoodi*, a pathogen of potato (*Solanum tuberosum* L.)

2.1 Abstract

Columbia root-knot nematode (CRKN, *Meloidogyne chitwoodi*) parasitizes potato plants in the Pacific Northwest (PNW) and causes small brown dots in the tuber flesh that dramatically reduce the market value of the crop. In the PNW two races of *M. chitwoodi,* Race 1 and Race 2 and a pathotype of Race 1, Race 1_{Roza} exist. The races of *M. chitwoodi* are primarily identified based on differential host tests. In order to understand the phylogeny of *M. chitwoodi* and develop molecular markers to identify the different races, we sequenced the genomes of *M. chitwoodi* Race 1, Race 2 and Race 1_{Roza} using Illumina and PacBio sequencing, assembled them and annotated them. The final assembly of PacBio sequencing was 47.47 Mb for Race 1 (30 contigs), 46.98 Mb for Race 2 (39 contigs) and 47.78 Mb for Race 1Roza (38 contigs). All three nematode genomes contain an average 25% GC content. Comparison of syntenies and orthologs elucidates the complex evolutionary history of this species and contributes to molecular marker development and analysis of host plant resistance to these root-knot nematodes.

2.2 Introduction

Columbia root-knot nematode (CRKN), *Meloidogyne chitwoodi* Golden, O'Bannon, Santo, and Finley is a soil-borne pathogen parasitizing a wide range of plants in the

Pacific Northwest (PNW). It is most abundant in the Columbia Basin potato growing region of Oregon and Washington, but is also found in California, Idaho, Colorado, New Mexico, and Texas (Powers et al., 2005). In potato (*Solanum tuberosum* L.), CRKN attacks roots and tubers causing visible small brown spots in the tuber flesh and dramatically reducing market value. Even low levels of damage can result in complete crop rejection. *Meloidogyne chitwoodi* has four juvenile stages (J1- J4) and an adult stage. The second-stage juveniles (J2) of CRKN invade the root elongation region and form feeding sites. Each feeding site of CRKN consists of four to eight transformed giant cells which provide nutrients for the nematodes (Kyndt et al., 2013). J2 develop into male and female adults after forming the feeding site. Adult female CRKN exude egg masses within the gall tissue (Davies et al., 2015). *M. chitwoodi* females can produce mictic and parthenogenesis eggs. The haploid chromosome number of CRKN is n = 18 (Van der Beek & Karssen, 1997) and in general they are diploids (pers. communication, Gleason)

In the Pacific Northwest, there are two races of *M. chitwoodi*: Races 1 and 2. Each infects a unique set of host plants. A key difference between these races is that Race 2 can reproduce on 'Thor' alfalfa, while Race 1 cannot and Race 1 can reproduce on carrot while Race 2 cannot (Mojtahedi et al., 1994). Race 1 was identified first, and it is more prevalent in the Columbia Basin. Race 2 is typically found where potatoes are grown in rotation with alfalfa (Mojtahedi et al., 1994). Both these Race 1 and Race 2 were believed to be unable to reproduce on a diploid wild potato species, *S. bulbocastanum* clone SB22 (P1275187). However, a newly emerged Race 1 population identified in Prosser, Washington named as Race 1_{Roza} can reproduce on

the clone SB22 and can break the resistance conditioned by the Race 1(*blb*) gene (Mojtahedi et al., 2007). By genome sequencing and comparison analyses, we will be able to develop molecular markers to distinguish those *M. chitwoodi* pathotypes. Genomic resource development of *M. chitwoodi* can promote on understanding its biology and epidemiology. Nematode genomes are relatively small; typical genome size for *Meloidogyne spp* is 50-250 Mb (Leroy et al., 2003). High through-put sequencing technology has reduced the cost of genomic analysis. Long reads with average reading length more than 10 kb from Pacific Biosciences ("PacBio") sequencing have improved the contiguity and completeness of assemblies. The *M. hapla* genome was first sequenced in 2008 and assembled at a size of 54 Mb (Opperman et al., 2008). The *M. floridensis* genome was sequenced in 2014 and assembled at a size of 99.8 Mb (Lunt et al., 2014). The *M. incognita* genome was sequenced in 2008 and assembled at a size of 86 Mb (Abad et al., 2008). The *M. gramninicola* genomes were sequenced in 2018 and assembled at a size of 38.18 Mb (Somvanshi et al., 2018). The *M. javanica* and *M. arenaria* genomes were sequenced in 2017 and assembled at sizes of 142.6 and 163.7Mb, respectively (Szitenberg et al., 2017).

In DNA, tandem repeats are sequences that have multiple identical or nearly-identical copies of a pattern (Benson, 1999). Because the patterns of tandem repeat and the numbers of copies in any specific tandem repeat could be different in different CRKN pathotypes, tandem repeats are useful for evolutionary analysis and DNA fingerprinting.

The term “synteny” describes a conserved region on a chromosome where genes with similar function and structure are in the same order. It is an important genome characteristic for comparison between species. Using Minimap2 (Li, 2018), an alignment program that can map a long DNA sequences against a large reference database, we can detect insertions and deletions and structural variations as well as syntenic regions among the genomes.

Orthologs are genes having the same function in different species; orthologs suggest an ancestor common to these species. The *Caenorhabditis elegans* pyr-1 gene encodes a trifunctional enzyme for *de novo* pyrimidine synthesis (Franks et al., 2006). Previous research show that the pry-1 gene was found to be in a conserved region in the *M. hapla* and *M. chitwoodi* genomes (Cha, 2016). Single orthologs of pyr-1 gene was found in in *M. chitwoodi* Race 1, Race 2, and Race 1_{Roza} and many *Meloidogyne* species. They are good to be used for orthologs comparison analysis. Finding the orthologs of pry-1 gene can provide support in generating a phylogenetic trees for CRKN pathotypes and other *Meloidogyne* species.

In this study, we assembled and annotated the *M. chitwoodi* Race 1, Race 2 and Race 1_{Roza} genomes. In addition, we observed the regions that differ among these three genomes based on genome alignment. Finally, we used BLAST (Altschul et al., 1990) to identify and compare the orthologs of these closely related genomes. The genomic information will be useful to develop markers to differentiate the races of *M. chitwoodi* and will aid in studying CRKN genetic resistance and its loss.

2.3 Materials and Methods

2.3.1 Nematode collection

Isolates of *M. chitwoodi* Race 1 (MC1), Race 2 (MC27) and Race 1_{Roza} (MC1 Roza) were obtained from Washington State University, Pullman. Nematode eggs were extracted from inoculated tomato plants using the hypochlorite method (Hussey & Barker, 1973). Eggs were hatched out at room temperature and the emerging second-stage juveniles (J2) were used for DNA extraction.

2.3.2 DNA extraction and high-throughput sequencing

DNA from nematode eggs of *M. chitwoodi* Race 1, Race 2 and Race 1_{Roza} was extracted and purified using a QIAamp DNA Mini Kit (Cat No. 51304, QIAGEN, Germany). DNA was sent to the Center for Genome Research and Biocomputing (CGRB), Oregon State University, Corvallis, Oregon for Illumina sequencing. For each nematode genotype, two replicate samples were barcoded, and genome libraries were constructed for separate runs through the HiSeq3000 (2 x 150 bp) and MiSeq (2 x 350 bp) instruments.

Highly repetitive nematode genomes challenge high-quality genome assembly using short read Illumina sequencing platforms. For improved quality, higher molecular weight nematode genomic DNA for PacBio sequencing was extracted by Amplicon Express (Pullman, WA, USA). Libraries were prepared according to the SMRTbell Library prep protocol (Pacific Biosciences) for PacBio Sequel RS II (Pacific

Biosciences) by the Center for Genome Research and Biocomputing (CGRB). DNA size selection with Blue Pippin (Sage Science) eliminated short insert templates.

2.3.3 Genome assembly

Illumina reads was generated from HiSeq3000. The quality of the data was checked using FASTQC. Genomic data generated from PacBio sequencing were assembled using the SMRT analysis pipeline, v.2.3.0. Illumina reads were aligned with PacBio data contigs using BWA (Li & Durbin, 2009) to check for the difference from different sequencing technologies.

2.3.4 Genome size estimation and completeness assessment

To evaluate the completeness of the genomic assembly, we used Jellyfish (Marçais & Kingsford, 2011)to extract and count canonical k-mers at k= 21, 31 and 71 nucleotides. For each k-mer value, we used GenomeScope (Vurture et al., 2017) to estimate heterozygosity, haploid genome length, repeat content, unique length and read error rate. We ran BUSCO v4 (Simão et al., 2015) with the Eukaryotic dataset eukaryota_odb10 (number of species = 70) to check genome completeness. Genome annotation was performed using Augustus (Stanke & Morgenstern, 2005) with *C. elegans* as the model.

2.3.5 Repeat analysis

Tandem Repeats Finder (Benson, 1999) software was used to identify tandem repeats. Total tandem repeats and the “perfect repeats” with 100% of matches between

adjacent copies overall and no indels between adjacent copies were compared across the three genomes. We summarized all the motif types for all tandem repeats and perfect tandem repeats for Race 1, Race 2 and Race 1_{Roza} and made a comparison. RepeatMasker (Tarailo-Graovac & Chen, 2009) was used to identify repetitive DNA sequences revealed in the previous step of gene annotation.

2.3.6 Gene prediction and annotation

MAKER2 (Holt & Yandell, 2011) was used to predict genes and annotate the genome. The masked sequence data generated by the RepeatMasker was used for gene prediction and annotation. The RNA seq transcriptome data from *M. chitwoodi* Race 1 was used as EST evidence (Zhang & Gleason, 2021). The transcriptome data from *M. arenaria, M. incognita,* and *M. hapla,* was used as alternative EST evidence. The proteome of *M. arenaria, M. incognita,* and *M. hapla* was used as protein evidence (Blanc-Mathieu et al., 2017; Opperman et al., 2008). A SNAP training file was prepared from the first round of annotation and used for further rounds of annotations.

2.3.7 Identification and clustering of orthologs in *M. chitwoodi* Race 1, Race 2, and Race 1_{Roza} and other *Meloidogyne* species.

The orthologs of *C. elegans* gene pyr-1 were identified in *M. javanica, M. arenaria, M. incognita, M. hapla, M. floridensis, and M. chitwoodi* proteomes using BLAST (Altschul et al., 1990) searches. All orthologs in *Meloidogyne* species were validated

and aligned with Clustal Omega (Madeira et al., 2019) ,which also generated the phylogenetic tree.

2.3.8 Genome alignment analysis.

We used Minimap2 (Li, 2018) to align the Race 1, Race 2 and Race 1_{Roza} genomes. We visualized the scaffold-to-scaffold alignment results with LINKVIEW (Yang, 2021)

.

2.3.9 Whole genome transcriptome and proteome BLAST

Transcriptomes and proteomes of Race 1, Race 2 and Race 1_{Roza} were entered into BLAST query databases. Race 1, Race 2 and Race 1_{Roza} transcriptomes and proteomes were aligned with each other using BLAST and orthologs with a high percentage of identical matches and alignment lengths were filtered out in each pairing between the three races.

2.4 Results

2.4.1 Sequencing statistics

The final assembly of PacBio sequencing resulted in 30, 39 and 38 highly polished contigs for Race 1, Race 2 and Race 1_{Roza}, respectively. The sum of all contig lengths was 47.47 Mb for Race 1 (30 contigs), 46.98 Mb for Race 2 (39 contigs) and 47.78 Mb for Race 1_{Roza} (38 contigs) (Table 1). The largest contig length was 3.04 Mb for

Race 1, 3.04 Mb for Race 2 and 2.94 Mb for Race 1_{Roza}. All three nematode genomes contain an average 25% GC content. The haploid genome size for Race 1, Race 2 and Race 1_{Roza} was estimated to be 43.03 Mb, 42.68 Mb and 42.66 Mb, respectively, at k-mer 21 (Table 2.1, Figures 2.1 through 2.3). This is close to the expected genome size recently reported for *M. gramninicola* (41.5 Mb) (Phan et al., 2020). The BUSCO score of the three genomes showed an average of 69.80% complete, 68.80% single copy, 0.93% duplicated, 12.67% fragmented and 17.53% missing. This result is similar to the published *M. hapla* genome (Table 2.2, Figure 2.2).

The annotated gene numbers for Race 1, Race 2 and Race 1_{Roza} are 12295, 12349, and 12534, respectively.

2.4.2 Repeat analysis

The tandem repeats with different motif sizes in Race 1, Race 2 and Race 1_{Roza} do not differ greatly (Table 2.3). By comparing motif types in Race 1, Race 2 and Race 1_{Roza}, the number of common motif types for all tandem repeats between Race 1 and Race 2 is 14867, greater than the number between Race 1 and Race 1_{Roza} (5124) or the number between Race 2 and Race 1_{Roza} (5411) (Figure 2.4-1). The number of common motif types for perfect tandem repeats between Race 1 and Race 2 (769) is also greater than the number between Race 1 and Race 1_{Roza} (377) or the number between Race 2 and Race 1_{Roza} (329) (Figure 2.4-2).

2.4.3 Genome alignment analysis

The results show that there are 1278 insertions and deletions longer than 1bp between Race 1 and Race 2 genomes, 705 insertions and deletions longer that 1bp between Race 1 and Race 1_{Roza} genomes, and 1400 insertions and deletions longer that 1bp between Race 2 and Race 1_{Roza} genomes. By visualizing the scaffold-to-scaffold Minimap results, we concluded that there are low alignment quality mapping regions in the left arm of Race 1 scaffold 8, which aligned with the right arm of Race 2 scaffold 8a, the right arm of Race 1_{Roza} scaffold 8a (Figure. 2.3-1), and the right arm of Race 1 scaffold 11. Race 1 scaffold 11 aligns with Race 2 scaffold 11b and Race 1_{Roza} scaffold 11b (Figure. 2.3-2). The low alignment quality mapping region between Race 1 scaffold 8 and Race 2 scaffold 8a has no overlap with the low alignment quality mapping region between Race 1 scaffold 8 and Race 1_{Roza} scaffold 8a. The low-quality mapping region between Race 1 scaffold 11 and Race 2 scaffold 11b includes but is not limited to the low-quality mapping region between Race 1 scaffold 11 and Race 1_{Roza} scaffold 11b.

2.4.4 Gene comparisons analysis

By using BLASTN (Altschul et al., 1990) on the Race 1, Race 2 and Race 1_{Roza} transcriptomes, we identified 4530 orthologs with greater than 90% matches and alignment length longer than 50% of the entire gene length between Race 1 and Race 2, 4159 orthologs between Race 2 and Race 1_{Roza}, 4144 orthologs between Race 1 and Race 1_{Roza}, and 3457 of the orthologs are among Race 1, Race 2 and Race 1_{Roza} (Table 2.6). By using BLASTP (Altschul et al., 1990) on the Race 1, Race 2, and

Race 1_{Roza} proteomes, we identified 7292 orthologs with 100% matches and alignment length longer than 80% of the entire protein length between Race 1 and Race 2, 6628 orthologs between Race 2 and Race 1_{Roza}, 6685 orthologs between Race 1 and Race 1_{Roza}, and 5203 of the orthologs are among Race 1, Race 2 and Race 1_{Roza} (Table 2.6).

To analyze the phylogeny of different *Meloidogyne* species and the pathotypes of *M. chitwoodi*, we use the pyr-1 gene to find orthologs. The pyr-1 gene is highly conserved across the *Meloidogyne* species studied here. We identified single orthologs in *M. arenaria, M. incognita, M. hapla, M. floridensis, M. chitwoodi* Race 1, Race 2, and Race $1_{Roza.}$ Two orthologs were found in *M. javanica.* We therefore performed a joint phylogenetic analysis of the orthogroup (Figure 2.5). This confirmed the close relationship of *M. chitwoodi* Race 1, Race 2, and Race 1_{Roza}.

2.5 Discussion

In our study, three CRKN genomes were first sequenced by Illumina sequencing and then by PacBio sequencing. The Illumina sequencing data was assembled into 7838 scaffolds (N50 =211) for Race 1, 5397 scaffolds (N50 = 154) for Race 2, 6660 scaffolds (N50 = 240) for Race 1_{Roza}. The genome of CRKN is highly repeated and "AT" rich, which increased the difficulty to assemble long scaffolds with Illumina sequencing data. PacBio sequencing was then used to get long sequenced reads and assemblage for long scaffolds. We aligned the Illumina sequencing and PacBio sequencing data to check the difference. The imperfect alignment (average of 15%)could be caused by heterozygosity or reading error. We further used the

alignment result to error check the PacBio sequenced genome using pilon v.1.22 (Walker et al. 2014).

Using Tandem repeats finder, we compared motif types that are most common in the genome of *M. chitwoodi* Race 1, Race 2, and Race 1_{Roza}. The numbers of common motif types for all tandem repeats and perfect tandem repeats between Race 1 and Race 2 are greater than the numbers between Race 1 and Race 1_{Roza} or the numbers between Race 2 and Race 1_{Roza}. This suggests that there is less diversity of tandem repeats between Race 1 and Race 2 compared with Race 1_{Roza}.

The overall genome alignment analysis shows that the insertions and deletions between Race 1 and Race 1_{Roza} are fewer than those between other groups. But we do not yet know whether the extra insertions and deletions are in the coding regions or noncoding region. From the scaffold-to-scaffold alignment, we see that the low alignment quality mapping regions between Race 1 and Race 2 and between Race 1 and Race 1_{Roza} are more separate than overlapping. More work need to be done in the future to compare the genes in low alignment quality mapping regions and unique genes we found in ortholog analysis among *M. chitwoodi* Race 1, Race 2 and Race $1_{Roza.}$

From the gene comparison analysis, we found that the number of orthologs between Race 1 and Race 2 that have highly similar DNA and protein sequences is larger than between any other groups of two. Using the highly conserved pyr-1 gene, we generated phylogenetic trees for *M. chitwoodi* Race 1, Race 2, and Race 1_{Roza} and other *Meloidogyne* species. The protein sequences in the three *M. chitwoodi*

pathotypes are identical. In the future, we will use more orthologs to generate the phylogenetic trees and elucidate the evolutionary history of this species.

2.6 Conclusion

The genomic annotations and comparisons of *M. chitwoodi* Race 1, Race 2, and Race 1_{Roza} elucidate the complex evolutionary history of this species. They also provide new evidence of horizontal gene transfer among different races and species. This is the most contiguous genome sequence available for any *Meloidogyne sp.* Furthermore, these genome analyses provide strong resources for future molecular marker development and analysis of the host plant's resistance to these root-knot nematodes.

2.7 References

Abad, P., Gouzy, J., Aury, J.-M., Castagnone-Sereno, P., Danchin, E. G., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., & Blok, V. C. (2008). Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology*, *26*(8), 909–915.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410.

Benson, G. (1999). Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Research*, *27*(2), 573–580. https://doi.org/10.1093/nar/27.2.573

Blanc-Mathieu, R., Perfus-Barbeoch, L., Aury, J.-M., Da Rocha, M., Gouzy, J., Sallet, E., Martin-Jimenez, C., Bailly-Bechet, M., Castagnone-Sereno, P., Flot, J.-F., Kozlowski, D. K., Cazareth, J., Couloux, A., Da Silva, C., Guy, J., Kim-Jo, Y.-J., Rancurel, C., Schiex, T., Abad, P., Wincker, P., Danchin, E. G. J. (2017). Hybridization and polyploidy enable genomic plasticity without sex in the most devastating plant-parasitic nematodes. *PLoS Genetics*, *13*(6), e1006777. https://doi.org/10.1371/journal.pgen.1006777

Cha, S. (2016). *Is the biology of M. chitwoodi and M. hapla reflected in their genomes?*

Davies, L. J., Brown, C. R., & Elling, A. A. (2015). Calcium is involved in the *R Mc1* (*blb*)-mediated hypersensitive response against *Meloidogyne chitwoodi* in potato. *Plant Cell Reports*, *34*(1), 167–177.

Franks, D. M., Izumikawa, T., Kitagawa, H., Sugahara, K., & Okkema, P. G. (2006). *C. elegans* pharyngeal morphogenesis requires both de novo synthesis of pyrimidines and synthesis of heparan sulfate proteoglycans. *Developmental Biology*, *296*(2), 409–420. https://doi.org/10.1016/j.ydbio.2006.06.008

Holt, C., & Yandell, M. (2011). MAKER2: An annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinformatics*, *12*(1), 491. https://doi.org/10.1186/1471-2105-12-491

Hussey, R. S., & Barker, K. R. (1973). Comparison of methods of collecting inocula of *Meloidogyne spp*., including a new technique. *Plant Disease Reporter*. https://agris.fao.org/agris-search/search.do?recordID=US201303126127

Kyndt, T., Vieira, P., Gheysen, G., & de Almeida-Engler, J. (2013). Nematode feeding sites: Unique organs in plant roots. *Planta*, *238*(5), 807–818.

Leroy, S., Duperray, C., & Morand, S. (2003). Flow cytometry for parasite nematode genome size measurement. *Molecular and Biochemical Parasitology*, *128*(1), 91–93. https://doi.org/10.1016/s0166-6851(03)00023-9

Li, H. (2018). Minimap2: Pairwise alignment for nucleotide sequences. Bioinformatics, 34(18), 3094–3100. https://doi.org/10.1093/bioinformatics/bty191

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics*, *25*(14), 1754–1760.

Lunt, D. H., Kumar, S., Koutsovoulos, G., & Blaxter, M. L. (2014). The complex hybrid origins of the root knot nematodes revealed through comparative genomics. *PeerJ*, *2*, e356.

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D., & Lopez, R. (2019). The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Research*, *47*(W1), W636–W641. https://doi.org/10.1093/nar/gkz268

Marçais, G., & Kingsford, C. (2011). A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics*, *27*(6), 764–770.

Mojtahedi, H., Brown, C. R., Riga, E., & Zhang, L. H. (2007). A new pathotype of *Meloidogyne chitwoodi* Race 1 from Washington State. *Plant Disease*, *91*(8), 1051–1051.

Mojtahedi, H., Santo, G. S., Brown, C. R., Ferris, H., & Williamson, V. (1994). A new host race of *Meloidogyne chitwoodi* from California. *Plant Disease*, *78*(10).

Opperman, C. H., Bird, D. M., Williamson, V. M., Rokhsar, D. S., Burke, M., Cohn, J., Cromer, J., Diener, S., Gajan, J., & Graham, S. (2008). Sequence and genetic map of *Meloidogyne hapla*: A compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences*, USA, *105*(39), 14802–14807.

Phan, N. T., Orjuela, J., Danchin, E. G. J., Klopp, C., Perfus‐Barbeoch, L., Kozlowski, D. K., Koutsovoulos, G. D., Lopez‐Roques, C., Bouchez, O., Zahm, M., Besnard, G., & Bellafiore, S. (2020). Genome structure and content of the rice root‐knot nematode (*Meloidogyne graminicola*). *Ecology and Evolution*, *10*(20), 11006–11021. https://doi.org/10.1002/ece3.6680

Powers, T. O., Mullin, P. G., Harris, T. S., Sutton, L. A., & Higgins, R. S. (2005). Incorporating molecular identification of *Meloidogyne spp*. into a large-scale regional nematode survey. *Journal of Nematology*, *37*(2), 226.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210–3212.

Somvanshi, V. S., Tathode, M., Shukla, R. N., & Rao, U. (2018). Nematode genome announcement: A draft genome for rice root-knot nematode, *Meloidogyne graminicola*. *Journal of Nematology*, *50*(2), 111–116. https://doi.org/10.21307/jofnem-2018-018

Stanke, M., & Morgenstern, B. (2005). AUGUSTUS: A web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Research*, *33*(Web Server issue), W465–W467. https://doi.org/10.1093/nar/gki458

Szitenberg, A., Salazar-Jaramillo, L., Blok, V. C., Laetsch, D. R., Joseph, S., Williamson, V. M., Blaxter, M. L., & Lunt, D. H. (2017). Comparative genomics of apomictic root-knot nematodes: Hybridization, ploidy, and dynamic genome change. *Genome Biology and Evolution*, *9*(10), 2844–2861.

Tarailo-Graovac, M., & Chen, N. (2009). Using RepeatMasker to identify repetitive elements in genomic sequences. *Current Protocols in Bioinformatics*, *25*(1), 4–10.

Van der Beek, J. G., & Karssen, G. (1997). Interspecific hybridization of meiotic parthenogenetic *Meloidogyne chitwoodi* and *M. fallax*. *Phytopathology*, *87*(10), 1061–1066.

Vurture, G. W., Sedlazeck, F. J., Nattestad, M., Underwood, C. J., Fang, H., Gurtowski, J., & Schatz, M. C. (2017). GenomeScope: Fast reference-free genome profiling from short reads. *Bioinformatics*, *33*(14), 2202–2204.

Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., & Young, S. K. (2014). Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PloS One*, *9*(11), e112963.

Yang, J. (2021). *LINKVIEW* [Python]. https://github.com/YangJianshun/LINKVIEW (Original work published 2019)

Zhang, L., & Gleason, C. (2021). Transcriptome Analyses of Pre-parasitic and Parasitic Meloidogyne Chitwoodi Race 1 to Identify Putative Effector Genes. *Journal of Nematology*, *53*, e2021-84. https://doi.org/10.21307/jofnem-2021-084

2.7 Tables

Table 2.1. Summary of statistics of *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Mc1 pathotype ($Mc1_{Roza}$) sequenced genomes.

Genotype	Assembly Size (bp)	Maximum Contig Length (bp)	N50 Contig Length (bp)	No. of Polished Contigs	Est. Genome Coverage (x)	Haploid Genome size (Mb)[1]
Race 1	47477280	3043654	2451023	30	172.04	43.03
Race 2	46924610	3043255	2317798	39	84.34	42.68
$Race\ 1_{Roza}$	47730107	2942887	2363161	38	168.05	42.66

[1]K-mer based estimation calculated using Jellyfish and GenomeScope (k-mer=21nt)

Table 2.2. BUSCO score estimation of *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Race 1Roza (Mc1Roza) sequenced genomes as compared to the publicly available *Meloidogyne hapla* genome. Scores were calculated using the eukaryote odb10 dataset (BUSCO v5.0.0).

	Complete BUSCOs	Complete and single-copy BUSCOs	Complete and duplicated BUSCOs	Fragmented BUSCOs	Missing BUSCOs	Total BUSCO groups searched
Race 1	C:68.6%	S:68.2%	D:0.4%	F:12.9%	M:18.5%	n:255
Race 2	C:71.0%	S:70.6%	D:0.4%	F:11.8%	M:17.2%	n:255
Race 1_{Roza}	C:69.8%	S:67.8%	D:2.0%	F:13.3%	M:16.9%	n:255
M. hapla	C:73.8%	S:72.2%	D:1.6%	F:12.2%	M:14.0%	n:255

Table 2.3. Summary of *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Race 1_{Roza} ($Mc1_{Roza}$) tandem repeats of different motif sizes.

	Total tandem repeats		Perfect tandem repeats	
	Motif size	Number of tandem repeats	Motif size	Number of tandem repeats
Race 1	Motifsizeunder10	2669	Motifsizeunder10	359
	Motifsize11to20	21791	Motifsize11to20	2328
	Motifsize21to30	11386	Motifsize21to30	218
	Motifsize31to40	2705	Motifsize31to40	65
	Motifsize41to50	1772	Motifsize41to50	36
	Motifsize51to100	1838	Motifsize51to100	44
	Motifsize101to250	902	Motifsize101to250	16
	Motifsize251to500	135	Motifsize251to500	0
Race 2	Motifsizeunder10	2633	Motifsizeunder10	364
	Motifsize11to20	21675	Motifsize11to20	2321
	Motifsize21to30	11345	Motifsize21to30	220
	Motifsize31to40	2740	Motifsize31to40	72
	Motifsize41to50	1701	Motifsize41to50	38
	Motifsize51to100	1825	Motifsize51to100	48
	Motifsize101to250	732	Motifsize101to250	15
	Motifsize251to500	121	Motifsize251to500	0
Race 1_{Roza}	Motifsizeunder10	2670	Motifsizeunder10	346
	Motifsize11to20	21881	Motifsize11to20	2316
	Motifsize21to30	11649	Motifsize21to30	224
	Motifsize31to40	2781	Motifsize31to40	65
	Motifsize41to50	1730	Motifsize41to50	32
	Motifsize51to100	1889	Motifsize51to100	48
	Motifsize101to250	888	Motifsize101to250	15
	Motifsize251to500	122	Motifsize251to500	0

Table 2.4. Insertions and deletions between *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Race 1_{Roza} ($Mc1_{Roza}$) detected using Mimimap2 alignment.

INDEL size	Race 1 & Race 2	Race 1 & Race 1_{Roza}	Race 2 & Race 1_{Roza}
1bp	1783	1622	2305
2bp	181	85	233
3-50bp	717	410	807
50-1000bp	336	183	310
>1000bp	44	27	50
Total (> 1bp)	1278	705	1400

Table 2.5. Orthologs in *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Race 1Roza (Mc1Roza) detected using BLASTN and BLASTP. Transcriptome orthologs have 90% or higher matches and longer than 50% alignment length of the entire gene. Proteomic orthologs are 100% identical and their alignment length is longer than 80% of the length of the entire protein length.

Transcriptome		Proteome	
	gene number		gene number
Race 1	12295	Race 1	12295
Race 2	12349	Race 2	12349
Race 1_{Roza}	12534	Race 1_{Roza}	12534
Orthologs in Race 2&Race 1_{Roza}	4159	Orthologs in Race 2&Race 1_{Roza}	6628
Orthologs in Race 1&Race 1_{Roza}	4144	Orthologs in Race 1&Race 1_{Roza}	6685
Orthologs in Race 2&Race 1	4530	Orthologs in Race 2&Race 1	7292
Orthologs in Race 1&Race 2&Race 1_{Roza}	3457	Orthologs in Race 1&Race 2&Race 1_{Roza}	5203

2.8 Figures

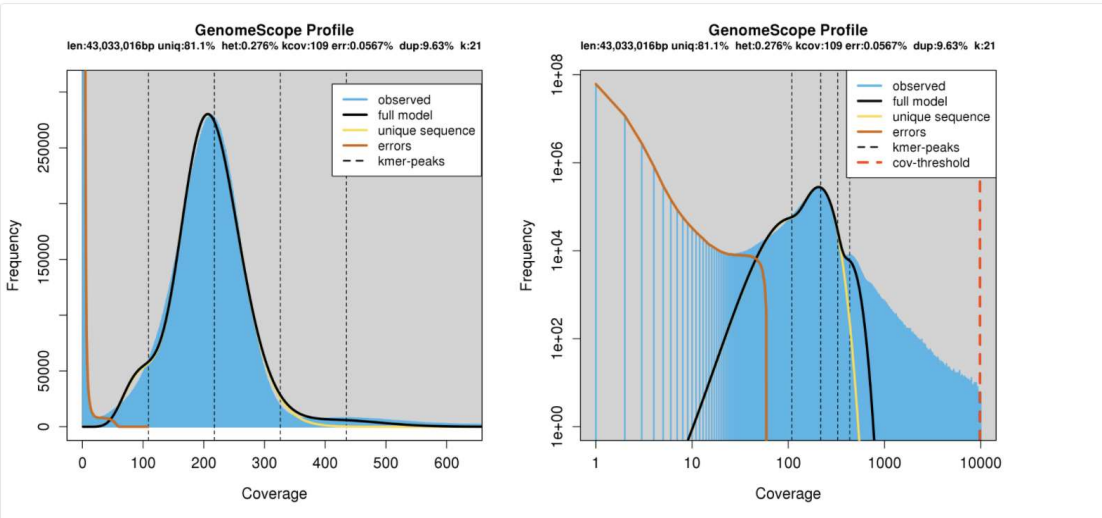


Figure 2.1-1. K-mer based estimation of the size of the *Meloidogyne chitwoodi* Race 1 (Mc1) genome calculated using Jellyfish and GenomeScope (k-mer=21nt).

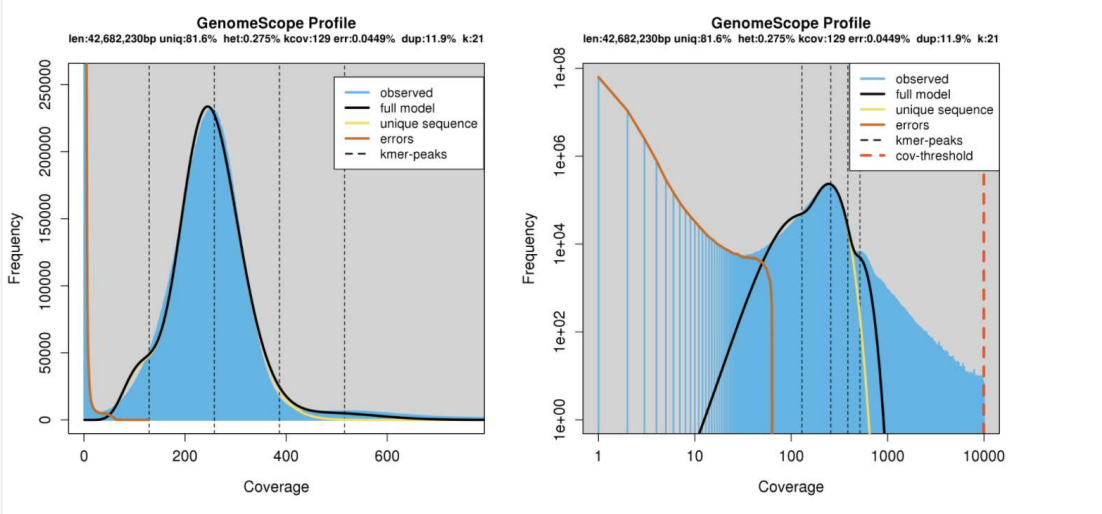


Figure 2.1-2. K-mer based estimation of the size of the *Meloidogyne chitwoodi* Race 2 (Mc27) genome calculated using Jellyfish and GenomeScope (k-mer=21nt).

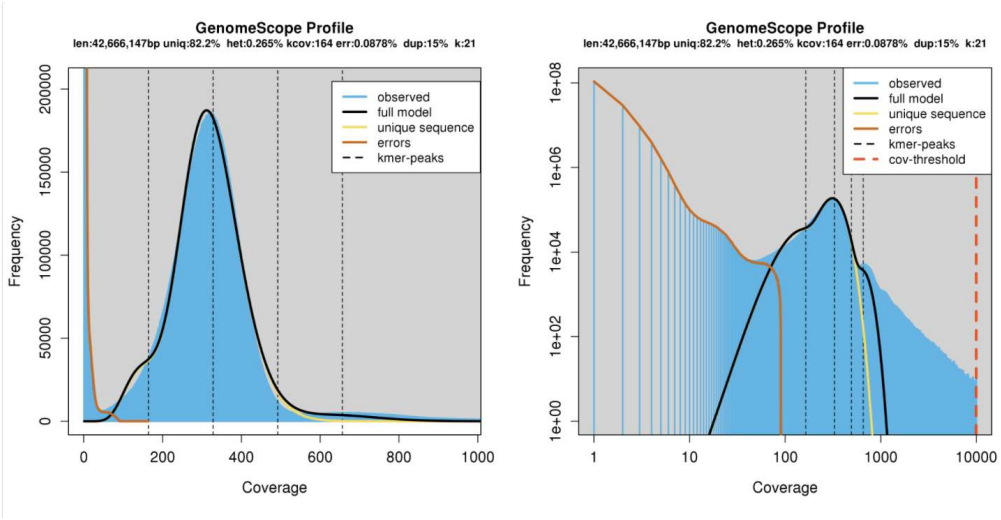


Figure 2.1-3. K-mer based estimation of the size of the *Meloidogyne chitwoodi* Race 1_{Roza} ($Mc1_{Roza}$) genome calculated using Jellyfish and GenomeScope (k-mer=21nt).

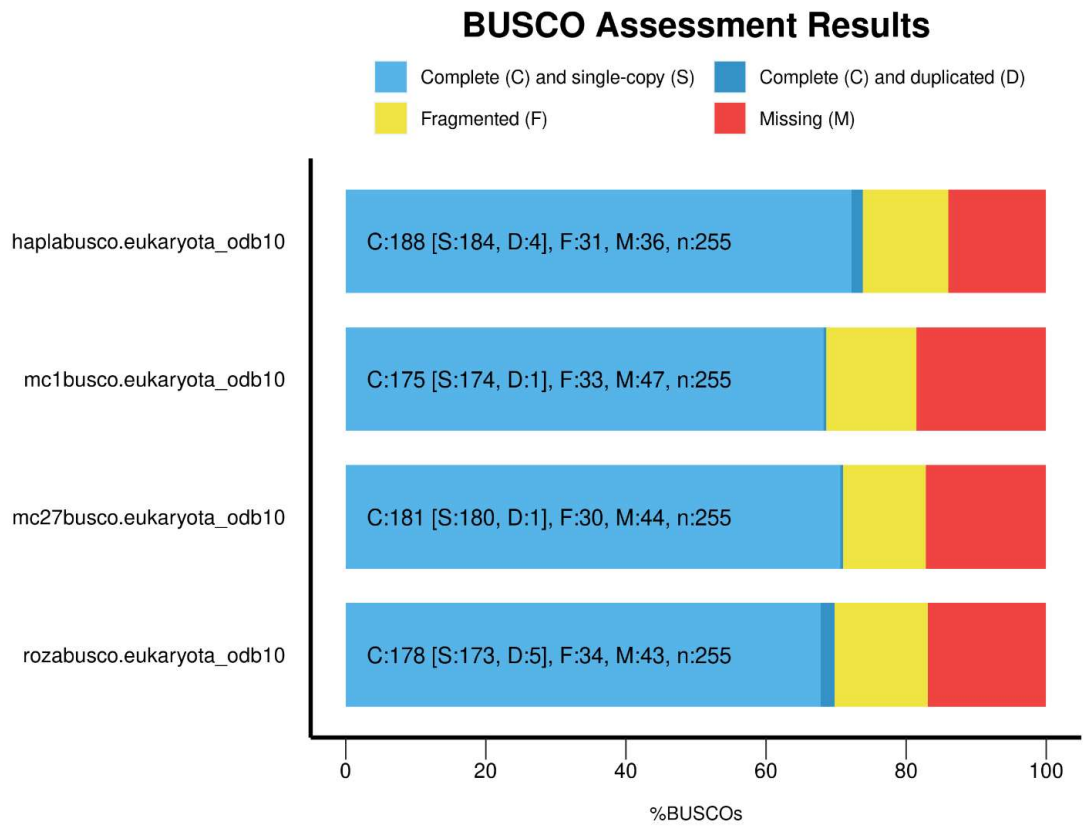


Figure 2.2. Summary of BUSCO analysis for *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Race 1_{Roza} (Mc1Roza) sequenced genomes using the eukaryota database. *Meloidogyne hapla* was used as a reference point.

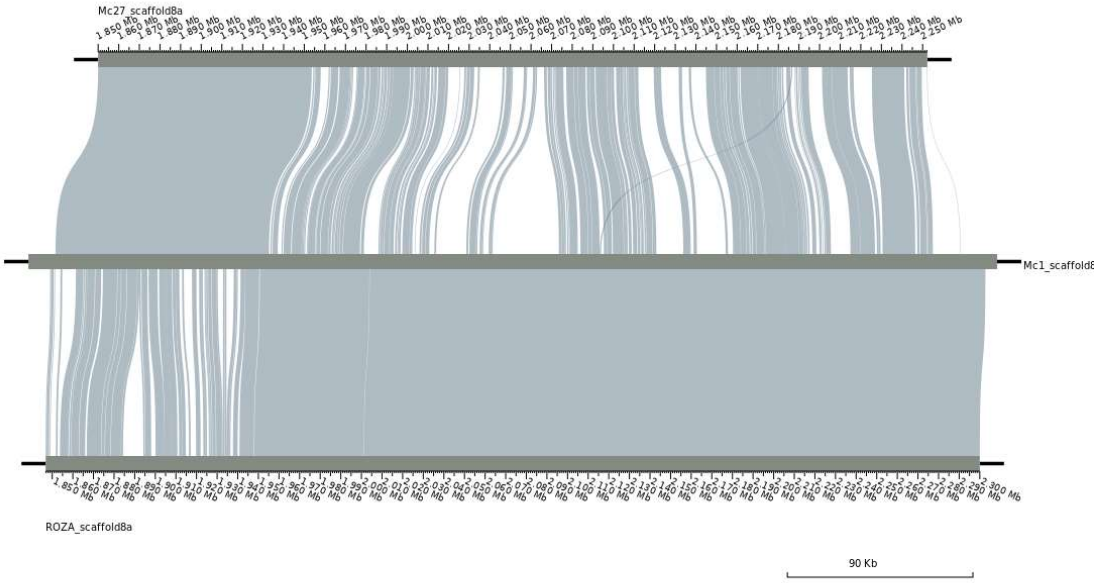


Figure 2.3-1. Low quality mapping regions in the left arm of *Meloidogyne chitwoodi* Race 1 (Mc1) scaffold 8 (reverse complement) aligned with the right arm of Race 2 (Mc27) scaffold 8a and right arm of Race 1_{Roza} ($Mc1_{Roza}$) scaffold 8a from scaffold-to-scaffold Minimap results and visualized using Linkview. The grey lines show high mapping quality regions, the void areas are low quality mapping regions.

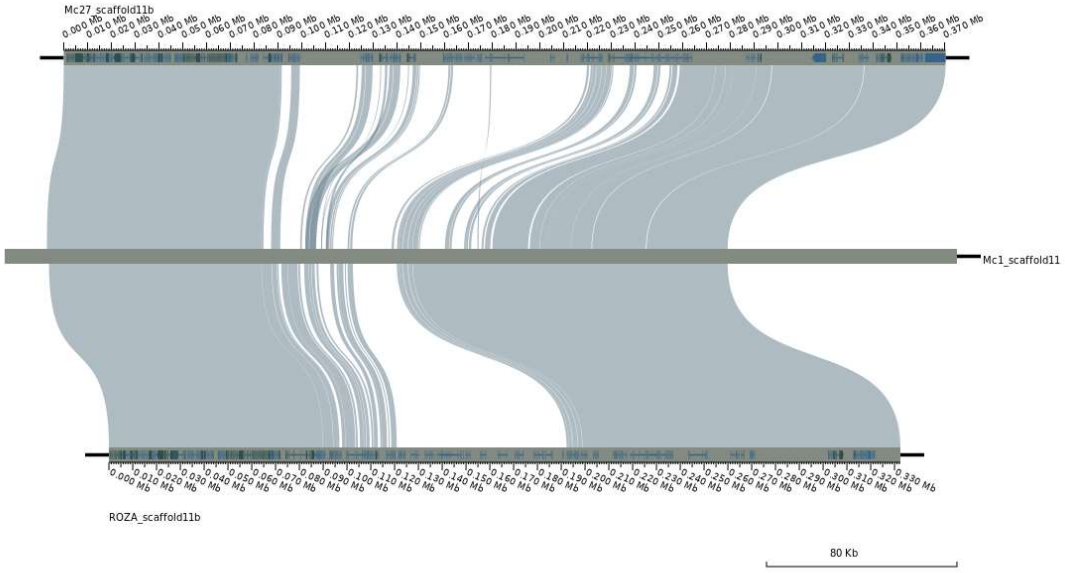


Figure 2.3-2. Low quality mapping regions in left arm of *Meloidogyne chitwoodi* Race 1 (Mc1) scaffold11 aligned with the right arm of Race 2 (Mc27) scaffold11b and the right arm of Race 1_{Roza} ($Mc1_{Roza}$) scaffold 11b from scaffold-to-scaffold Minimap results visualized using Linkview. The grey lines show high quality mapping regions, the void areas are low quality mapping regions.

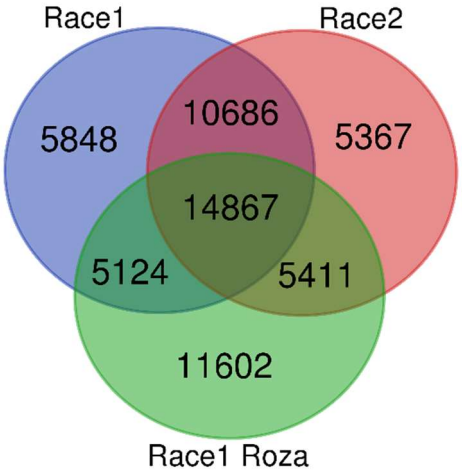


Figure 2.4-1. Venn diagram shows the common and unique motif type for all tandem repeats in *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Race 1_{Roza} ($Mc1_{Roza}$)

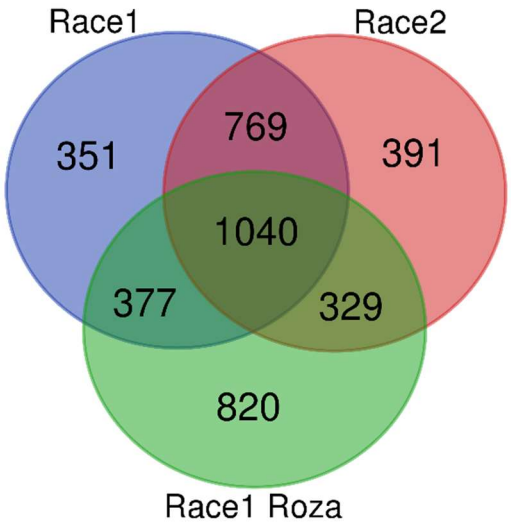


Figure 2.4-2. Venn diagram shows the common and unique motif type for perfect tandem repeats in *Meloidogyne chitwoodi* Race 1 (Mc1), Race 2 (Mc27) and Race 1_{Roza} ($Mc1_{Roza}$)

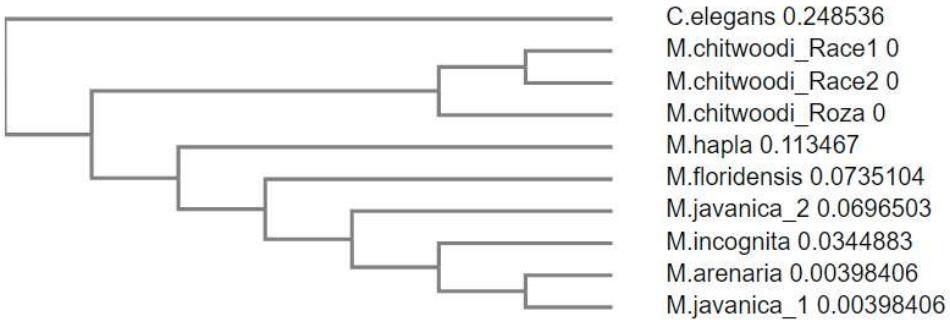


Figure 2.5. Phylogenetic tree of pyr-1 gene in *M. arenaria, M. incognita, M. hapla, M. floridensis, M. chitwoodi* Race 1, Race 2, and Race $1_{Roza.}$

3. Molecular marker development to identify different pathotypes of *Meloidogyne chitwoodi*

3.1 Abstract

Columbia root-knot nematode parasitizes potato plants in the Pacific Northwest (PNW) and causes visible small brown dots in the tuber flesh that dramatically reduce the market value of the crop. In the PNW two races of *M. chitwoodi,* Race 1 and Race 2 and a pathotype Race 1_{Roza} exist. The races of *M. chitwoodi* are primarily differentiated based on host tests. Currently, *M. chitwoodi* can be differentiated from *M. hapla* or other *Meloidogyne* Sp. based on morphology and by molecular markers but we cannot differentiate different pathotypes of *M. chitwoodi* based on morphology. Based on the genome comparison of *M. chitwoodi* Race 1, Race 2 and Race $1_{Roza,}$ we developed 36 pairs of PCR primers for SSR regions and 17 pairs of PCR primers for INDEL regions. Among those, HSINDEL8, HSINDEL9 and HSINDEL10 successfully differentiate all the three pathotypes of *M. chitwoodi* and has potential application in plant disease diagnostics.

3.2 Introduction

Columbia root-knot nematode (CRKN), *Meloidogyne chitwoodi* Golden, O'Bannon, Santo, and Finley is a soil-borne pathogen parasitizing a wide range of plants in the Pacific Northwest (PNW). In potato *(Solanum tuberosum* L.), CRKN attacks roots and tubers causing small visible brown spots in the tuber flesh, dramatically reducing their market value. It is most abundant in the Columbia Basin potato growing region

of Oregon and Washington, but is also found in California, Idaho, Colorado, New Mexico, and Texas (Powers et al. 2005). *Meloidogyne chitwoodi* has four juvenile stages (J1- J4) and an adult stage. The second-stage juveniles (J2) of CRKN invade the root elongation region and form a feeding site. Each CRKN feeding site consists of four to eight transformed giant cells which provide nutrients for the nematodes (Kyndt et al., 2013). J2 develop into male and female adults after forming the feeding site. Adult female CRKN exude egg masses within the gall tissue. (Davies et al., 2015).

Meloidogyne chitwoodi control is difficult because of its wide host range (e.g., wheat, corn, and carrot) (Brown et al. 2006). Alternate host species suffer less damage, but support population increase. The population of *M. chitwoodi* supported by alternate hosts could increase sharply by the end of the growing season and result in serious economic losses in a subsequent potato crop. The low level of damage to several host species compounds the difficulty in identifying any potential threat to a subsequent potato crop. Fumigation before planting a potato crop is an effective method to control the nematodes, although it is costly and may lead to negative environmental impacts (Brown et al. 2009). Because of the environmental concerns of using harmful fumigants and the broad host range of the nematode species, host resistance is viewed as the best control alternative for this pathogen.

In the Pacific Northwest, two contrasting populations of *M. chitwoodi* exist: Race 1 and Race 2. These are differentiated by the host plants they are able to infect. Race 1 can reproduce on carrot while Race 2 cannot (Mojtahedi et al. 1994). Race 2 can reproduce on ‘Thor’ alfalfa while Race 1 cannot (Santo et al. 1985). There is a

pathotype of *M. chitwoodi,* WAMCRoza, which was identified in experimental plots planted repeatedly with potato clones resistant to Race 1. This isolate has been named Race 1_{Roza}. Race 1Roza can reproduce on *Solanum bulbocastanum* (P1275187, SB22) and can break resistance conditioned by the *Race 1(blb)* gene (Mojtahedi et al. 2007). *Meloidogyne chitwoodi, M. hapla, Trichodorus obtusus*, *Pratylenchus vulnus* and some other plant-pathogenic nematodes can be found in soil samples and differentiated by their morphological characteristics. The methods to identify nematodes from soil samples require experience and an understanding of nematode morphology. Accurate identification of race and host range is key to control of *M. chitwoodi*. Currently, we cannot differentiate *M. chitwoodi* races based on morphology. *M. chitwoodi* can be differentiated from *M. hapla* based on morphology and by using polymerase chain reaction (PCR) after identifying DNA-based molecular markers (Zhang et al. 2019). Host testing of CRKN races is tedious and time consuming. Development of molecular markers that can differentiate *M. chitwoodi* races holds promise of positive economic impact for potato production in the Columbia Basin.

3.3 Materials and Methods

3.3.1 Nematode isolates

Isolates of *M. chitwoodi* Race 1, Race 2 and Race 1_{Roza} were obtained from USDA-ARS, Prosser WA, while *M. hapla* isolates were obtained from the Dr. Gleason's lab at Washington State University.

3.3.2 Extraction of nematode eggs from root tissue

Fifty-five days after inoculation, tomato stems were cut above the soil level. The roots were washed gently to remove all soil. Roots were cut into 1 cm sections and put into a labeled shaking canister. Roots were covered in a 10% Clorox® and shaken for four minutes, at which point water was added to each canister until it was 75 % full to avoid damage to the eggs. The canisters were emptied of roots and all detritus onto #200 and #500 sieves and eggs were collected. The #200 sieve was sprayed 10 times with water so that the eggs passed through the #200 sieve and remained on the #500 sieve. The eggs were then transferred from the #500 sieve to 100 mL bottles by placing a funnel in the top of the bottle and rinsing the screen with 50-70 mL of water. It was necessary to carefully and gradually pour the water into the 100 mL bottles, which were then refrigerated.

3.3.3 DNA extraction for nematode eggs

The three *M. chitwoodi* isolates Race 1, Race 2, and Race 1_{Roza} were maintained on tomato plants; eggs were harvested from the roots as described in 3.3.2. Genomic DNA was extracted from nematode eggs using E.Z.N.A Tissue DNA kit (OMEGA, Norcross, GA). The previously extracted nematode eggs were transferred to a 1.5 mL microcentrifuge tube, to which were added 200 µL TL Buffer and 25 µL Proteinase K Solution. The microcentrifuge tube was vortexed to mix thoroughly. It was then incubated at 55 °C in a shaking water bath for 2 hours and vortexed every 20-30 minutes. After incubation, the sample was centrifuged at maximum speed for 5

minutes. The supernatant was transferred to a new sterile 1.5 mL microcentrifuge tube, mixed with 220 µL BL Buffer and incubated at 70 °C for 10 minutes. Next, 220 µL 100% ethanol was added to the sample and mixed thoroughly. The entire sample was transferred to a HiBind® DNA Mini Column inserted into a 2 mL collection tube, and then centrifuged at maximum speed for 30 seconds. Filtrate and the collection tube were discarded. The HiBind® DNA Mini Column was inserted into a new 2 mL collection tube and washed twice by adding 700 µL DNA wash buffer diluted with 100% ethanol, centrifuging at maximum speed for 30 seconds, discarding the filtrate, and reusing the collection tube. After washing, the empty HiBind® DNA Mini Column was centrifuged at maximum speed for 2 minutes to dry the column, which was then transferred into a nuclease-free 1.5 mL microcentrifuge tube. Next, 100-200 µL 70 °C elution buffer was added to the HiBind® DNA Mini Column. The column was allowed to rest at room temperature for 2 minutes, then the HiBind® DNA Mini Column with microcentrifuge tube was centrifuged at maximum speed for 1 minute. The empty HiBind® DNA Mini Column was discarded. Eluted DNA in the microcentrifuge tube was stored at -20 °C.

3.3.4 Soil sample collection

Five soil samples, each consisting of 10 sub-samples from different locations in a CRKN infested field at the Hermiston Agricultural Research and Extension Center, were collected in January 2020. CRKN has been maintained in this field for years (Abawi et al. 2007). Soil samples were placed in labeled plastic bags and sent to a

commercial lab (AGNEMA, Pasco, WA) for nematode analysis. In addition, soil samples with known CRKN were obtained from AGNEMA for marker validation.

3.3.5 Extraction of nematodes from soils

A 200-cc soil sample was placed into a 500-cc cup, which was filled with tap water and stirred. The supernatant was slowly poured onto a #400 mesh sieve, and carefully shaken until the water drained away. The sample retained on the sieve was washed to one side with water and then transferred into a 100 mL centrifuge tube. The tube was centrifuged at 3500 rpm for 3 minutes. All supernatant and any soil or detritus clinging to the tube lid were carefully discarded. A sugar solution (454g sucrose liter^{-1}) was added to within one-half inch of the top of the tube, gently mixed with the sample and centrifuged at 3500 rpm for 3 minutes. After centrifuging, the supernatant was poured onto a small #500 sieve, washed to one side of the sieve to remove the sugar solution and the sample retained on the sieve was transferred into a 100 mL centrifuge tube for storage under refrigeration at 4 °C until analysis.

3.3.6 DNA extraction from nematodes isolated from soil samples

Soil samples containing *M. chitwoodi* were used for DNA extraction. Microorganism DNA was extracted from soil samples using the DNeasy PowerSoil Pro Kit (QIAGEN, Germantown, MD). Briefly, the nematode sample extracted from soil that included different kinds of nematodes and other microorganisms was centrifuged and concentrated into 100-200 µL in a 1.5 mL microcentrifuge tube. The sample was then mixed with 800 µL of Solution CD1 and poured into a PowerBead Pro Tube. The

PowerBead Pro Tube was then set on a vortex adapter for 1.5–2 mL tubes and vortexed at maximum speed for 10 minutes. The PowerBead Pro Tube was next centrifuged at 15,000 x g for 1 min. Supernatant was then transferred to a clean 2 mL microcentrifuge tube. To the supernatant was added 600 µL of Solution CD3 and mixed. The mixed supernatant was passed through the MB Spin Column by loading 650 µL of lysate onto an MB Spin Column, centrifuging at 15,000 x g for 1 min, and twice discarding the flow-through. The MB Spin Column was then inserted into a clean 2 mL collection tube. Five hundred µl of Solution EA flowed through the MB Spin Column by centrifuging at 15,000 x g for 1 min and discarding the flow-through. Next, 500 µL of Solution C5 flowed through the MB Spin Column by centrifuging at 15,000 x g for 1 min and discarding the flow-through. The MB Spin Column was then centrifuged at 16,000 x g for 2 min to remove all Solution C5 and then carefully placed into a new 1.5 mL elution tube. To the center of the white filter membrane in the MB Spin Column was added 50–100 µl of Solution C6. After sitting at room temperature for 2 min, the MB Spin Column with the elution tube was centrifuged at 15,000 x g for 1 min. The MB Spin Column was discarded. The DNA remaining in the elution tube was stored at -20 °C.

3.3.7 Marker development:

The genome sequences of *M. chitwoodi* isolates Race 1 (MC1), Race 2 (MC27) and Race 1_{Roza} ($MC1_{Roza}$) (Bali et al., 2021) were used for primer design and subsequent marker development. Tandem Repeats Finder (TRF) (Benson, 1999) identified the simple sequence repeats (SSRs) from the genome sequence of Race 1.

Minimap2 (Li, H. 2018) was used to align the sequences of the *M. chitwoodi* isolate Race 1, Race 2 and Race 1_{Roza} genomes to reveal insertions and deletions between genomes. We then filtered the insertions and deletions and used Primer3 (https://bioinfo.ut.ee/primer3-0.4.0/) to design the PCR primers (Untergasser et al. 2012, Koressaar et al. 2007, Koressaar et al. 2018).

3.3.8 Molecular marker analysis

Thirty-six pairs of SSR primers,17 pairs of INDEL primers and primer for *M.hapla* DNA validation were tested with *M. chitwoodi* isolates Race 1, Race 2, Race 1_{Roza} and *M.hapla* egg DNA. A 10 µL PCR reaction mixture contained 2 µL 5X Mytaq Reaction Buffer (Bioline), 0.2 µL MyTaq DNA Polymerase (Bioline), 0.5 µL DMSO, 1 µL DNA template, 0.3 µL forward and reverse primers and 6 µL of molecular grade water. PCR cycling conditions used to amplify SSR markers were initially 95 °C for 90 seconds, following by 38 cycles: denaturation at 95 °C for 20 seconds, annealing at 56 °C for 15 seconds, extension at 72 °C for 15 seconds and a final step at 72 °C for 5 minutes. PCR cycling conditions used for amplifying INDEL markers and *M.hapla* DNA validation markers were initial 95 °C for 90 seconds followed by 38 cycles: denaturation at 95 °C for 20 seconds, annealing at 58 °C for 15 seconds, extension at 72 °C for 15 seconds and a final step at 72 °C for 5 min. The amplification product sizes were determined by comparison with the 100 bp molecular marker ladder (Promega, USA) following electrophoresis of 10 µL PCR product on a 2% agarose gel in TBE buffer.

3.4 Results

3.4.1 Nematode morphological identification

Soil samples collected from farms in the potato producing regions of the Columbia Basin were used for nematode morphological identification and future molecular identification. For each sample, nematodes were identified and counted under the microscopy by a commercial lab AGNEMA (Pasco, WA). Samples containing *M. chitwoodi* were kept for DNA extraction and molecular identification. The sample name and CRKN counts are shown (Table 3.1).

3.4.2 Development and screening of polymorphic markers

The TRF identified 216 SSRs with the motif length shorter than 10 nucleotide repeats that included "G" or "C" in the Race 1 genome. Thirty-six of them showed potential polymorphism based on the BLAST of target SSR region between Race 1, Race 2 and Race 1_{Roza} genomes. Thirty-six pairs of SSR primers were designed from the result. Seventeen pairs of INDEL primers were designed based on the Minimap2 results. Thirty-six pairs of SSR primers and 17 pairs of INDEL primers were tested for polymorphism on agarose gel using Race 1, Race 2, and Race 1_{Roza} egg DNA. The DNA of Race 1, Race 2, and Race 1_{Roza} are validated by PCR based markers (Wishart et al., 2002; Zijlstra, 2000) (Table 3.2, Figure 3.9). Of the 36 SSR markers, two (HS04FSSR4 and HS07FSSR3) showed polymorphism between Race 1, Race 1_{Roza} and Race 2. Of the 17 INDEL markers, one (HSINDEL5) showed polymorphism of Race 2 against Race 1, Race $1_{Roza;}$ four INDEL markers (HSINDEL6, HSINDEL7,

HSINDEL9, HSINDEL10) showed polymorphism of Race 1_{Roza} against Race 1 and Race 2 and one INDEL marker (HSINDEL8) showed polymorphism of Race 1 against Race 2 and Race $1_{Roza.}$ A total of eight markers showed polymorphism against the three isolates of CRKN (Table 3.3).

3.4.3 Validation of polymorphic markers on soil samples

Two SSR and eight INDEL markers displaying initial polymorphism were validated with a mixture of Race 1, Race 2 and Race 1_{Roza}, *M. hapla* and nematode DNA extracted from various soil samples. Four INDEL markers (HSINDEL5, HSINDEL8 HSINDEL9 and HSINDEL10) showed polymorphism across all samples. INDEL marker HSINDEL5 (Figure 3.3) differentiated *M. chitwoodi* Race 2 from Race 1 and Race 1_{Roza} , while marker HSINDEL8 (Figure 3.4) easily differentiated *M. chitwoodi* Race 1 from Race 2 and Race 1_{Roza}, and markers HSINDEL9 and HSINDEL10 (Figure 3.4, Figure 3.5) differentiated *M. chitwoodi* Race 1_{Roza} from Race 1 and Race 2. The marker analyses found that *M. chitwoodi* Race 1 and Race 2 were identified in the soil samples from the Hermiston Agricultural Research and Extension Center (Table 3.3, Figure 3.6). *M. chitwoodi* pathotype Race 1_{Roza} was identified in some soil samples from Washington state (Table 3.1, Figure 3.7, Figure 3.8). INDEL marker HSINDEL5 did not identify any *M. chitwoodi* Race 2 from soil samples. None of the INDEL and SSR markers were amplified from *M. hapla* DNA while the *M. hapla* specific markers amplified (Figure 3.9).

3.5 Discussion

DNA extracted from soil samples was of low concentration; for that reason, we required sensitive PCR markers and more PCR cycles to create sufficient amplification products for clear electrophoresis agarose gel images. Testing nematode DNA extracted from soil samples is more challenging than testing DNA from nematode eggs. DNA extracted from soil samples includes DNA of different nematodes and other soil-borne microorganism, which require highly sensitive and specific PCR markers. Markers HSINDEL8, HSINDEL9 and HSINDEL10 showed good sensitivity and no false positives were displayed in our soil sample validations. The primer design method used for SSR markers filters the simple sequence repeats in the Race 1 genome and blasts the target sequences with the Race 2 and Race 1_{Roza} genomes. The *M. chitwoodi* genomes are highly AT rich and have many repeating sequences. The blast result could be disturbed by similar repeating regions among genomes, reducing the accuracy of blast identification of different SSR regions. Also, the highly AT rich and repeating genome of *M. chitwoodi* may reduce the sensitivity and specificity of SSR primers. Most of the SSR primers were designed from short repeating sequences that failed to identify the nematode races. Though we identified two SSR markers to be polymorphic in the initial screening, those two markers are not sufficiently robust to differentiate *M. chitwoodi* from soil samples.

Of 17 INDEL markers, six showed polymorphisms for at least one pathotype of *M. chitwoodi* under agarose gel electrophoresis. This provides a reliable way to identify short insertions and deletions using Minimap2 whole genome alignment. The same method could be used to find smaller INDELs and predict SNP markers that could

identify *M. chitwoodi* races using High Resolution Melting (HRM) analysis (Demeler et al., 2013).

Identifying *M. chitwoodi* races is an important step in controlling CRKN by host range. By developing molecular markers, we successfully differentiated *M. chitwoodi* Race 1 from Race 2 and pathotype Race 1_{Roza} with a small amount of DNA extracted from soil samples. In our study, the INDEL primers that can identify *M. chitwoodi* races did not amplify in *M. hapla* DNA and other nematode DNA from soil samples, indicating that our primers are stable and reliably identify *M. chitwoodi.* As we know that *M. fallax* is a close relative of *M. chitwoodi* (Zhang, 2019), we plan to apply these primers to *M. fallax* DNA to determine whether they differentiate *M. chitwoodi* from this closely related species.

Introgression of resistance genes from wild potato species is an important control method for CRKN. We identified Race 1_{Roza}, which breaks the resistance from *S. bulbocastanum* clone SB22, in the soil sample taken from different locations in Washington State. There is an urgent need to introgress additional source(s) of resistance to CRKN Race 1_{Roza}. Graebner (2018) screened and identified four clones, PI239424hou-2mc, PI239424hou-6mc, PI283107hou5mc, and PI283107hou-9mc from *S. hougasii* (6X) which have significantly high levels of resistance against the three isolates of *M. chitwoodi*. Future studies should include identification and distribution of CRKN pathotypes in the Columbia Basin area.

3.6 Conclusion

We successfully developed PCR based molecular markers that can differentiate *M. chitwoodi* Race 1, Race 2 and Race $1_{Roza.}$ INDEL markers HSINDEL8, HSINDEL9 and HSINDEL10 are robust and differentiate *M. chitwoodi* races from DNA extracted from soil samples; they are an effective tool for diagnostic labs.

3.7 References

Abawi, G. S., & Gugino, B. K. (2007). Soil sampling for plant-parasitic nematode assessment.

Bali, S., Hu, S., Vining, K., Brown, C. R., Majtahedi, H., Zhang, L., Gleason, C., & Sathuvalli, V. (2021). Nematode Genome Announcement: Draft genome of *Meloidogyne chitwoodi*, an economically important pest of potato in the Pacific Northwest. Molecular Plant-Microbe Interactions®, MPMI-12-20-0337-A. https://doi.org/10.1094/MPMI-12-20-0337-A

Benson, G. (1999). Tandem repeats finder: a program to analyze DNA sequences. Nucleic acids research, 27(2), 573-580.

Brown, C. R., Mojtahedi, H., James, S., Novy, R. G., & Love, S. (2006). Development and evaluation of potato breeding lines with introgressed resistance to Columbia root-knot nematode (*Meloidogyne chitwoodi*). *American Journal of Potato Research*, *83*(1), 1-8.

Brown, C. R., Mojtahedi, H., Zhang, L.-H., & Riga, E. (2009). Independent resistant reactions expressed in root and tuber of potato breeding lines with

introgressed resistance to *Meloidogyne chitwoodi*. *Phytopathology*, *99*(9), 1085–1089.

Davies, L. J., Brown, C. R., & Elling, A. A. (2015). Calcium is involved in the $R_{Mc1\ (blb)}$-mediated hypersensitive response against *Meloidogyne chitwoodi* in potato. *Plant Cell Reports*, *34*(1), 167–177.

Demeler, J., Ramünke, S., Wolken, S., Ianiello, D., Rinaldi, L., Gahutu, J. B., Cringoli, G., von Samson-Himmelstjerna, G., & Krücken, J. (2013). Discrimination of gastrointestinal nematode eggs from crude fecal egg preparations by inhibitor-resistant conventional and real-time PCR. *PloS One*, *8*(4), e61285.

Graebner, R. C. (2018). Breeding qualitative and quantitative traits for potatoes in the Columbia Basin. Oregon State University.

Koressaar, T., & Remm, M. (2007). Enhancements and modifications of primer design program Primer3. *Bioinformatics*, *23*(10), 1289–1291.

Kõressaar, T., Lepamets, M., Kaplinski, L., Raime, K., Andreson, R., & Remm, M. (2018). Primer3_masker: integrating masking of template sequence with primer design software. *Bioinformatics*, *34*(11), 1937–1938.

Kyndt, T., Vieira, P., Gheysen, G., & de Almeida-Engler, J. (2013). Nematode feeding sites: Unique organs in plant roots. Planta, 238(5), 807–818.

Li, H. (2018). Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics*, *34*(18), 3094-3100.

Mojtahedi, H., Brown, C. R., Riga, E., & Zhang, L. H. (2007). A new pathotype of *Meloidogyne chitwoodi* Race 1 from Washington State. *Plant Disease*, *91*(8), 1051–1051.

Mojtahedi H, Santo GS, Brown CR, Ferris H, Williamson V (1994) A new host race of *Meloidogyne chitwoodi* from California. *Plant Disease*, *78*, 1010.

Powers, T. O., Mullin, P. G., Harris, T. S., Sutton, L. A., & Higgins, R. S. (2005). Incorporating molecular identification of *Meloidogyne spp*. into a large-scale regional nematode survey. *Journal of Nematology*, *37*(2), 226.

Santo, G. S., & Pinkerton, J. N. (1985). A second race of *Meloidogyne chitwoodi* discovered in Washington State. *Plant Disease*, *69*(4), 361.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3--new capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115. https://doi.org/10.1093/nar/gks596

Wishart, J., Phillips, M. S., & Blok, V. C. (2002). Ribosomal intergenic spacer: A polymerase chain reaction diagnostic for *Meloidogyne chitwoodi, M. fallax,* and *M. hapla*. *Phytopathology*, *92*(8), 884–892.

Zhang, L., & Gleason, C. (2019). Loop-mediated isothermal amplification for the diagnostic detection of *Meloidogyne chitwoodi* and *M. fallax*. *Plant Disease*, *103*(1), 12-18.

Zijlstra, C. (2000). Identification of *Meloidogyne chitwoodi, M. fallax* and *M. hapla* based on SCAR-PCR: A powerful way of enabling reliable identification of populations or individuals that share common traits. *European Journal of Plant Pathology*, *106*(3), 283–290.

3.8 Tables

Table 3.1. Nematode morphological identification and validation of Polymorphic Markers results on Soil Samples.

Soil Sample	Marker screening ID	Location	CRKN count	HSINDEL8	HSINDEL9	HSINDEL10	Race Identify
1804941	J	WA	224	263bp	264&285bp	300bp	Race 1_{Roza}
1807338	K	Othello, WA	610	263bp	264&285bp	338bp	Race 1_{Roza}
1807339	L	Othello, WA	270	263bp	264&285bp	338bp	Race 1_{Roza}
1807352*	O	Othello, WA	16	263bp	264&285bp	338bp	Race 1_{Roza}
1807353*	P	Othello, WA	4	263bp	264&285bp	338bp	Race 1_{Roza}
1807372	S	HAREC	6	263bp	264bp	300bp	Race 2
1807372	T	HAREC	232	263&238bp	264bp	300bp	Race 1
1807372	U	HAREC	48	263&238bp	264bp	300bp	Race 1
1807372	V	HAREC	12	263&238bp	264bp	300bp	Race 1
1807372	W	HAREC	18	263&238bp	264bp	300bp	Race 1

* Soil sample 1807352 and 1807353 extract DNA with isolated CRKNs.

Table 3.2. Molecular marker primers used to validate the DNA of *Meloidogyne chitwoodi* and *Meloidogyne hapla* (Wishart et al., 2002; Zijlstra, 2000).

Name	Primers Sequence
JMV1	GGATGGCGTGCTTTCAAC
JMV hapla	TTTCCCCTTATGATGTTTACCC
JMV2	AAAAATCCCCTCGAAAAATCCACC
Ff2	CCATTTCTGCTAAATGCCAAACTA
Rf	GGACACAGTAATTCATGAGCTAG
Fh	TGACGGCGGTGAGTGCGA
Rh	TGACGGCGGTACCTCATAG

Table 3.3. Molecular marker primers that showed polymorphism among the three isolates of CRKN.

	Forward primer	Reverse primer	Annealing temperature
HS04FSSR4	CTTACCTTCCTTTCCCTTTTCC	AATTGCTCACAGACAACAGCA	56 ºC
HS07FSSR3	ACTCTGTGTGGGGTGATTCTTT	CATTCCGGTTATTCCGGTTA	56 ºC
HSINDEL5	ACTTGTAGTTTTAATTTTGTGATGC	AATGAGAAATTTGAGAAGGTCTCG	58 ºC
HSINDEL6	GGATAATAAAGATGGGGGATTGAT	GTTGCTCATTCACAAACACTTTTC	58 ºC
HSINDEL7	CCAAATAAATATACACCGCTGGTT	CGAAGAAAAGGAAAAGAAATTGAG	58 ºC
HSINDEL8	CAAAACGTCATTCCTTAGTTGTCA	TGCTCCGACAGTTTGTTTTATATT	58 ºC
HSINDEL9	CTTTGGAAATAATTTTGGAGGTGT	CAGCAAGTACTTCTCATTGACAAAA	58 ºC
HSINDEL10	CCGCTTATACTATTTTTCTCTTCACTG	ATTCAAAGGGGTAACGGAAAA	58 ºC

3.9 Figures

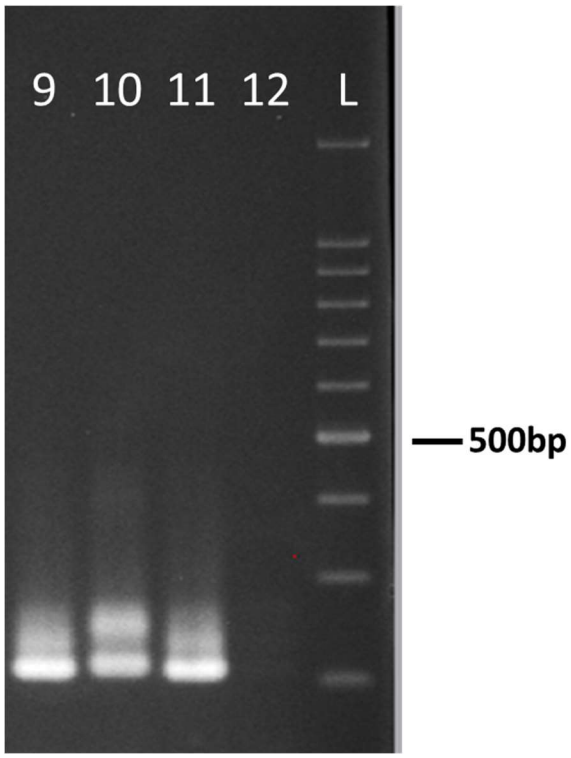


Figure 3.1. Agarose gel electrophoresis of SSR primer HS04FSSR4 products. Lane 9 was PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; lane 10 was PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; lane 11 was PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; lane 12 was PCR assays on *M. hapla* DNA extracted from eggs. L was 100 bp DNA ladder. Lanes 9-12 were amplified with annealing temperature 56 ℃.

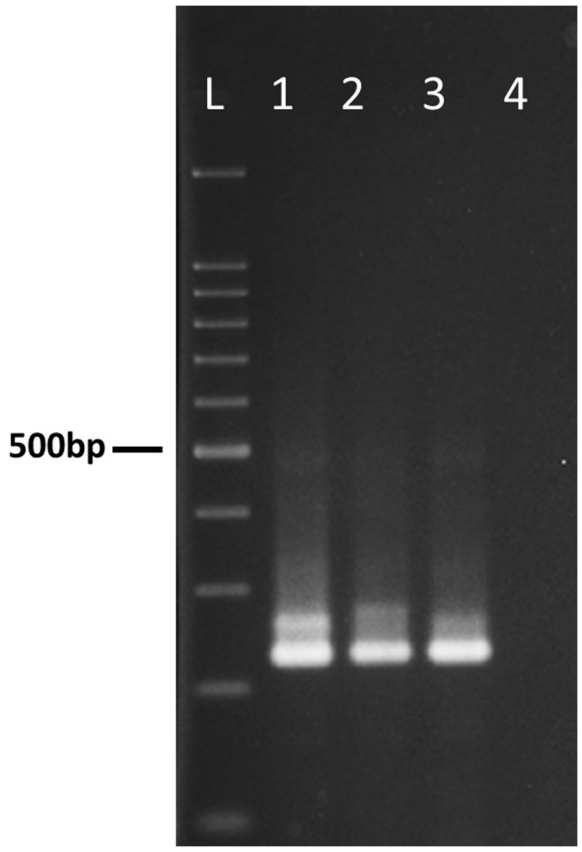


Figure 3.2. Agarose gel electrophoresis of SSR primer HS07FSSR3 products. Lane 1 was PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; lane 2 was PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; lane 3 was PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; lane 4 was PCR assays on *M. hapla* DNA extracted from eggs. L was 100 bp DNA ladder. Lanes 1-4 were amplified with annealing temperature 56 ℃.

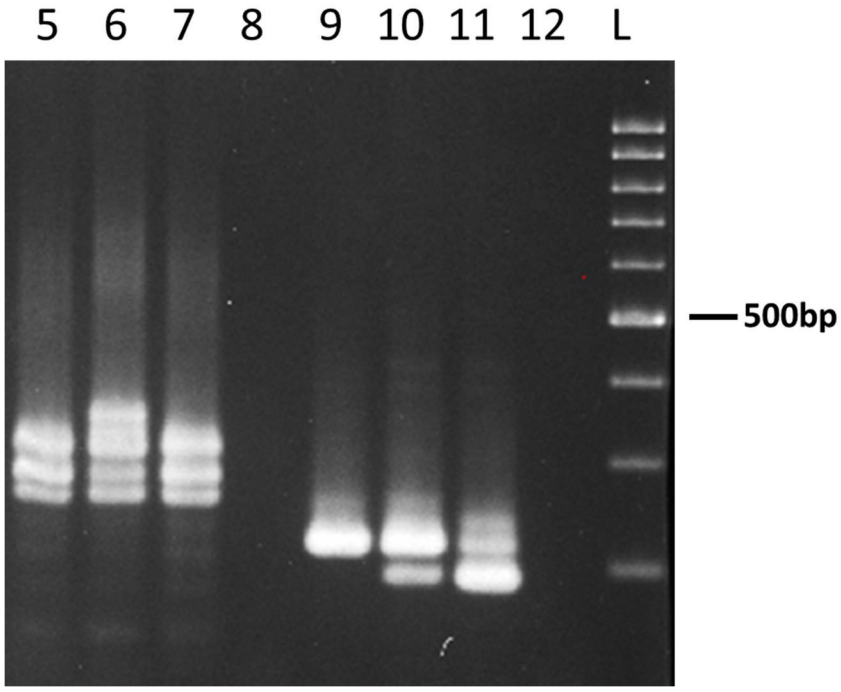


Figure 3.3. Agarose gel electrophoresis of INDEL primers HSINDEL5, HSINDEL6 products with annealing temperature of 58℃. Lanes 5, 9 were PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; 6, 10 were PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; 7, 11 were PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; 8, 12 were PCR assays on *M. hapla* DNA extracted from eggs. L was 100 bp DNA ladder. Lanes 5-8 are PCR products using INDEL primers HSINDEL5; 9-12 are PCR products using INDEL primers HSINDEL6.

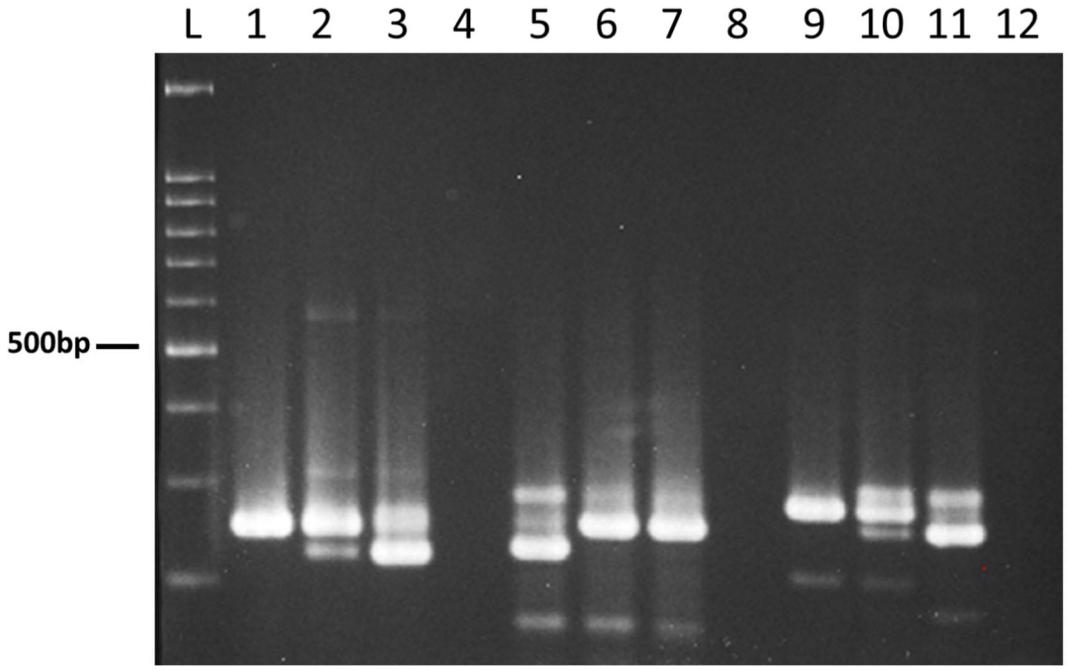


Figure 3.4. Agarose gel electrophoresis of INDEL primers HSINDEL7, HSINDEL8, HSINDEL9 products with annealing temperature of 58°C. Lanes 1, 5, 9 were PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; 2, 6, 10 were PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; 3, 7, 11 were PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; 4, 8, 12 were PCR assays on *M. hapla* DNA extracted from eggs. L was 100 bp DNA ladder. Lanes 1-4 are PCR products using INDEL primers HSINDEL7; 5-8 are PCR products using INDEL primers HSINDEL8; 9-12 are PCR products using INDEL primers HSINDEL9.

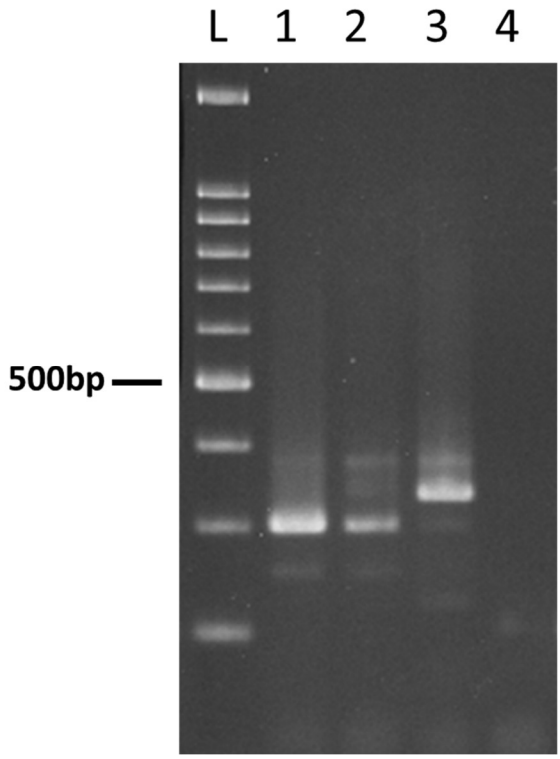


Figure 3.5. Agarose gel electrophoresis of INDEL primers HSINDEL10 products with annealing temperature of 58°C. Lane 1 was PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; 2 was PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; 3 was PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; 4 was PCR assays on *M. hapla* DNA extracted from eggs. L was 100 bp DNA ladder.

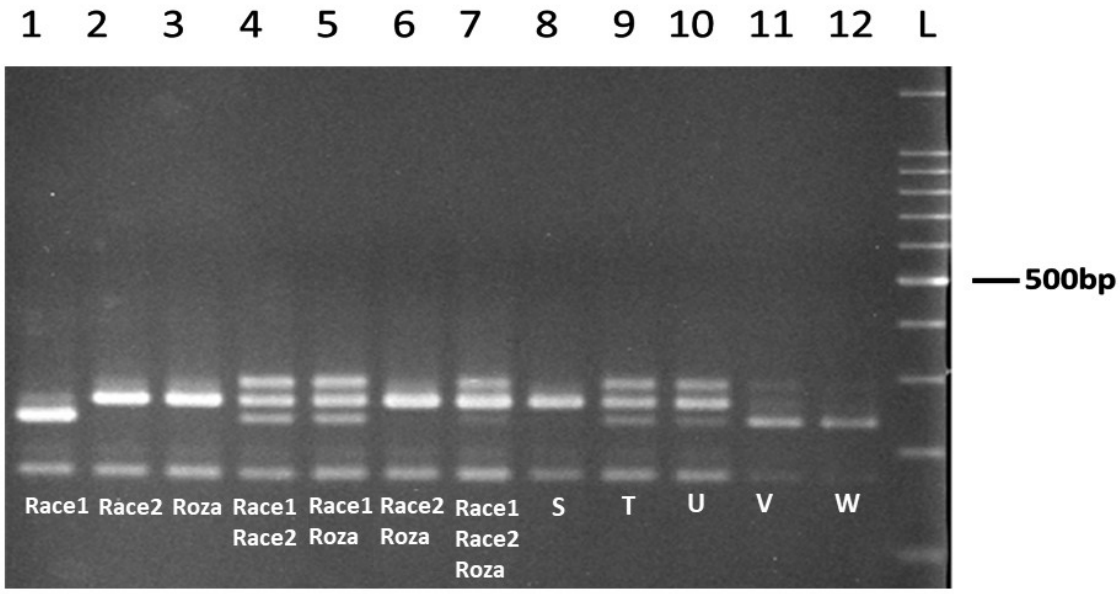


Figure 3.6. Agarose gel electrophoresis of INDEL primers HSINDEL8 products with annealing temperature of 58℃. Lane 1 was PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; 2 was PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; 3 was PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; 4 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA and *M. chitwoodi* Race 2 DNA; 5 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 6 was PCR assays on DNA mixture by *M. chitwoodi* Race 2 DNA and *M. chitwoodi* Race 1_{Roza} DNA;. 7 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA, *M. chitwoodi* Race 2 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 8-12 were PCR products using DNA extracted from soil samples collected at the Hermiston Agricultural Research and Extension Center. L was 100 bp DNA ladder.

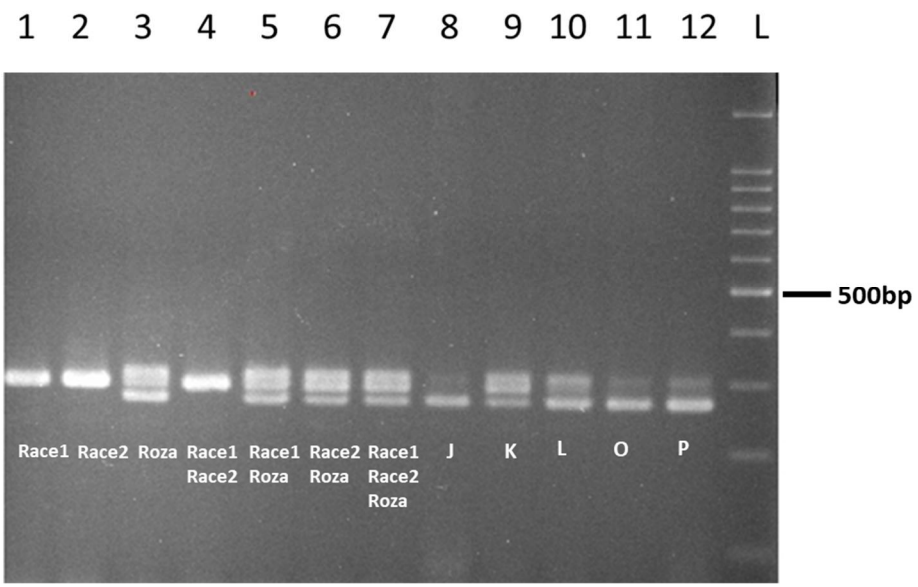


Figure 3.7. Agarose gel electrophoresis of PCR production of INDEL primers HSINDEL9 products with annealing temperature of 58 ℃. Lane 1 was PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; 2 was PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; 3 was PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; 4 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA and *M. chitwoodi* Race 2 DNA; 5 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 6 was PCR assays on DNA mixture by *M. chitwoodi* Race 2 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 7 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA, *M. chitwoodi* Race 2 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 8-12 were PCR products using DNA extracted from soil samples collected from AGNEMA. L was 100 bp DNA ladder.

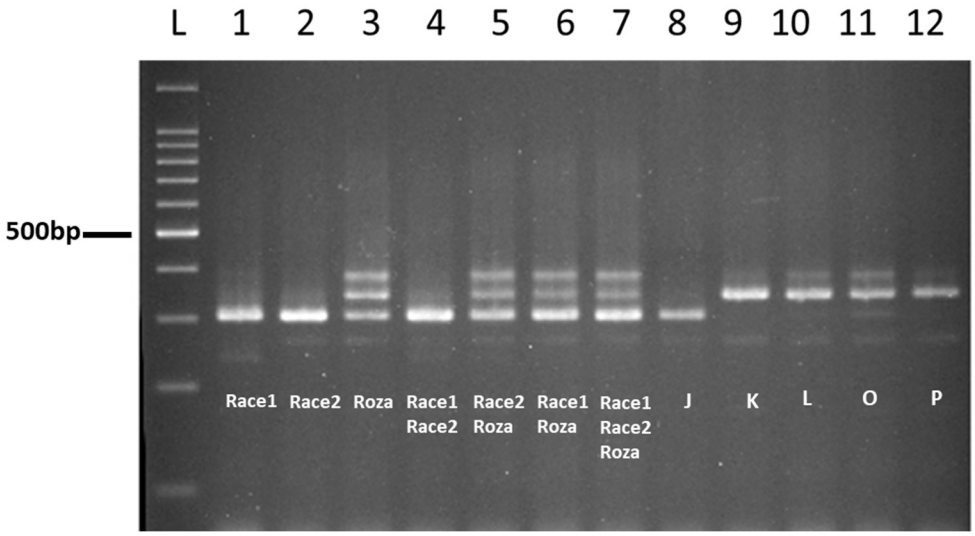


Figure 3.8. Agarose gel electrophoresis of INDEL primers HSINDEL10 products with annealing temperature of 58°C. Lane 1 was PCR assays on *M. chitwoodi* Race 1 DNA extracted from eggs; 2 was PCR assays on *M. chitwoodi* Race 2 DNA extracted from eggs; 3 was PCR assays on *M. chitwoodi* Race 1_{Roza} DNA extracted from eggs; 4 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA and *M. chitwoodi* Race 2 DNA; 5 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 6 was PCR assays on DNA mixture by *M. chitwoodi* Race 2 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 7 was PCR assays on DNA mixture by *M. chitwoodi* Race 1 DNA, *M. chitwoodi* Race 2 DNA and *M. chitwoodi* Race 1_{Roza} DNA; 8-12 were PCR products using DNA extracted from soil samples collected from AGNEMA ; L was 100 bp DNA ladder;

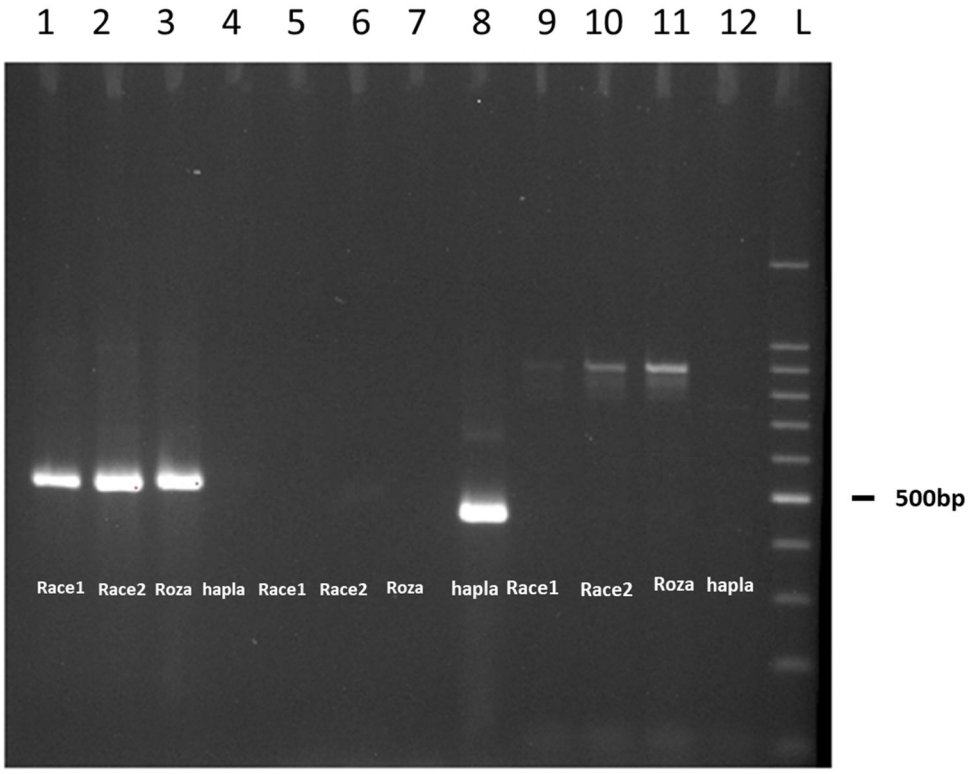


Figure 3.9. Agarose gel electrophoresis of primers validating DNA of *M. chitwoodi* Race 1, Race 2, Race 1Roza and *M. hapla* with annealing temperature of 58℃. Lane 1, 2, 3, 4 was PCR assays using primers JMV1 and JMV2, which are *M. chitwoodi* and *M. fallax*-specific primers. Lane 5, 6, 7, 8 was PCR assays using primers JMV1 and JMV-Hapla, which are *M. hapla*-specific primers. Lane 9, 10, 11, 12 was PCR assays using Ff2/Rf/Fh/Rh, which can specifically amplify *M. chitwoodi* and *M. hapla.* Lane 1, 5, 9 was PCR assays using *M. chitwoodi* Race 1 DNA, Lane 2, 6, 10 was PCR assays using *M. chitwoodi* Race 2 DNA, Lane 3, 7, 11 was PCR assays using *M. chitwoodi* Race 1_{Roza} DNA, Lane 4, 8, 12 was PCR assays using *M. hapla* DNA, L was 100 bp DNA ladder.

4. Developing Molecular Markers Linked to Corky ringspot resistance in *Solanum tuberosum* from Castle Russet

4.1 Abstract

Corky ringspot (CRS) disease caused by tobacco rattle virus (TRV) and vectored by stubby root nematodes (*Paratrichodorus* spp. and *Trichodorus* spp.), can render 6-55% of potatoes in an infested field unmarketable. Previous studies identified 22 SNP markers significantly associated with CRS resistance from 'Castle Russet' using 48 seedlings. In this study we developed 44 pairs of PCR primers around these previously identified SNPs. SNP marker PotVar0108448 on chromosome 9 shows polymorphisms on agarose gel electrophoresis and explains the highest percentage of phenotypic variance. Based on the initial marker screening result, we designed 36 pairs of primers for SSRs, 72 for short INDELs (1bp to 50 bp)and 36 for long INDELs (larger than 50bp) upstream and downstream of SNP marker PotVar0108448 and screened them on 48 seedlings of POR15V001 and 170 seedlings of POR16V001. Markers INDEL20, INDEL490-7, and Potvar008448 are linked to CRS resistance from 'Castle Russet'. Marker INDEL490-7 is robust and able to amplify in diverse CRS resistant germplasm and has potential for use in the marker assisted selection (MAS).

4.2 Introduction

Corky ringspot (CRS) is an economically important disease of potato in the Columbia Basin caused by tobacco rattle virus (TRV) and vectored by stubby root nematodes

(*Trichodorus* sp. and *Paratrichodorus* sp.). In potato (*Solanum tuberosum* L.), CRS is characterized by necrotic rings in the tuber flesh, which render unmarketable 6 to 55% of potatoes in an infested field (Hafez & Sundararaj, 2009). Because the vector of CRS is a nematode, damage caused by TRV is most effectively reduced by control of the nematode vector with nematicides and soil fumigants. Before 1990, fumigants and aldicarb were used to control nematodes. Aldicarb has not been used since 1989 and CRS populations have increased in Columbia Basin (Weingartner & Shumaker, 1990). Introgression of resistance into potato cultivars is the most efficient and environmentally sustainable method to control corky ringspot.

In United States, stubby-root nematode is found in Florida, Iowa, Kansas, Michigan, New York, South Carolina, North Dakota, South Dakota, Texas, and Virginia. Stubby-root nematodes are ectoparasitic nematodes, which live in the soil and feed on root tips. They feed by puncturing plant cells with a dagger-like onchiostyle, injecting saliva into the punctured cell and ingesting its contents (Crow, 2019). This feeding behavior makes the stubby-root nematode a natural vector to spread TRV, pea early browning virus and pepper ringspot virus. US seed certification programs have classified CRS as a zero-tolerance disease (Brown & Mojtahedi, 2005) due to its potential to reduce marketable potato yield.

Tobacco rattle virus, a member of the genus *Tobravirus*, has two parts of single-stranded, positive sense RNA. Genomic RNA1 encodes the replicase protein, cell-to-cell movement protein and silencing suppressor protein, while RNA2 encodes the coat protein and nematode-transmission factor (Donaire et al., 2008). ‘Castle Russet’, a recent release from the Northwest Potato Variety Development Program, has

improved agronomic performance and resistance to *potato virus Y* and CRS (Figure 4.1). Based on marker association analysis, 'Castle Russet' resistance to CRS was mapped to a major peak on chromosome 9 and two minor peaks on chromosomes 1 and 10 (Graebner, 2018). Twenty-two SNP markers were identified as significantly associated with CRS severity. Developing efficient breeder-friendly molecular markers linked to CRS resistance from 'Castle Russet' would aid in future marker assisted breeding. In this study, we developed PCR-based markers from previously identified SNPs on chromosome 9 for use in marker-assisted selection (MAS).

4.3 Materials and methods

4.3.1 Plant materials

For the mapping of CRS resistance and marker development, population POR15V001 was generated by making controlled crosses between resistant 'Castle Russet' and susceptible selection POR08BD1-3. Population POR16V001 was generated by making a controlled crosses between resistant 'Castle Russet' and susceptible selection A06084-2TE. Disease inoculations and mapping studies were carried out on 48 individuals of POR15V001 and 170 individuals of POR16V001.

4.3.2 Evaluation for Corky ringspot resistance

All clones of POR15V001 and POR16V001 were planted in fields infested with TRV and the stubby root nematode at Prosser, WA for disease inoculation and evaluation. Tubers of each clone were harvested separately, stored for three months, then evaluated for CRS. To evaluate CRS disease, up to 20 tubers (if available) were cut

length- and widthwise into four pieces and scored on a scale of 0-8 based on the number of wedge sides that showed internal browning evidence of CRS. A disease severity index was calculated for each selection using the following equation:

$$DSI = (\Sigma S)/(T*8)*100$$

Where "S" is the score assigned to each tuber from a specific plot, and "T" is the number of tubers scored for that plot (Graebner, 2018). For this analysis, DSIs were averaged across the six plots of POR15V001 planted in 2016 and 2017 and for POR16V001 in 2018, 2019 and 2020.

4.3.3 Potato DNA extraction

The Dellapotra (Dellaporta et al., 1983) nucleic acid extraction method was used in this study. For each clone, 100 mg of leaf tissue was ground in a 2 mL extraction buffer (100 mM Tris, pH 8.0, 50 mM EDTA, 500 mM NaCl, 10 mM 2-mercaptoethanol) using BIOREBA extraction bags and a homogenizer. Of the resultant slurry, 750 µL was placed into a 1.5 mL microcentrifuge tube and mixed with 100 µL 10% SDS. The microcentrifuge tube was incubated in a 65 °C water bath for 10 mins. After incubation, 200 µL of 5 M potassium acetate was added and mixed with the slurry. The microcentrifuge tube was then placed on ice for 5-15 mins and centrifuged for 8-10 mins. Next, 800 µL supernatant was carefully transferred into a new 1.5 mL microcentrifuge tube, mixed with 400 µL cold isopropanol, held on ice for 5 mins and centrifuged for 8-10 min. The supernatant was discarded, and the nucleic acid pellet held in the bottom of the tube was cleaned by adding 750 µL cold 70% ethanol, mixing gently, centrifuging for 3 min and discarding the ethanol. After

cleaning twice, the pellets were allowed to dry in room temperature, dissolved in 400 µL of sterile water, and stored in the microcentrifuge tube at -20 °C until further analyses.

4.3.4 Marker development:

Forty-four pairs of primers were designed based on the reference genome sequences of The Potato Genome Sequencing Consortium pseudomolecule v4.03 (Sharma et al., 2013; Xu et al., 2011), to flank 22 previously identified SNP markers that are significantly associated with CRS resistance (Graebner, 2018) using Primer 3 (Koressaar & Remm, 2007; Untergasser et al., 2012). Thirty-six pairs of SSR marker primers were developed in the same region from the reference genome on the upstream and downstream sides of SNP marker PotVar0108448 (Chr09: 59677060). Using Minimap2, we aligned the newly sequenced 'Castle Russet' genome with the potato reference genome pseudomolecule v4.03 chromosome 9 from 57177000bp to 61540751bp and filtered the insertions and deletions (INDEL) between the genomes. Seventy-two pairs of primers were designed for small INDELs (1bp to 50 bp) and 36 pairs of primer for large INDELs (larger than 50bp).

4.3.5 Molecular marker analysis

Primers pairs potentially linked to CRS resistance were screened against five resistant and five susceptible seedlings from population POR15V001 along with the parents. A total of 44 SNP markers, 36 SSR markers and 108 INDEL markers were screened. PCRs were in 10-µL volume containing 2 µL 5X Mytaq Reaction Buffer (Bioline),

0.2 µL MyTaq DNA Polymerase (Bioline), 0.5 µL DMSO, 1 µL DNA template, 0.3 µL forward and reverse primers and 6 µL of molecular grade water was subjected to PCR analysis. PCR amplification cycling conditions were 95 °C for 90 sec, following by 38 cycles: denaturation at 95 °C for 20 sec, annealing at 55 – 60 °C (based on the primer) for 15 seconds, extension at 72 °C for 15 sec, the final step extension at 72 °C for 5 minutes. Amplification product size was determined by comparison with the 100bp ladder following electrophoresis of 10 µL on a 2% *w/v* agarose gel in TBE buffer, stained with ethidium bromide (Sigma-Aldrich Co., St. Louis, MO) and photographed using an ultraviolet imaging system (BioRad, Hercules, California).

4.3.6 Data analysis and linkage map construction

Segregation for disease resistance and molecular markers in the two populations POR15V001 and POR16V001 was analyzed using Chi-square goodness-of-fit tests. The markers that segregated in the two populations were scored 1 or h for showing the presence of a band, and 0 or a for the absence of a band. Resistance to CRS was similarly scored. As we could identify no natural break for CRS resistance and susceptibility in the average DSI scores, we assigned resistance when DSI was less than five. The data were imported into JoinMap 5.0 using population type “BC1”

4.3.7 Validation of markers:

The molecular markers developed in this study were further validated on a set of 23 potato clones with known resistance or susceptibility to CRS (Table 4.4)

4.4 Results

4.4.1 Segregation for Corky ringspot resistance

Of the 48 clones of progeny POR15V001, 23 showed resistances to CRS, and 25 clones showed susceptibility. The segregation fit the expected 1:1 resistance: susceptibility ratio (Table 4.2), indicating that CRS resistance from 'Castle Russet' is controlled by a dominant allele at a single locus. Some clones of the progeny POR16V001 showed resistance to CRS, some showed susceptibility, and some have an unknown CRS response (Table 4.2). The lack of phenotyping data for both years increases the difficulty of scoring a resistant clone compared to a susceptible clone; a valid resistance score assignment requires two years of phenotyping data.

4.4.2 DNA markers linked to Corky ringspot resistance.

The DNA of five resistant and five susceptible clones in progeny POR15V001 and of the two parents was used to search for potential molecular markers linked to CRS resistance. Of 44 SNP primer pairs, two developed from Solcap SNP PotVar0108448 showed polymorphism between resistant and susceptible clones. Of 36 SSR primer pairs, five (SSR571B, SSR576, SSR582, SSR601B and SSR610) showed polymorphism between resistant and susceptible clones. Of 72 small INDEL primer pairs that are on the outside of the small INDELs, eight (INDEL9, INDEL10, INDEL15, INDEL20, INDEL30, INDEL41, INDEL55, and INDEL61) showed polymorphism between resistant and susceptible clones. Of 36 large INDEL primer

pairs that are in the large INDELs, two showed polymorphisms on resistant and susceptible clones (Table 4.1).

To validate the markers identified in the initial screening, all the polymorphic markers were scored in 48 clones in progeny POR15V001. Marker INDEL9, SSR571B, SSR 582, INDEL20, Potvar008448, INDEL55, INDEL61, SSR610, INDEL490-7, and INDEL910-5 showed potential linkage to CRS resistance, and all segregated in the expected 1:1 ratio (Table 4.3). A linkage map was constructed with these and previously scored markers using JoinMap 5.0. All markers were placed in a single group at LOD 10 (Figure 4.2). The map spanned 41.9 cM with markers in the order: INDEL61, SSR610, INDEL55, INDEL9, SSR571B, SSR 582, Potvar008448, INDEL20, INDEL490-1, and INDEL910-5. The markers INDEL61 and SSR610 were most closely linked to CRS resistance.

These ten markers linked to CRS resistance were validated in a larger population of 170 clones in progeny POR16V001. Markers INDEL9, SSR571B, SSR 582 and SSR610 did not segregate in the expected 1:1 ratio (Table 4.3). A linkage map was constructed with marker INDEL20, Potvar008448, INDEL55, INDEL61, INDEL490-15, and INDEL490-7 using JoinMap 5.0. All markers were placed in a single group at LOD 10 (Figure 4.3) and spanned 15.9 cM with markers in the order: Potvar008448, INDEL490-7/ INDEL490-15, INDEL20, INDEL61, and INDEL55. The marker Potvar008448 was closest to the source of resistance.

All these markers were validated in diverse European potato germplasm with known CRS resistance. Marker INDEL490-15 and INDEL490-7, primer pairs in the same

INDEL, amplified in all the resistant clones and is a good candidate for MAS (Table 4.4).

4.5 Discussion

Based on previous research, we designed different types of PCR-based molecular markers on chromosome 9 to map the CRS resistance gene from 'Castle Russet'. For the two populations used, the progeny POR15V001 is a small population of 48 individuals and the resistance segregation fit the 1:1 ratio. High-quality and precision mapping of resistance requires a large population; hence our use of POR16V001, which has 170 clones. Though we had a large population of seedlings, only ~120 clones have two years of phenotyping data, increasing the difficulty of validly scoring resistant clones. Clones with a DSI below 5.0 for two years were designated as resistant. More complete phenotyping data would permit us to improve the map of progeny POR16V001.

Based on the linkage map of progenies POR16V001 and POR15V001, it is evident that CRS resistance does not fall between the markers we screened. This may have been due to the inaccuracy of the phenotyping data and the limited population size. Another possible reason is that more molecular markers over a larger range on the upstream side of our mapping area are required. Based on the linkage map using progeny POR16V001, marker INDEL20 is closest to the resistance gene. Marker INDEL20 has potential for marker-assisted selection for CRS resistance from 'Castle Russet'. Another improvement would be to screen our molecular markers against other clones that may carry the same source of CRS resistance. Our results show that

INDEL marker INDEL490-15 and INDEL490-7, primer pairs in the same INDEL, can describe most of the CRS resistance in these clones. The molecular markers we designed in this project have potential to contribute to marker-assisted selection of other resistant parents. By genotyping the progenies with molecular markers, we can avoid the cost of phenotyping CRS resistance in large populations.

4.6 Conclusion

With the whole genome resources for ‘Castle Russet’ and based on association analysis, we developed PCR-based molecular markers linked to CRS resistance from ‘Castle Russet’. Markers INDEL490-7 and INDEL490-15 were robust and identified resistance in diverse germplasm; they have the potential for use in MAS.

4.7 Reference

Brown, C. R., & Mojtahedi, H. (2005). Breeding for resistance to Meloidogyne species and trichodorid-vectored virus. *Genetic Improvement of Solanaceous Crops*, *1*, 267–292.

Crow, W. T. (2019). Stubby-root nematode, *Trichodorus obtusus cobb* (*Nematoda*: *Adenophorea*: *Triplonchida*: *Diphtherophorina*: *Trichodoridea*: *Trichodoridae*). *EDIS*, *2005*(5). https://doi.org/10.32473/edis-in617-2004

Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A plant DNA minipreparation: Version II. *Plant Molecular Biology Reporter*, *1*(4), 19–21. https://doi.org/10.1007/BF02712670

Donaire, L., Barajas, D., Martínez-García, B., Martínez-Priego, L., Pagán, I., & Llave, C. (2008). Structural and genetic requirements for the biogenesis of tobacco rattle virus-derived small interfering RNAs. *Journal of Virology*, *82*(11), 5167–5177.

Graebner, R. C. (2018). *Breeding qualitative and quantitative traits for potatoes in the Columbia Basin*. Oregon State University.

Hafez, S. L., & Sundararaj, P. (2009). Management of corky ringspot disease of potatoes in the Pacific Northwest. *University of Idaho. Extension*, 1–6.

Koressaar, T., & Remm, M. (2007). Enhancements and modifications of primer design program Primer3. *Bioinformatics*, *23*(10), 1289–1291.

Sharma, S. K., Bolser, D., Boer, J. de, Sønderkær, M., Amoros, W., Carboni, M. F., D'Ambrosio, J. M., Cruz, G. de la, Genova, A. D., Douches, D. S., Eguiluz, M., Guo, X., Guzman, F., Hackett, C. A., Hamilton, J. P., Li, G., Li, Y.,

Lozano, R., Maass, A., Marshall, D., Martinez, D., McLean, K., Mejía, N., Milne, L., Munive, S., Nagy, I., Ponce, O., Ramirez, M., Simon, R., Thomson, S. J., Torres, Y., Waugh, R., Zhang, Z., Huang, S., Visser, R. G. F., Bachem, C. W. B., Sagredo, B., Feingold, S. E., Orjeda, G., Veilleux, R. E., Bonierbale, M., Jacobs, J. M. E., Milbourne, D., Martin, D. M. A.,Bryan, G. J. (2013). Construction of Reference Chromosome-Scale Pseudomolecules for Potato: Integrating the Potato Genome with Genetic and Physical Maps. *G3: Genes, Genomes, Genetics*, *3*(11), 2031–2047. https://doi.org/10.1534/g3.113.007153

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115–e115.

Weingartner, D. P., & Shumaker, J. R. (1990). Control of nematodes and soil-borne diseases in Florida potatoes with aldicarb and 1, 3-D. *Journal of Nematology*, *22*(4S), 775.

Xu, X., Pan, S., Cheng, S., Zhang, B., Mu, D., Ni, P., Zhang, G., Yang, S., Li, R., Wang, J., Orjeda, G., Guzman, F., Torres, M., Lozano, R., Ponce, O., Martinez, D., De la Cruz, G., Chakrabarti, S. K., Patil, V. U., Wageningen University & Research Centre. (2011). Genome sequence and analysis of the tuber crop potato. *Nature*, *475*(7355), 189–195. https://doi.org/10.1038/nature10158

4.8 Tables

Table 4.1. List of primers used for mapping CRS resistance on chromosome 9.

	Forward primer	Reverse primer	Annealing temperature
PotVar0108448-1	TCTGTTTCACTATTCCCTCCGT	TTCCCCGCCTTTGATCATCA	59 ℃
SSR571B	CCTCCTCTTCCTACTTCTCCTTCT	CCGACCAACTCAAAATATCCTCTA	57 ℃
SSR582	GGGAAACTAGACAAAACAGGCA	GTCATCTTATCCCCTTGGAGTG	57 ℃
SSR610	GAATACATGGGTTTGGCATCTT	CACACAAGTGGTAAGGGGAAA	57 ℃
INDEL9	CTCTTCACATGTACGAACCATCTC	AGCTTATGATTGTCACAAAGTCCA	57 ℃
INDEL20	CCTTCTACAAATGTGTGAAACCTG	GTTGTTGAGTCCGACAACAAAATA	59 ℃
INDEL55	ACTTAGGATGAAAACCACCAGAAG	ATGCAAGTGAGAAACTTGATTCAT	59 ℃
INDEL61	CAGGAAAATGATACAACTTTGTGC	TCTATTTCCAAGCTCTACGTTTGA	59 ℃
INDEL490-7	ACTTCATAAAATGCGGAAAACAAT	TATTCCCCAAAATCAATGATAACC	59 ℃
INDEL490-15	ACTCATCCACCGTGTATAGGATCT	TTTGTGAATGAATTTTGATTTTGC	59 ℃
INDEL920-5	TTGCTAAACAGTTGAAGGATCAAA	GCTTCGAATGGATTAAAGGATCTA	59 ℃

Table 4.2. Segregation of corky ringspot resistance markers for two populations: POR15V001 and POR16V001.

Progeny	Parentage	Number of plants			χ2	
		Resistant	Susceptible	unknown	Value	*P*
POR15V001	Castle Russet × POR08BD1-3	23	25	0	0.08	0.78
POR16V001	Castle Russet × A06084-2TE	97	72	1	3.7	0.05

Table 4.3. Segregation at marker loci linked to corky ringspot resistance in two populations: POR15V001 and POR16V001

Progeny	Marker	Observed frequency (Present:absent)	χ2	
			Value	*P*
POR15V001	SSR610	23:25	0.08	0.78
	INDEL61	23:25	0.08	0.78
	Potvar008448-1	23:25	0.08	0.78
	INDEL490-7	21:26	0.53	0.47
	INDEL490-15	21:26	0.53	0.47
	INDEL920-5	20:27	1.04	0.31
	INDEL20	21:25	0.35	0.55
	SSR582	23:24	0.02	0.89
	SSR571B	20:24	0.36	0.55
	INDEL9	23:24	0.02	0.89
	INDEL55	24:23	0.02	0.89
POR16V001	INDEL20	99:71	4.61	0.03
	INDEL490-7	96:74	2.85	0.09
	INDEL490-15	96:74	2.85	0.09
	INDEL55	93:77	1.51	0.21
	INDEL61	96:73	3.13	0.08
	Potvar08448-1	97:71	4.02	0.04

Table 4.4. Markers screening results on other potato clones

	PotVar0108448-1	SSR610	INDEL9	INDEL20	INDEL55	INDEL61	INDEL490-15	INDEL490-7	INDEL920-3	Score of resistance
Etana	a	h	a	a	a	a	a	a	a	2
Allians	a	a	a	a	h	a	h	h	a	6
Belana	a	a	a	a	a	a	a	a	a	5
Hirta	a	h	h	h	a	a	h	h	h	6
Ronea	h	h	h	h	h	h	h	h	h	8
E77/330	h	h	h	a	h	h	h	h	h	7
L619/87/4796	a	h	h	a	a	a	a	a	a	3
E88/110	a	h	h	h	a	a	a	a	a	5
Bintje	h	h	h	a	h	h	h	a	h	8
Gladiator	a	a	h	h	a	a	a	a	a	unknown
Castle Russet	h	h	h	h	h	h	h	h	h	9
VR12-33	h	h	a	a	a	h	h	h	h	unknown
VR12-34	a	h	h	a	a	a	a	a	h	unknown
Mia	h	a	a	a	h	a	h	h	h	unknown
Regina	a	h	a	h	a	a	h	h	a	8
Fontane	h	h	h	a	h	a	h	a	h	7
Krone	a	h	a	h	a	a	a	a	a	6
Lilly	h	h	h	h	h	a	h	h	h	9
Albertine	h	h	h	a	h	h	h	h	h	4
Gala	h	h	a	a	h	a	h	h	h	7
B14/216/109	h	a	h	h	h	h	h	h	h	9
Corinna	h	a	h	a	h	a	h	h	h	6
Marion	a	h	a	a	a	a	h	h	a	8
E12/42/89	h	a	h	h	h	h	h	h	h	8

For marker PotVar0108448-1, having band size 320bp as “h”, band size 350bp as “a”. For marker SSR610, having band size 210bp as “h”, band size 197bp as “a”. For marker INDEL9, having band size 215bp as “h”, band size 270bp as “a”. For marker INDEL20, having band size 273bp as “h”, band size 300bp as “a”. For marker INDEL55, having band size 200bp as “h”, band size 225bp as “a”. For marker INDEL61, having band size 274bp as “h”, band size 300bp as “a”. For marker INDEL490-15, band amplified as “h”, no band amplified as “a”. For marker INDEL490-7, band amplified as “h”, no band amplified as “a”. For marker INDEL920-3, band amplified as “h”, no band amplified as “a”. For the score of resistance, “1” is highly susceptible to CRS, “9” is highly resistant to CRS.

4.9 Figures

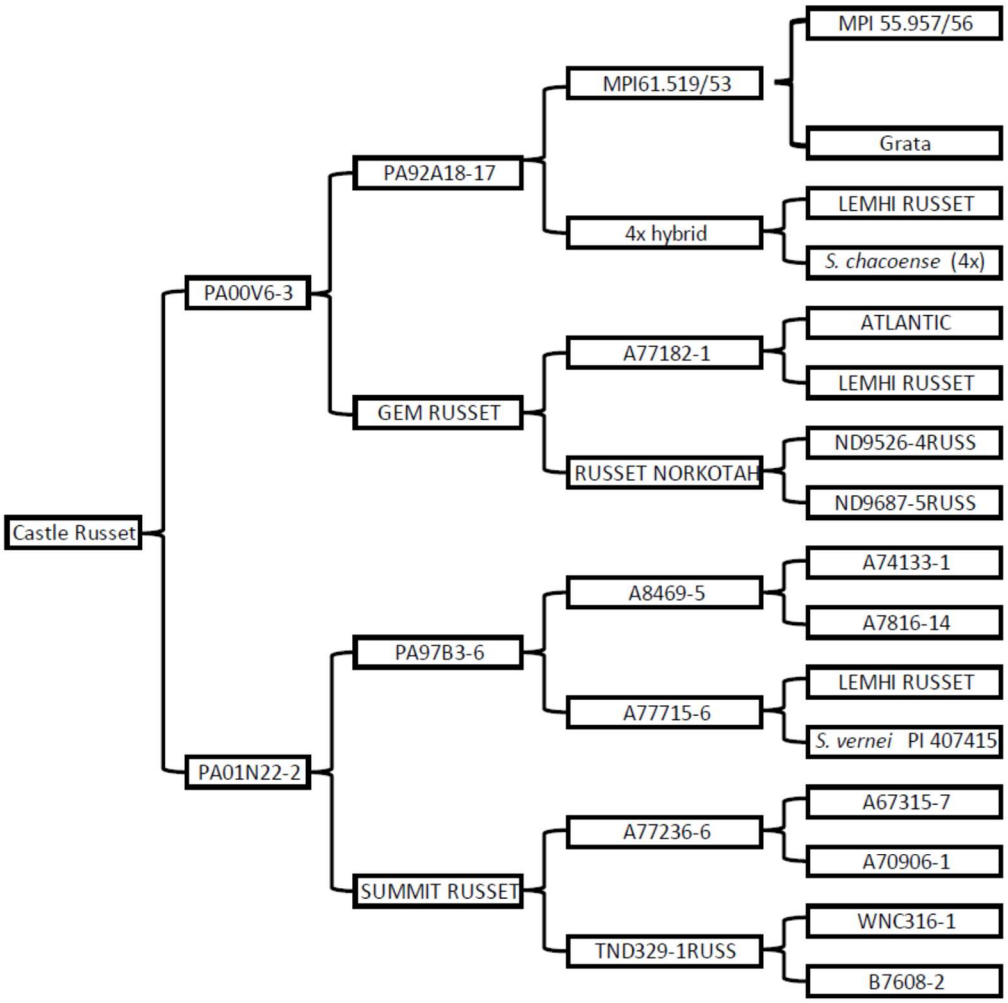


Figure 4.1. Pedigree of 'Castle Russet' potato.

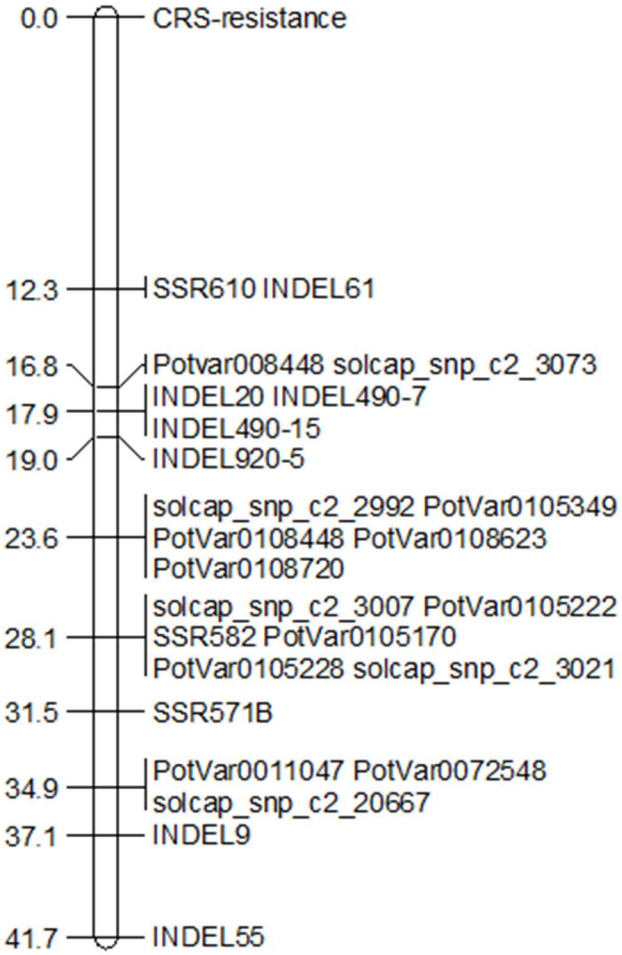


Figure 4.2. Map of DNA markers and CRS resistance locus in progeny POR15V001

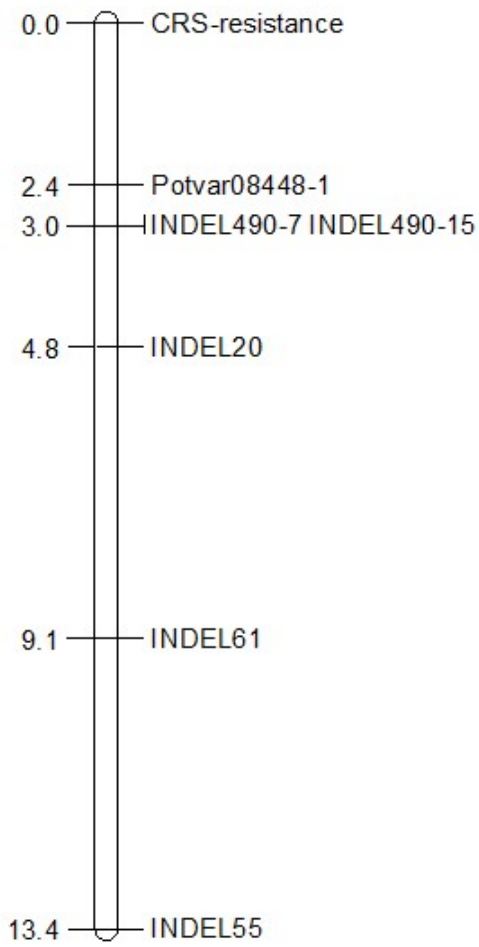
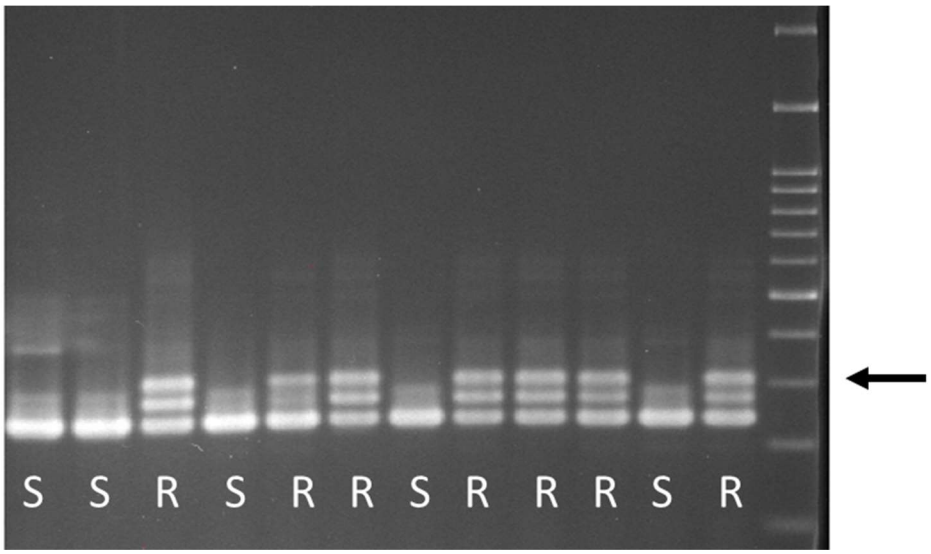
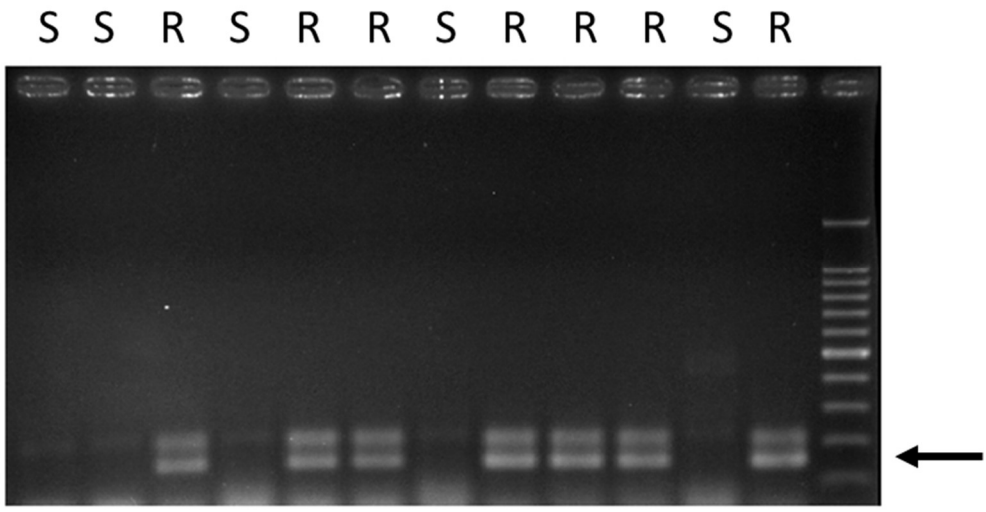


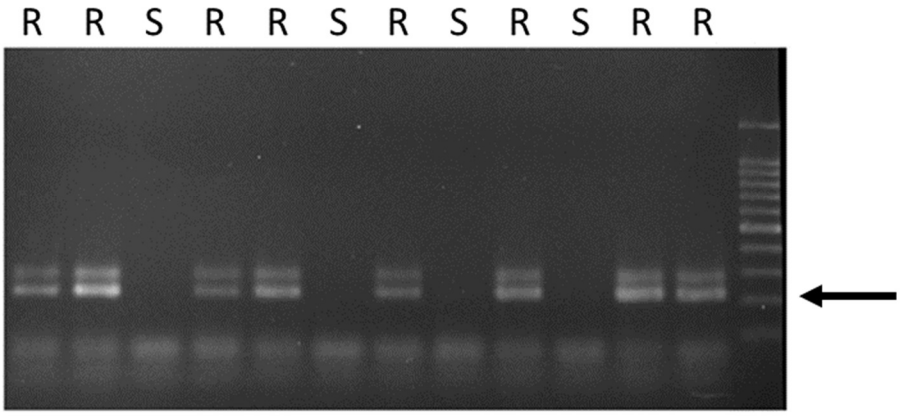
Figure 4.3. Map of DNA markers and CRS resistance locus in progeny POR16V001



INDEL20



INDEL490-7



INDEL490-15

Figure 4.4. Molecular marker INDEL20, INDEL490-7 and INDEL490-15 have potential in marker-assisted selection for corky ringspot resistance in potato.

5. Conclusion

Potato (*Solanum tuberosum* L.) plays an important role in tackling the threat of global food insecurity due to its high yield and broad global acceptance. However, several pathogens threaten potato production, and cause direct yield loss by rendering potatoes tubers unmarketable. For major pathogens in potatoes, identifying pathogen type and breeding new varieties that carry multiple resistances is an efficient way for sustaining potato production.

In this study, we sequenced and annotated the genomes of three pathotypes of *M. chitwoodi* Race 1, Race 2 and Race 1_{Roza}. Based on the genome comparisons, we developed molecular markers that successfully differentiated all three pathotypes of *M. chitwoodi*. In addition, we developed molecular markers linked to resistance to Corky ringspot from 'Castle Russet'.

Columbia root-knot nematode (CRKN, *Meloidogyne chitwoodi*) parasitizes potato plants in the Pacific Northwest (PNW). It causes small brown dots in the tuber flesh and dramatically reduces the market value of the crop. In the PNW, two races of *M. chitwoodi*, Race 1 and Race 2 and a pathotype of Race 1, Race 1_{Roza} exist. The races of *M. chitwoodi* are primarily identified based on a differential host test. The genomes we sequenced and assembled are the most contiguous genome sequences available for any *Meloidogyne sp.* The genomic annotations and comparisons of *M. chitwoodi* Race 1 (12295 genes annotated), Race 2 (12349 genes annotated), and Race 1_{Roza} (12534 genes annotated)elucidate the complex evolutionary history of this species. These genome analyses will be good contributions to molecular marker development and analysis of the host plant's resistance to these root-knot nematodes.

Currently, *M. chitwoodi* can be differentiated from *M. hapla* based on morphology and by molecular markers, but we cannot differentiate *M. chitwoodi* races based on morphology. Based on the genome comparisons of *M. chitwoodi* Race 1, Race 2 and Race $1_{Roza,}$ we developed 36 pairs of PCR primers for SSR markers and 17 pairs of PCR primers for INDEL markers. A total of eight markers (HS04FSSR4, HS07FSSR3, HSINDEL5, HSINDEL6, HSINDEL7, HSINDEL8, HSINDEL9, and HSINDEL10) showed polymorphism among the three isolates of CRKN on agarose gels. Among those molecular markers, four successfully differentiate all three pathotypes of *M. chitwoodi* examined in this study. Identification of the pathotype of CRKN can be used to control CRKN in crop ratations based on the different pathotype host range. At the same time, introgression of resistance genes from wild potato species is an important control method for CRKN. We identified Race 1_{Roza}, which breaks the resistance from *S. bulbocastanum* clone SB22, in the soil sample taken from different locations in Washington State. There is an urgent need to introgress additional sources of resistance to CRKN Race 1_{Roza}.

Corky ringspot (CRS) disease, caused by tobacco rattle virus (TRV) and vectored by stubby root nematodes, can render 6-55% of potatoes in an infested field unmarketable. The newly released potato variety ‘Castle Russet’ has genetic resistance to CRS. Previous studies identified 22 SNP markers using 48 seedlings that are significantly associated with CRS resistance from ‘Castle Russet’. In this study, we developed 44 pairs of PCR primers around previously identified significant SNPs. SNP marker PotVar0108448 on chromosome 9 shows polymorphisms in agarose gel electrophoresis and explains the highest percentage of phenotypic variance. Based on

the initial marker screening, we developed 36 pairs of SSR primers, 72 pairs of primers for short INDELs, and 36 pairs of primers for long INDELs on the upstream and downstream sides of SNP marker PotVar0108448. Those markers were screened on 48 seedlings of POR15V001 and 170 seedlings of POR16V001. In progeny POR16V001 markers INDEL20, INDEL490-7, Potvar008448 are linked to CRS resistance at 2.4 to 4.8 cM. Marker INDEL490-7 is robust in identifying resistance from diverse germplasm. These markers have the potential for use in marker-assisted selection (MAS). By genotyping the seedlings with molecular markers, we can avoid the cost of phenotyping CRS resistance in large populations.

Bibliography

Abad, P., Gouzy, J., Aury, J.-M., Castagnone-Sereno, P., Danchin, E. G., Deleury, E., Perfus-Barbeoch, L., Anthouard, V., Artiguenave, F., & Blok, V. C. (2008). Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology*, *26*(8), 909–915.

Abawi, G. S., & Gugino, B. K. (2007). Soil sampling for plant-parasitic nematode assessment.

Alfthan, G., Laurinen, M. S., Valsta, L. M., Pastinen, T., & Aro, A. (2003). Folate intake, plasma folate and homocysteine status in a random Finnish population. *European Journal of Clinical Nutrition*, *57*(1), 81–88. https://doi.org/10.1038/sj.ejcn.1601507

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410.

Aversano, R., Contaldi, F., Ercolano, M. R., Grosso, V., Iorizzo, M., Tatino, F., Xumerle, L., Molin, A. D., Avanzato, C., Ferrarini, A., Delledonne, M., Sanseverino, W., Cigliano, R. A., Capella-Gutierrez, S., Gabaldón, T., Frusciante, L., Bradeen, J. M., & Carputo, D. (2015). The Solanum commersonii Genome Sequence Provides Insights into Adaptation to Stress Conditions and Genome Evolution of Wild Potato Relatives. *The Plant Cell*, *27*(4), 954–968. https://doi.org/10.1105/tpc.114.135954

Bali, S., Sathuvalli, V., Brown, C., Novy, R., Ewing, L., Debons, J., Douches, D., Coombs, J., Navarre, D., & Whitworth, J. (2017). Genetic fingerprinting of potato varieties from the northwest potato variety development program. *American Journal of Potato Research*, *94*(1), 54–63.

Barrell, P. J., Meiyalaghan, S., Jacobs, J. M., & Conner, A. J. (2013). Applications of biotechnology and genomics in potato improvement. *Plant Biotechnology Journal*, *11*(8), 907–920.

Benson, G. (1999). Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Research*, *27*(2), 573–580. https://doi.org/10.1093/nar/27.2.573

Blanc-Mathieu, R., Perfus-Barbeoch, L., Aury, J.-M., Da Rocha, M., Gouzy, J., Sallet, E., Martin-Jimenez, C., Bailly-Bechet, M., Castagnone-Sereno, P., Flot, J.-F., Kozlowski, D. K., Cazareth, J., Couloux, A., Da Silva, C., Guy, J., Kim-Jo, Y.-J., Rancurel, C., Schiex, T., Abad, P., Wincker, P., Danchin, E. G. J. (2017). Hybridization and polyploidy enable genomic plasticity without sex in the most devastating plant-parasitic nematodes. *PLoS Genetics*, *13*(6), e1006777. https://doi.org/10.1371/journal.pgen.1006777

Bradshaw, J. E. (2007). Potato-breeding strategy. In *Potato Biology and Biotechnology* (pp. 157–177). Elsevier.

Brevik, A., Vollset, S. E., Tell, G. S., Refsum, H., Ueland, P. M., Loeken, E. B., Drevon, C. A., & Andersen, L. F. (2005). Plasma concentration of folate as a biomarker for the intake of fruit and vegetables: The Hordaland Homocysteine Study. *The American Journal of Clinical Nutrition*, *81*(2), 434–439.

Brodie, B. B., Evans, K., & Franco, J. (1993a). Nematode parasites of potatoes. *Plant Parasitic Nematodes in Temperate Agriculture.*, 87–132.

Brown, C. R. (2008). Breeding for phytonutrient enhancement of potato. *American Journal of Potato Research*, *85*(4), 298–307.

Brown, C. R., & Mojtahedi, H. (2005). Breeding for resistance to *Meloidogyne species* and *trichodorid*-vectored virus. *Genetic Improvement of Solanaceous Crops*, *1*, 267–292.

Brown, C. R., Mojtahedi, H., Zhang, L.-H., & Riga, E. (2009). Independent resistant reactions expressed in root and tuber of potato breeding lines with introgressed resistance to *Meloidogyne chitwoodi*. *Phytopathology*, *99*(9), 1085–1089.

Brown, C. R., Mojtahedi, H., James, S., Novy, R. G., & Love, S. (2006). Development and evaluation of potato breeding lines with introgressed resistance to Columbia root-knot nematode (*Meloidogyne chitwoodi*). *American Journal of Potato Research*, *83*(1), 1-8.

Brown, C. R., Yang, C.-P., Mojtahedi, H., Santo, G. S., & Masuelli, R. (1996). RFLP analysis of resistance to Columbia root-knot nematode derived from *Solanum bulbocastanum* in a BC 2 population. *Theoretical and Applied Genetics*, *92*(5), 572–576.

Celebi-Toprak, F., Slack, S. A., & Jahn, M. M. (2002). A new gene, Ny_{tbr}, for hypersensitivity to Potato virus Y from Solanum tuberosum maps to chromosome IV. *Theoretical and Applied Genetics*, *104*(4), 669–674.

Cernák, I., Decsi, K., Nagy, S., Wolf, I., Polgár, Z., Gulyás, G., Hirata, Y., & Taller, J. (2008). Development of a locus-specific marker and localization of the Ry_{sto} gene based on linkage to a catalase gene on chromosome XII in the tetraploid potato genome. *Breeding Science*, *58*(3), 309–314.

Cha, S. (2016). *Is the biology of M. chitwoodi and M. hapla reflected in their genomes?*

Chow, F. W.-N., Koutsovoulos, G., Ovando-Vázquez, C., Neophytou, K., Bermúdez-Barrientos, J. R., Laetsch, D. R., Robertson, E., Kumar, S., Claycomb, J. M., Blaxter,

M., Abreu-Goodger, C., & Buck, A. H. (2019). Secretion of an Argonaute protein by a parasitic nematode and the evolution of its siRNA guides. *Nucleic Acids Research*, *47*(7), 3594–3606. https://doi.org/10.1093/nar/gkz142

Consortium, P. G. S. (2011). Genome sequence and analysis of the tuber crop potato. *Nature*, *475*(7355), 189.

Crow, W. T. (2019). Stubby-root nematode, *Trichodorus obtusus cobb* (*Nematoda*: *Adenophorea*: *Triplonchida*: *Diphtherophorina*: *Trichodoridea*: *Trichodoridae*). *EDIS*, *2005*(5). https://doi.org/10.32473/edis-in617-2004

Davies, L. J., Brown, C. R., & Elling, A. A. (2015). Calcium is involved in the R *Mc1 (blb*)-mediated hypersensitive response against *Meloidogyne chitwoodi* in potato. *Plant Cell Reports*, *34*(1), 167–177.

Dellaporta, S. L., Wood, J., & Hicks, J. B. (1983). A plant DNA minipreparation: Version II. *Plant Molecular Biology Reporter*, *1*(4), 19–21. https://doi.org/10.1007/BF02712670

Demeler, J., Ramünke, S., Wolken, S., Ianiello, D., Rinaldi, L., Gahutu, J. B., Cringoli, G., von Samson-Himmelstjerna, G., & Krücken, J. (2013). Discrimination of gastrointestinal nematode eggs from crude fecal egg preparations by inhibitor-resistant conventional and real-time PCR. *PloS One*, *8*(4), e61285.

Doležel, J., Greilhuber, J., & Suda, J. (2007). Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols*, *2*(9), 2233.

Donaire, L., Barajas, D., Martínez-García, B., Martínez-Priego, L., Pagán, I., & Llave, C. (2008). Structural and genetic requirements for the biogenesis of tobacco rattle virus-derived small interfering RNAs. *Journal of Virology*, *82*(11), 5167–5177.

Douches, D., Hirsch, C. N., Manrique-Carpintero, N. C., Massa, A. N., Coombs, J., Hardigan, M., Bisognin, D., De Jong, W., & Buell, C. R. (2014). The contribution of the Solanaceae coordinated agricultural project to potato breeding. *Potato Research, 57*(3), 215–224.

Drewnowski, A., & Rehm, C. D. (2013). Vegetable cost metrics show that potatoes and beans provide most nutrients per penny. *PloS One*, *8*(5), e63277.

Endelman, J., & Jansky, S. H. (2015). Genotyping-by-sequencing of a diploid potato F2 population. *Plant and Animal Genome XXIII, San Diego, CA*.

Franks, D. M., Izumikawa, T., Kitagawa, H., Sugahara, K., & Okkema, P. G. (2006). C. elegans pharyngeal morphogenesis requires both de novo synthesis of pyrimidines and synthesis of heparan sulfate proteoglycans. *Developmental Biology*, *296*(2), 409–420. https://doi.org/10.1016/j.ydbio.2006.06.008

Gasparyan, L., Mazo, I., Simonyan, V., & Gasparyan, F. (2019). DNA Sequencing: Current State and Prospects of Development. *Open Journal of Biophysics*, *09*(03), 169–197. https://doi.org/10.4236/ojbiphy.2019.93013

Gebhardt, C., Blomendahl, C., Schachtschabel, U., Debener, T., Salamini, F., & Ritter, E. (1989). Identification of 2n breeding lines and 4n varieties of potato (*Solanum tuberosum, ssp. Tuberosum*) with RFLP-fingerprints. *Theoretical and Applied Genetics*, *78*(1), 16–22.

Goyer, A., & Sweek, K. (2011). Genetic diversity of thiamin and folate in primitive cultivated and wild potato (Solanum) species. *Journal of Agricultural and Food Chemistry*, *59*(24), 13072–13080.

Graebner, R. C. (2018). *Breeding qualitative and quantitative traits for potatoes in the Columbia Basin*. Oregon State University.

Hafez, S. L., & Sundararaj, P. (2009). Management of corky ringspot disease of potatoes in the Pacific Northwest. *University of Idaho. Extension*, 1–6.

Hämäläinen J. H., KN Watanabe, JPT Valkonen, A Arihara, RL Plaisted, E Pehu, L Miller, and SA Slack. (1997). Mapping and marker-assisted selection for a gene for extreme resistance to potato virus Y. *Theoretical and Applied Genetics*, *94*, 192-197.

Hämäläinen, J. H., Sorri, V. A., Watanabe, K. N., Gebhardt, C., & Valkonen, J. P. T. (1998). Molecular examination of a chromosome region that controls resistance to potato Y and A potyviruses in potato. *Theoretical and Applied Genetics*, *96*(8), 1036–1043.

Hamilton, J. P., Hansey, C. N., Whitty, B. R., Stoffel, K., Massa, A. N., Van Deynze, A., De Jong, W. S., Douches, D. S., & Buell, C. R. (2011). Single nucleotide polymorphism discovery in elite North American potato germplasm. *BMC Genomics*, *12*(1), 1–12.

Hirsch, C. D., Hamilton, J. P., Childs, K. L., Cepela, J., Crisovan, E., Vaillancourt, B., Hirsch, C. N., Habermann, M., Neal, B., & Buell, C. R. (2014). Spud DB: A resource for mining sequences, genotypes, and phenotypes to accelerate potato breeding. *The Plant Genome*, *7*(1), plantgenome2013-12.

Holt, C., & Yandell, M. (2011). MAKER2: An annotation pipeline and genome-database management tool for second-generation genome projects. *BMC Bioinformatics*, *12*(1), 491. https://doi.org/10.1186/1471-2105-12-491

Hussey, R. S., & Barker, K. R. (1973). Comparison of methods of collecting inocula of *Meloidogyne spp*., including a new technique. *Plant Disease Reporter*. https://agris.fao.org/agris-search/search.do?recordID=US201303126127

Jiang, G.-L. (2013). Molecular markers and marker-assisted breeding in plants. *Plant Breeding from Laboratories to Fields*, 45–83.

Kardolus, J. P., van Eck, H. J., & van den Berg, R. G. (1998). The potential of AFLPs in biosystematics: A first application in Solanum taxonomy (Solanaceae). *Plant Systematics and Evolution*, *210*(1), 87–103.

Kasai, K., Morikawa, Y., Sorri, V. A., Valkonen, J. P. T., Gebhardt, C., & Watanabe, K. N. (2000). Development of SCAR markers to the PVY resistance gene Ry_{adg} based on a common feature of plant disease resistance genes. *Genome*, *43*(1), 1–8.

Koressaar, T., & Remm, M. (2007). Enhancements and modifications of primer design program Primer3. *Bioinformatics*, *23*(10), 1289–1291.

Kõressaar, T., Lepamets, M., Kaplinski, L., Raime, K., Andreson, R., & Remm, M. (2018). Primer3_masker: integrating masking of template sequence with primer design software. *Bioinformatics*, *34*(11), 1937–1938.

Kyndt, T., Vieira, P., Gheysen, G., & de Almeida-Engler, J. (2013). Nematode feeding sites: Unique organs in plant roots. *Planta*, *238*(5), 807–818.

Leisner, C. P., Hamilton, J. P., Crisovan, E., Manrique-Carpintero, N. C., Marand, A. P., Newton, L., Pham, G. M., Jiang, J., Douches, D. S., & Jansky, S. H. (2018). Genome sequence of M6, a diploid inbred clone of the high-glycoalkaloid-producing tuber-bearing potato species *Solanum chacoense*, reveals residual heterozygosity. *The Plant Journal*, *94*(3), 562–570.

Leroy, S., Duperray, C., & Morand, S. (2003). Flow cytometry for parasite nematode genome size measurement. *Molecular and Biochemical Parasitology*, *128*(1), 91–93. https://doi.org/10.1016/s0166-6851(03)00023-9

Li, H. (2018). Minimap2: Pairwise alignment for nucleotide sequences. *Bioinformatics*, *34*(18), 3094–3100. https://doi.org/10.1093/bioinformatics/bty191

Lindqvist-Kreuze, H., Gastelo, M., Perez, W., Forbes, G. A., de Koeyer, D., & Bonierbale, M. (2014). Phenotypic stability and genome-wide association study of late blight resistance in potato genotypes adapted to the tropical highlands. *Phytopathology*, *104*(6), 624–633.

Lunt, D. H., Kumar, S., Koutsovoulos, G., & Blaxter, M. L. (2014). The complex hybrid origins of the root knot nematodes revealed through comparative genomics. *PeerJ*, *2*, e356.

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gur, T., Madhusoodanan, N., Basutkar, P., Tivey, A. R. N., Potter, S. C., Finn, R. D., & Lopez, R. (2019). The EMBL-EBI search and sequence analysis tools APIs in 2019. *Nucleic Acids Research*, *47*(W1), W636–W641. https://doi.org/10.1093/nar/gkz268

Marçais, G., & Kingsford, C. (2011). A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. *Bioinformatics*, *27*(6), 764–770.

Mojtahedi, H., Brown, C. R., Riga, E., & Zhang, L. H. (2007). A new pathotype of *Meloidogyne chitwoodi* Race 1 from Washington State. *Plant Disease*, *91*(8), 1051–1051.

Mojtahedi, H., Santo, G. S., Brown, C. R., Ferris, H., & Williamson, V. (1994). A new host race of *Meloidogyne chitwoodi* from California. *Plant Disease*, *78*(10).

Mojtahedi, H., Santo, G. S., & Wilson, J. H. (1988). Host tests to differentiate *Meloidogyne chitwoodi* races 1 and 2 and *M. hapla*. *Journal of Nematology*, *20*(3), 468.

Mori, K., Sakamoto, Y., Mukojima, N., Tamiya, S., Nakao, T., Ishii, T., & Hosaka, K. (2011). Development of a multiplex PCR method for simultaneous detection of diagnostic DNA markers of five disease and pest resistance genes in potato. *Euphytica*, *180*(3), 347–355.

Nadeem, M. A., Nawaz, M. A., Shahid, M. Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., & Labhane, N. (2018). DNA molecular markers in plant breeding: Current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, *32*(2), 261–285.

National Agricultural Statistics Service. 2020, September 17. Potato Annual Summary. Retrieved from https://downloads.usda.library.cornell.edu/usda-esmis/files/fx719m44h/ks65j252d/wm118c91g/pots0920.pdf

Navarre, D. A., Brown, C. R., & Sathuvalli, V. R. (2019). Potato vitamins, minerals and phytonutrients from a plant biology perspective. *American Journal of Potato Research*, *96*(2), 111–126.

Onkendi, E. M., Kariuki, G. M., Marais, M., & Moleleki, L. N. (2014). The threat of root-knot nematodes (*Meloidogyne spp*.) in Africa: A review. *Plant Pathology*, *63*(4), 727–737.

Opperman, C. H., Bird, D. M., Williamson, V. M., Rokhsar, D. S., Burke, M., Cohn, J., Cromer, J., Diener, S., Gajan, J., & Graham, S. (2008b). Sequence and genetic map of

Meloidogyne hapla: A compact nematode genome for plant parasitism. *Proceedings of the National Academy of Sciences*, *105*(39), 14802–14807.

Pham, G. M., Hamilton, J. P., Wood, J. C., Burke, J. T., Zhao, H., Vaillancourt, B., Ou, S., Jiang, J., & Buell, C. R. (2020). Construction of a chromosome-scale long-read reference genome assembly for potato. *GigaScience*, *9*(9), giaa100. https://doi.org/10.1093/gigascience/giaa100

Phan, N. T., Orjuela, J., Danchin, E. G. J., Klopp, C., Perfus-Barbeoch, L., Kozlowski, D. K., Koutsovoulos, G. D., Lopez-Roques, C., Bouchez, O., Zahm, M., Besnard, G., & Bellafiore, S. (2020). Genome structure and content of the rice root-knot nematode (*Meloidogyne graminicola*). *Ecology and Evolution*, *10*(20), 11006–11021. https://doi.org/10.1002/ece3.6680

Powers, T. O., Mullin, P. G., Harris, T. S., Sutton, L. A., & Higgins, R. S. (2005). Incorporating molecular identification of *Meloidogyne spp*. Into a large-scale regional nematode survey. *Journal of Nematology*, *37*(2), 226.

Provan, J., Powell, W., & Waugh, R. (1996). Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). *Theoretical and Applied Genetics*, *92*(8), 1078–1084.

Ramakrishnan, A. P., Ritland, C. E., Blas Sevillano, R. H., & Riseman, A. (2015). Review of Potato Molecular Markers to Enhance Trait Selection. *American Journal of Potato Research*, *92*(4), 455–472. https://doi.org/10.1007/s12230-015-9455-7

Rhoads, A., & Au, K. F. (2015). PacBio sequencing and its applications. *Genomics, Proteomics & Bioinformatics*, *13*(5), 278–289.

Sahebani, N., & Hadavi, N. (2008). Biological control of the root-knot nematode *Meloidogyne javanica* by Trichoderma harzianum. *Soil Biology and Biochemistry*, *40*(8), 2016–2020. https://doi.org/10.1016/j.soilbio.2008.03.011

Santo, G. S., O'bannon, J. H., Finley, A. M., & Golden, A. M. (1980). Occurrence and host range of a new root-knot nematode (*Meloidogyne chitwoodi*) in the Pacific Northwest. *Plant Disease*, *64*(10), 951–952.

Santo, G. S., and J. N. Pinkerton. (1985). A second race of *Meloidogyne chitwoodi* discovered in Washington. *Plant Disease*, *69*, 631.

Sato, M., Nishikawa, K., Komura, K., & Hosaka, K. (2006). Potato virus Y resistance gene, Ry chc, mapped to the distal end of potato chromosome 9. *Euphytica*, *149*(3), 367–372.

Schreiber, L., Nader-Nieto, A. C., Schönhals, E. M., Walkemeier, B., & Gebhardt, C. (2014). SNPs in genes functional in starch-sugar interconversion associate with natural variation of tuber starch and sugar content of potato (*Solanum tuberosum L.*). *G3: Genes, Genomes, Genetics*, *4*(10), 1797–1811.

Sharma, S. K., Bolser, D., Boer, J. de, Sønderkær, M., Amoros, W., Carboni, M. F., D'Ambrosio, J. M., Cruz, G. de la, Genova, A. D., Douches, D. S., Eguiluz, M., Guo, X., Guzman, F., Hackett, C. A., Hamilton, J. P., Li, G., Li, Y., Lozano, R., Maass, A., Marshall, D., Martinez, D., McLean, K., Mejía, N., Milne, L., Munive, S., Nagy, I., Ponce, O., Ramirez, M., Simon, R., Thomson, S. J., Torres, Y., Waugh, R., Zhang, Z., Huang, S., Visser, R. G. F., Bachem, C. W. B., Sagredo, B., Feingold, S. E., Orjeda, G., Veilleux, R. E., Bonierbale, M., Jacobs, J. M. E., Milbourne, D., Martin, D. M. A.,Bryan, G. J. (2013). Construction of Reference Chromosome-Scale

Pseudomolecules for Potato: Integrating the Potato Genome with Genetic and Physical Maps. *G3: Genes, Genomes, Genetics*, *3*(11), 2031–2047. https://doi.org/10.1534/g3.113.007153

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O., & Spiegel, Y. (2001). Biological control of the root-knot nematode *Meloidogyne javanica* by Trichoderma harzianum. *Phytopathology*, *91*(7), 687–693.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., & Zdobnov, E. M. (2015). BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*, *31*(19), 3210–3212.

Simko, I., Haynes, K. G., & Jones, R. W. (2006). Assessment of linkage disequilibrium in potato genome with single nucleotide polymorphism markers. *Genetics*, *173*(4), 2237–2245.

Solomon-Blackburn, R. M., & Barker, H. (2001). Breeding virus resistant potatoes (Solanum tuberosum): A review of traditional and molecular approaches. *Heredity*, *86*(1), 17–35.

Somvanshi, V. S., Tathode, M., Shukla, R. N., & Rao, U. (2018). Nematode Genome Announcement: A Draft Genome for Rice Root-Knot Nematode, *Meloidogyne graminicola*. *Journal of Nematology*, *50*(2), 111–116. https://doi.org/10.21307/jofnem-2018-018

Song, Y.-S., Hepting, L., Schweizer, G., Hartl, L., Wenzel, G., & Schwarzfischer, A. (2005). Mapping of extreme resistance to PVY (Ry_{sto}) on chromosome XII using anther-culture-derived primary dihaploid potato lines. *Theoretical and Applied Genetics*, *111*(5), 879–887.

Song, Y.-S., & Schwarzfischer, A. (2008). Development of STS markers for selection of extreme resistance (Ry sto) to PVY and maternal pedigree analysis of extremely resistant cultivars. *American Journal of Potato Research*, *85*(2), 159–170.

Spooner, D. M., Ghislain, M., Simon, R., Jansky, S. H., & Gavrilenko, T. (2014). Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. *The Botanical Review*, *80*(4), 283–383.

Spooner, D. M., & Hetterscheid, W. L. (2006). 13. Origins, Evolution, and Group Classifi cation of Cultivated Potatoes. In *Darwin's Harvest* (pp. 285–307). Columbia University Press.

Stanke, M., & Morgenstern, B. (2005). AUGUSTUS: A web server for gene prediction in eukaryotes that allows user-defined constraints. *Nucleic Acids Research*, *33*(Web Server issue), W465–W467. https://doi.org/10.1093/nar/gki458

Szajko, K., Chrzanowska, M., Witek, K., Strzelczyk-Żyta, D., Zagórska, H., Gebhardt, C., Hennig, J., & Marczewski, W. (2008). The novel gene Ny-1 on potato chromosome IX confers hypersensitive resistance to Potato virus Y and is an alternative to *Ry* genes in potato breeding for PVY resistance. *Theoretical and Applied Genetics*, *116*(2), 297–303.

Szitenberg, A., Salazar-Jaramillo, L., Blok, V. C., Laetsch, D. R., Joseph, S., Williamson, V. M., Blaxter, M. L., & Lunt, D. H. (2017a). Comparative genomics of apomictic root-knot nematodes: Hybridization, ploidy, and dynamic genome change. *Genome Biology and Evolution*, *9*(10), 2844–2861.

Tarailo-Graovac, M., & Chen, N. (2009). Using RepeatMasker to identify repetitive elements in genomic sequences. *Current Protocols in Bioinformatics*, *25*(1), 4–10.

The Potato Genome Sequencing Consortium. 2011. Genome sequence and analysis of the tuber crop potato. *Nature*, *475*, 189–195.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3—New capabilities and interfaces. *Nucleic Acids Research*, *40*(15), e115–e115.

USDA - National Agricultural Statistics Service—Washington—Potatoe Reports. (n.d.). Retrieved July 6, 2021, from https://www.nass.usda.gov/Statistics_by_State/Washington/Publications/Potatoes/index.php

Valkonen, J. P. (2015). Elucidation of virus-host interactions to enhance resistance breeding for control of virus diseases in potato. *Breeding Science*, *65*(1), 69–76.

Valkonen, J. P. T., Jones, R. A. C., Slack, S. A., & Watanabe, K. N. (1996). Resistance specificities to viruses in potato: Standardization of nomenclature. *Plant Breeding*, *115*(6), 433–438.

Van der Beek, J. G., & Karssen, G. (1997). Interspecific hybridization of meiotic parthenogenetic *Meloidogyne chitwoodi* and *M. fallax*. *Phytopathology*, *87*(10), 1061–1066.

van den Berg, R. G., & Jacobs, M. M. J. (2007). Chapter 4—Molecular Taxonomy. In D. Vreugdenhil, J. Bradshaw, C. Gebhardt, F. Govers, D. K. L. Mackerron, M. A. Taylor, & H. A. Ross (Eds.), *Potato Biology and Biotechnology* (pp. 55–76). Elsevier Science B.V. https://doi.org/10.1016/B978-044451018-1/50046-4

Vurture, G. W., Sedlazeck, F. J., Nattestad, M., Underwood, C. J., Fang, H., Gurtowski, J., & Schatz, M. C. (2017). GenomeScope: Fast reference-free genome profiling from short reads. *Bioinformatics*, *33*(14), 2202–2204.

Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., & Young, S. K. (2014). Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PloS One*, *9*(11), e112963.

Wang, W., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, *218*(1), 1–14.

Watanabe, K. N., Kikuchi, A., Shimazaki, T., & Asahina, M. (2011). Salt and drought stress tolerances in transgenic potatoes and wild species. *Potato Research*, *54*(4), 319–324.

Weber, J. L. (1990). Informativeness of human (dC-dA) n·(dG-dT) n polymorphisms. *Genomics*, *7*(4), 524–530.

Weingartner, D. P., & Shumaker, J. R. (1990). Control of nematodes and soil-borne diseases in Florida potatoes with aldicarb and 1, 3-D. *Journal of Nematology*, *22*(4S), 775.

Wetterstrand, K. (2020). *The Cost of Sequencing a Human Genome*. Genome.Gov. https://www.genome.gov/about-genomics/fact-sheets/Sequencing-Human-Genome-cost

Williams, C. E., Wielgus, S. M., Haberlach, G. T., Guenther, C., Kim-Lee, H., & Helgeson, J. P. (1993). RFLP analysis of chromosomal segregation in progeny from

an interspecific hexaploid somatic hybrid between *Solanum brevidens* and *Solanum tuberosum*. *Genetics*, *135*(4), 1167–1173.

Witek, K., Strzelczyk-Żyta, D., Hennig, J., & Marczewski, W. (2006). A multiplex PCR approach to simultaneously genotype potato towards the resistance alleles Ry-f sto and Ns. *Molecular Breeding*, *18*(3), 273–275.

Wishart, J., Phillips, M. S., & Blok, V. C. (2002). Ribosomal intergenic spacer: A polymerase chain reaction diagnostic for *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla*. *Phytopathology*, *92*(8), 884–892.

Xu, X., Pan, S., Cheng, S., Zhang, B., Mu, D., Ni, P., Zhang, G., Yang, S., Li, R., Wang, J., Orjeda, G., Guzman, F., Torres, M., Lozano, R., Ponce, O., Martinez, D., De la Cruz, G., Chakrabarti, S. K., Patil, V. U., Wageningen University & Research Centre. (2011a). Genome sequence and analysis of the tuber crop potato. *Nature*, *475*(7355), 189–195. https://doi.org/10.1038/nature10158

Zhang, L., & Gleason, C. (2019). Loop-mediated isothermal amplification for the diagnostic detection of *Meloidogyne chitwoodi* and *M. fallax*. *Plant Disease*, *103*(1), 12–18.

Zhang, L., & Gleason, C. (2021). Transcriptome Analyses of Pre-parasitic and Parasitic *Meloidogyne chitwoodi* Race 1 to Identify Putative Effector Genes. *Journal of Nematology*, *53*, e2021-84. https://doi.org/10.21307/jofnem-2021-084

Zhang, Z., Schwartz, S., Wagner, L., & Miller, W. (2000). A greedy algorithm for aligning DNA sequences. *Journal of Computational Biology: A Journal of Computational Molecular Cell Biology*, *7*(1–2), 203–214. https://doi.org/10.1089/10665270050081478

Zijlstra, C. (2000). Identification of *Meloidogyne chitwoodi, M. fallax* and *M. hapla* based on SCAR-PCR: A powerful way of enabling reliable identification of populations or individuals that share common traits. *European Journal of Plant Pathology*, *106*(3), 283–290.

Appendix A. Supplemental analysis of gene annotation and comparison analysis of *Meloidogyne chitwoodi*

Supplementary 1.1. Illumina reads were used align with PacBio data contigs using BWA and Samtools.

SGE_Batch -c "samtools sort -T /nfs0/ROOTS/Sathuvalli_Lab/hushen/bwa/Mc27miseq/Mc27.sorted -o Mc27_miseq_bwa_aln.sorted.bam Mc27_miseq_bwa_aln.bam" -r Mc27_miseq_bwa_aln.sorted -P 8
SGE_Batch -c "samtools sort -T /nfs0/ROOTS/Sathuvalli_Lab/hushen/bwa/Mc27hiseq/Mc27.sorted -o Mc27_hiseq_bwa_aln.sorted.bam Mc27_hiseq_bwa_aln.bam" -r Mc27_hiseq_bwa_aln.sorted -P 8
SGE_Batch -c "samtools sort -T /nfs0/ROOTS/Sathuvalli_Lab/hushen/bwa/Mc1hiseq/Mc27.sorted -o Mc1_hiseq_bwa_aln.sorted.bam Mc1_hiseq_bwa_aln.bam" -r Mc1_hiseq_bwa_aln.sorted -P 8
SGE_Batch -c "samtools sort -T /nfs0/ROOTS/Sathuvalli_Lab/hushen/bwa/Rozahiseq/Roza.sorted -o ROZA_hiseq_bwa_aln.sorted.bam ROZA_hiseq_bwa_aln.bam" -r ROZA_hiseq_bwa_aln.sorted -P 8
SGE_Batch -c "samtools flagstat Mc27_hiseq_bwa_aln.bam" -r Mc27flsgstat -P 8

samtools sort -T /nfs0/ROOTS/Sathuvalli_Lab/hushen/bwa/Rozahiseq/Roza.sorted -o ROZA_hiseq_bwa_aln.sorted.bam ROZA_hiseq_bwa_aln.bam
SGE_Batch -c "samtools flagstat ROZA_miseq_bwa_aln.sorted | > ROZA_miseqflsgstat.txt" -r ROZAmiflsgstat -P 8
SGE_Batch -c "samtools flagstat ROZA_hiseq_bwa_aln.sorted > ROZA_hiseqflsgstat.txt" -r ROZAhiflsgstat -P 8

SGE_Batch -c "samtools mpileup -C50 Mc1_hiseq_bwa_aln.sorted.bam Mc1_miseq_bwa_aln.sorted.bam -o Mc1.mpileup.sorted.bam" -r Mc1_mpileup -P 8
SGE_Batch -c "samtools mpileup -C50 Mc27_hiseq_bwa_aln.sorted.bam Mc27_miseq_bwa_aln.sorted.bam -o Mc27.mpileup.sorted.bam" -r Mc27_mpileup -P 8
SGE_Batch -c "samtools mpileup -C50 ROZA_hiseq_bwa_aln.sorted.bam ROZA_miseq_bwa_aln.sorted.bam -o ROZA.mpileup.sorted.bam" -r ROZA_mpileup -P 8

SGE_Batch -c "samtools view -h -o Mc1_hiseq_bwa_aln.sorted.sam Mc1_hiseq_bwa_aln.sorted.bam" -r Mc1hiview -P 8

grep -v "@" ROZA_hiseq_bwa_aln.sorted.sam | awk '{ print $5}' | less -S
SGE_Batch -c "grep -v "@" ROZA_hiseq_bwa_aln.sorted.sam | awk '{if($6 == "151m") print $0}' | -o rozasum.txt" -r rozasum -P 8

SGE_Batch -c "grep -v "@" ROZA_hiseq_bwa_aln.sorted.sam | awk '{ print $6}' > roza.txt" -r roza6 -P 8

wc -l Mc1.txt
215526570 Mc1.txt

grep -c "151M" Mc1.txt
184256162

wc -l Mc27.txt

```
252706007 Mc27.txt

grep -c "151M" Mc27.txt
214448216

 wc -l roza.txt
268729460 roza.txt

grep -c "151M" roza.txt
228189027
```

Supplementary table 1.1. Total alignment and perfect alignment between Illumina and PacBio sequencing for *M. chitwoodi* Race 1, Race 2, and Race 1_{Roza}.

	Total alignment	Perfect alignment	Percentage of Perfect alignment
Race 1	215526570	184256162	85.49%
Race 2	252706007	214448216	84.86%
Race 1_{Roza}	268729460	228189027	84.91%

Supplementary 1.2. Genome size estimation using Jellyfish to extract and count canonical k-mers at k= 21, 31 and 71 nucleotides.

```
jellyfish count -t 8 -C -m 21 -s 2G -o 21mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc1/lane2-s012-indexRPI20-GTGGCC-
Mc1_S12_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc1/lane2-s012-
indexRPI20-GTGGCC-Mc1_S12_L002_R2_001.fastq
jellyfish histo -o Mc1_21mer_out.histo 21mer_out

jellyfish count -t 8 -C -m 31 -s 2G -o 31mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc1/lane2-s012-indexRPI20-GTGGCC-
Mc1_S12_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc1/lane2-s012-
indexRPI20-GTGGCC-Mc1_S12_L002_R2_001.fastq
jellyfish histo -o Mc1_31mer_out. histo 31mer_out

jellyfish count -t 8 -C -m 71 -s 2G -o 71mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc1/lane2-s012-indexRPI20-GTGGCC-
Mc1_S12_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc1/lane2-s012-
indexRPI20-GTGGCC-Mc1_S12_L002_R2_001.fastq
jellyfish histo -o Mc1_71mer_out. histo 71mer_out
jellyfish count -t 8 -C -m 21 -s 2G -o 21mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc27/ lane2-s011-indexRPI9-GATCAG-
Mc27_S11_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc27/ lane2-s011-
indexRPI9-GATCAG-Mc27_S11_L002_R2_001.fastq
jellyfish histo -o Mc27_21mer_out.histo 21mer_out

jellyfish count -t 8 -C -m 31 -s 2G -o 31mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc27/ lane2-s011-indexRPI9-GATCAG-
Mc27_S11_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc27/ lane2-s011-
indexRPI9-GATCAG-Mc27_S11_L002_R2_001.fastq
```

```
jellyfish histo -o Mc27_31mer_out. histo 31mer_out

jellyfish count -t 8 -C -m 71 -s 2G -o 71mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc27/ lane2-s011-indexRPI9-GATCAG-
Mc27_S11_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/mc27/ lane2-s011-
indexRPI9-GATCAG-Mc27_S11_L002_R2_001.fastq
jellyfish histo -o Mc27_71mer_out. histo 71mer_out


jellyfish count -t 8 -C -m 21 -s 2G -o 21mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/roza/ lane2-s013-indexRPI21-GTTTCG-
Roza_S13_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/roza/ lane2-s013-
indexRPI21-GTTTCG-Roza_S13_L002_R2_001.fastq
jellyfish histo -o Roza_21mer_out.histo 21mer_out

jellyfish count -t 8 -C -m 31 -s 2G -o 31mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/roza/ lane2-s013-indexRPI21-GTTTCG-
Roza_S13_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/roza/ lane2-s013-
indexRPI21-GTTTCG-Roza_S13_L002_R2_001.fastq
jellyfish histo -o Roza_31mer_out. histo 31mer_out

jellyfish count -t 8 -C -m 71 -s 2G -o 71mer_out --min-qual-char=?
/nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/roza/ lane2-s013-indexRPI21-GTTTCG-
Roza_S13_L002_R1_001.fastq /nfs0/ROOTS/Sathuvalli_Lab/hushen/illumina/roza/ lane2-s013-
indexRPI21-GTTTCG-Roza_S13_L002_R2_001.fastq
jellyfish histo -o Roza_71mer_out. histo 71mer_out
```

Supplementary 1.3. Evaluation of the completeness of the genomic assembly using BUSCO v4

```
busco -i Meloidogyne_chitwoodi_MC1_final_01092020.fasta -f -m genome --auto-lineage-euk -o
mc1busco --augustus --config /dfs/ROOTS/Sathuvalli_Lab/hushen2/genomebusco/Mc1config.ini


busco -i Meloidogyne_chitwoodi_MC27_final_01092020.fasta -f -m genome --auto-lineage-euk -o
mc27busco --augustus --config /dfs/ROOTS/Sathuvalli_Lab/hushen2/genomebusco/Mc1config.ini


busco -i Meloidogyne_chitwoodi_ROZA_final_01092020.fasta -f -m genome --auto-lineage-euk -o
rozabusco --augustus --config /dfs/ROOTS/Sathuvalli_Lab/hushen2/genomebusco/Mc1config.ini
```

Supplementary 1.4. Tandem repeats identification using Tandem Repeats Finder (Benson, 1999).

```
SGE_Batch -c "trf MC1.fasta 2 7 7 80 10 50 500 -f -d -m" -r Mc1trf -P 8
SGE_Batch -c "trf MC27.fasta 2 7 7 80 10 50 500 -f -d -m" -r Mc27trf -P 8
SGE_Batch -c "trf ROZA.fasta 2 7 7 80 10 50 500 -f -d -m" -r ROZAtrf -P 8
```

Supplementary 1.5. Gene annotation using MAKER2 (Holt & Yandell, 2011).

```
SGE_Batch -c "/local/cluster/MAKER/bin/maker -g
/dfs/ROOTS/Sathuvalli_Lab/hushen2/annotation/mc1maskre2/masked_sequencesMC1.fasta -c 4 -base
Mc1" -q hoser -r Mc1_maker1 -P 4
/local/cluster/maker-2.31.10/bin/gff3_merge -n -d
/dfs/ROOTS/Sathuvalli_Lab/hushen2/annotation/mc1maskre2/Mc1.maker.output/Mc1_master_datasto
re_index.log

SGE_Batch -c "/local/cluster/MAKER/bin/maker -g
/dfs/ROOTS/Sathuvalli_Lab/hushen2/annotation/mc27maskre2/masked_sequencesMC27.fasta -c 4 -
base Mc27" -q hoser -r Mc27_maker1 -P 4
/local/cluster/maker-2.31.10/bin/gff3_merge -n -d
/dfs/ROOTS/Sathuvalli_Lab/hushen2/annotation/mc27maskre2/Mc27.maker.output/Mc27_master_dat
astore_index.log


SGE_Batch -c "/local/cluster/MAKER/bin/maker -g
/dfs/ROOTS/Sathuvalli_Lab/hushen2/annotation/rozamaskre2/masked_sequencesROZA.fasta -c 4 -
base Roza" -q hoser -r Roza_maker1 -P 4
/local/cluster/maker-2.31.10/bin/gff3_merge -n -d
/dfs/ROOTS/Sathuvalli_Lab/hushen2/annotation/rozamaskre2/Roza.maker.output/Roza_master_datast
ore_index.log
```

Supplementary 1.6. Genome alignment analyze using Minimap2 (Holt & Yandell, 2011)

```
minimap2 -cx asm5 --cs Meloidogyne_chitwoodi_MC1_final_01092020.fasta
Meloidogyne_chitwoodi_MC27_final_01092020.fasta > Mc1Mc27.paf
minimap2 -cx asm5 --cs Meloidogyne_chitwoodi_MC1_final_01092020.fasta
Meloidogyne_chitwoodi_ROZA_final_01092020.fasta > Mc1Roza.paf
minimap2 -cx asm5 --cs Meloidogyne_chitwoodi_MC27_final_01092020.fasta
Meloidogyne_chitwoodi_ROZA_final_01092020.fasta > Mc27Roza.paf
sort -k6,6 -k8,8n Mc1Mc27.paf > Mc1Mc27.srt.paf
sort -k6,6 -k8,8n Mc1Roza.paf > Mc1Roza.srt.paf
sort -k6,6 -k8,8n Mc27Roza.paf > Mc27Roza.srt.paf
/nfs0/ROOTS/Vining_Lab/bin/minimap2/misc/paftools.js call Mc1Mc27.srt.paf > Mc1Mc27.var.txt
44150502 reference bases covered by exactly one contig
4027 substitutions; ts/tv = 0.841
1024 1bp deletions
759 1bp insertions
99 2bp deletions
82 2bp insertions
352 [3,50) deletions
365 [3,50) insertions
189 [50,1000) deletions
```

147 [50,1000) insertions
23 >=1000 deletions
21 >=1000 insertions
nfs0/ROOTS/Vining_Lab/bin/minimap2/misc/paftools.js call Mc1Roza.srt.paf > Mc1Roza.var.txt
45026099 reference bases covered by exactly one contig
1725 substitutions; ts/tv = 0.851
887 1bp deletions
735 1bp insertions
41 2bp deletions
44 2bp insertions
189 [3,50) deletions
221 [3,50) insertions
93 [50,1000) deletions
90 [50,1000) insertions
9 >=1000 deletions
18 >=1000 insertions
/nfs0/ROOTS/Vining_Lab/bin/minimap2/misc/paftools.js call Mc27Roza.srt.paf > Mc27Roza.var.txt
43923554 reference bases covered by exactly one contig
4134 substitutions; ts/tv = 0.868
1068 1bp deletions
1237 1bp insertions
95 2bp deletions
138 2bp insertions
412 [3,50) deletions
395 [3,50) insertions
138 [50,1000) deletions
172 [50,1000) insertions
21 >=1000 deletions
29 >=1000 insertions

Supplementary 1.7. Transcriptomes and proteomes BLAST of Race 1, Race 2 and Race 1_{Roza}

makeblastdb -in Mc1.all.maker.transcripts.fasta -input_type fasta -dbtype nucl -out Mc1
makeblastdb -in Mc27.all.maker.transcripts.fasta -input_type fasta -dbtype nucl -out Mc2
makeblastdb -in Roza.all.maker.transcripts.fasta -input_type fasta -dbtype nucl -out Roza
blastn -query Mc27.all.maker.transcripts.fasta -db Roza -evalue 1e-5 -perc_identity 90 -outfmt 6 > resultsMc27Roza.txt
blastn -query Mc27.all.maker.transcripts.fasta -db Mc1 -evalue 1e-5 -perc_identity 90 -outfmt 6 > resultsMc27Mc1.txt
blastn -query Mc1.all.maker.transcripts.fasta -db Roza -evalue 1e-5 -perc_identity 90 -outfmt 6 > resultsMc1ROza.txt
makeblastdb -in Mc1.all.maker.proteins.fasta -dbtype prot -parse_seqids -out Mc1.protein
makeblastdb -in Mc27.all.maker.proteins.fasta -dbtype prot -parse_seqids -out Mc27.protein
makeblastdb -in Roza.all.maker.proteins.fasta -dbtype prot -parse_seqids -out Roza.protein
blastp -query Mc27.all.maker.proteins.fasta -db Roza.protein -out resultsproteinMc27Roza.txt -evalue 1e-10 -num_threads 4 -outfmt 6 -num_alignments 5
blastp -query Mc27.all.maker.proteins.fasta -db Mc1.protein -out resultsproteinMc27Mc1.txt -evalue 1e-10 -num_threads 16 -outfmt 6 -num_alignments 5
blastp -query Mc1.all.maker.proteins.fasta -db Roza.protein -out resultsproteinMc1Roza.txt -evalue 1e-10 -num_threads 16 -outfmt 6 -num_alignments 5

Appendix B. Supplemental tables for molecular markers primers to identify CRKN races

Supplementary table 2.1. List of SSR markers primers to identify CRKN races.

	Forward primer	Reverse primer
HS00FSSR11	GGAAGAGGAATGGAGTGAAAA	GGTTGGAAAATCGTACCAAA
HS01FSSR8	GTAGGTCTTGGTCTTGGGTCTT	TATTAACCATTTTCAGCGCC
HS02FSSR1	CGCAACACTTCGTCATCAAT	CTCAGTTTAGCATCGGTGGTG
HS02FSSR2-1	GTTCGGTCATTTTCGGTCAT	ATCCGTAATCCAGTGTTTTCGT
HS02FSSR2-2	AAATGACCTGACCTGACCTGAC	ATCCGTAATCCAGTGTTTTCGT
HS02FSSR7	GATGATGAAGAGGAAGAAGATGAAG	CCAAGATGTCAAACTCCCAAAT
HS02FSSR8-1	TTAATTGTGACCAGTGCTTGGA	GCTCGTACTTATGCCTCGTACC
HS02FSSR8-2	TTGGAAGCTCGTACCAAAATTC	GCTCGTACTTATGCCTCGTACC
HS02FSSR9	GTCCTTTGACTGACCAGAAGGT	GAGAGAAGAGAAGAGAGAAGAGAGAAA
HS04FSSR14	TTTCTCCTTTCTGCTTGCTCTT	AAATGTCGTCTCAACCTTCCTC
HS04FSSR4	CTTACCTTCCTTTCCCTTTTCC	AATTGCTCACAGACAACAGCA
HS05FSSR4	AAAATACTTCCTCCACCACCG	CGGATTTCCAATGATGATGAG
HS05FSSR9	GTTGTTGTGATTGTTGTTGTGG	ATCAGGCAATAAATCTGGACCT
HS07FSSR3	ACTCTGTGTGGGGTGATTCTTT	CATTCCGGTTATTCCGGTTA
HS08FSSR5	CTGAAATGAGAGGGGATATTGG	TTGGTATGCCTGTAAAGATTGG
HS09FSSR4	ACCTCATACTCATACTCATACCTCG	AATTTATTGCCCTCTAGTTGCC
HS10FSSR10-1	AATAACCAAGACCCAAGACCAA	TGTGAAAAGTAGAGCTGTTCCAAG
HS10FSSR10-2	AGACCCAAGACCAAGACCTACA	TGTGAAAAGTAGAGCTGTTCCAAG
HS10FSSR12	TTTGAATTATTCCTTCCCCCTAA	GCTGAATCGAATGAGCTATGAA
HS10FSSR13	TTTGAATTATTCCTTCCCCCTAA	GCTGAATCGAATGAGCTATGAA
HS10FSSR4	GCACAACCACTCCCAATTTT	TGGTCCTTCTTTCCTTGTATGG
HS10FSSR5	GTAAATCAGGGTTGCATCGG	GCAAAAGGTTCTGGGAAGTAAA
HS10FSSR7	CTTGGAAGCTCGTACCAAAAT	GTGAGATGAGGAATTGAGTGGA
HS10FSSR9-1	GGTCTTCCATAGCTTACCTTACAAA	TTTTGGTCTTGGTCTTGGTCTT
HS10FSSR9-2	AAATAACCAAGACCCAAGACCC	CGGCTTTCTTTTAGTAGCACTCAT
HS13FSSR10	TTCAAAGTTATCGAAAATTGAACG	TTATTTACTTTATTTAATTGTGAAGAACG
HS13FSSR2	CGGCTTTCTTTTAGTAGCACTCAT	AAATAACCAAGACCCAAGACCC
HS13FSSR7	AAATAACCAAGACCCAAGACCC	CGGCTTTCTTTTAGTAGCACTCAT
HS17FSSR1-1	AAATAACCAAGACCCAAGACCC	CGGCTTTCTTTTAGTAGCACTCAT
HS17FSSR1-2	AAGACCAAGACCAAGACCAAAA	GTTCACCACCAAGCACAGTAGA
HS17FSSR2-1	AAGACCAAGACCAAGACCAAAA	GCGCACAGCTTAACTTTCATC

HS17FSSR2-2	AGACCCAAGACCAAGACCTACA	GCGCACAGCTTAACTTTCATC
HS20FSSR1-1	ATTCCTTTTGAGGTGTCTGAGG	CTTCTCCTTCTCCTCCTCCTTC
HS20FSSR1-2	GGAGGAGAAATAGGAGGAGGAG	CCTCAGACACCTCAAAAGGAAT
HS20FSSR2-1	CCATCCAGCGATAGGTTGAAA	TAATACTGAAAGGGTCGGGTCG
HS20FSSR2-2	GTTACCCGAAACCCGATACC	CGAACCCACATTTCCTAAAGAG

Supplementary table 2.2. List of INDEL markers primers to identify CRKN races.

	Forward primer	Reverse primer
HSINDEL1	TTTATTTCCCTCTTTAAAGGACCA	CGAGTTTTAACCCTTGACTGAGTT
HSINDEL2	TAGTTAACCATACGGGTATGTCGT	CCCCTTCACCCTCTACTCTCTT
HSINDEL3	TTTTCTTGACTCCTAGAACCTTGG	GCTACACTAACGGAGGAAGCTCTA
HSINDEL4	GGGAAATATTTAACCCACTATCCA	ATTTCGTATAATTCTGCGGTGGT
HSINDEL5	ACTTGTAGTTTTAATTTTGTGATGC	AATGAGAAATTTGAGAAGGTCTCG
HSINDEL6	GGATAATAAAGATGGGGGATTGAT	GTTGCTCATTCACAAACACTTTTC
HSINDEL7	CCAAATAAATATACACCGCTGGTT	CGAAGAAAAGGAAAAGAAATTGAG
HSINDEL8	CAAAACGTCATTCCTTAGTTGTCA	TGCTCCGACAGTTTGTTTTATATT
HSINDEL9	CTTTGGAAATAATTTTGGAGGTGT	CAGCAAGTACTTCTCATTGACAAAA
HSINDEL10	CCGCTTATACTATTTTTCTCTTCACTG	ATTCAAAGGGGTAACGGAAAA
HSINDEL11	ATTCCACAGCTTGAAAAACAATTA	CGATTTAAGCACTATATGAACACG
HSINDEL12	CTATCTTAAAACCGCCTACAACAA	CGATTTAAGCACTATATGAACACG
HSINDEL13	AATAATTTTATGGGACGAATTGTG	AAATAAAAGAGGGGAAATGTTCAG
HSINDEL14	ATATTTAGTAGTGACCGCGGGTAT	AGTTTGCATAAAAATAGGCAGTCG
HSINDEL15	GGGAAATATTTAACCCACTATCCA	ATTTCGTATAATTCTGCGGTGGT
HSINDEL16	CCTATTAAATTCGGTGGGGTACTA	GGGACTGAAACGTCTCCTTTT
HSINDEL17	TTACACCTCCTATGGTTTTCCAAT	TTTTCGTGAAAATTTAGCTGCTATT

Appendix C. Supplemental tables for molecular markers primers design and map for Corky ringspot resistance

Supplementary table 3.1. List of SNP primers on chromosome 1, chromosome 9 and chromosome 10.

	Forward primer	Reverse primer	Polymorphic on agarose gel*
PotVar0050687-1	TCCTCAACTGGGTTCTCCTG	TCCTCGCCAGGTACTTGAAC	NP
PotVar0072548-1	GAGCACCGTATCAGTCGTCA	ACACAATGGTGCCAGTCTCA	NP
solcap_snp_c2_20667-1	GAGGGGTCAACATCGGTCAT	TGAAGCTTGCACATTTCGCT	NP
PotVar0011047-1	TCCATACCAGGTTAGCATGCA	TCTGTGCCAAATTTACCGCC	NP
solcap_snp_c2_3021-1	CGCCTCAGTAACAGACCCAT	TGCAGTTCAGGTGTGTTTCG	NP
solcap_snp_c2_3007-1	CGGATTTGTGCTTCTGAGGG	GTACTCAAATGCAGGTGGGG	NP
PotVar0105170-1	GGCTTGTCGTTCACTGGATC	CCAACGGCAGAGTACCAAAC	NP
PotVar0105222-1	GCATCCAACAAAATACCAAAGGC	TAGGGGTGCTTTATGGTGCT	NP
PotVar0105228-1	CCAATCTCAAGAAACCAGCCAT	GGGAGTATGGAAATTTGGTGCA	NP
PotVar0105349-1	AAGCGTTACAAACAGGTCACAA	ACGTTGTGCTTCATGTCTGC	NP
solcap_snp_c2_3073-1	GTGGTTCTACGCGAGGAAAC	AGAATCGAATGGACAAAGCACC	NP
solcap_snp_c2_2992-1	AGCCACCTCCTTTTCCATCA	CAAGAGCAAAGCAACCAAGC	NP
PotVar0108720-1	ATCCACCTTACTGCGATCCTT	CAGTGGGAGCGAAGTGTTTT	NP
PotVar0108623-1	GTCTTCCCCAAGGTCCGTAA	CACAACCTGCAATAGTCTGGG	NP
PotVar0108448-1	TCTGTTTCACTATTCCCTCCGT	TTCCCCGCCTTTGATCATCA	P
solcap_snp_c1_12229-1	TCAGAAGACAAAGAGGGCCA	AGCTGACATGTGGAGTATTGGT	NP
solcap_snp_c1_12236-1	TCTTTGGTGGGTTGGTTCCT	ACCTTCACTGTGACCACTCC	NA
PotVar0122870-1	TGGACATTAGAACAAGAACTTTGGA	GCTGCACTTGAGCCAAAGG	NP
PotVar0122753-1	CTTGCAAAATGTGTGGTTGGTT	ACTCTCTGGCCCAAGAACTT	NP
PotVar0122751-1	CTTGCAAAATGTGTGGTTGGT	GGAAGTGAAGATAAGAAACCATTGG	NP
PotVar0122709-1	TGCCATCCAACATAGTGCAAC	AGTAAAACAAAACAAAGTGCAGGAA	NP
PotVar0122699-1	ATTGTTAGTGACCTACGCCAAAT	CCTGGCTGAATGGTGCTTTT	NP
PotVar0050687-2	CACACAGCTGCATCAGCATA	CTGCAACCCTGAAAATAGGG	NP
PotVar0072548-2	CAATTGAAAAGAAGAGAGGCAAT	GAGACACAATGGTGCCAGTC	NP
solcap_snp_c2_20667-2	TTCTCTGCCACTCCTTCCAG	GGTGACACGTTGATACAGCTAT	NP
PotVar0011047-2	TCGATTCCATACCAGGTTAGCA	CCATTGGCCTAACATCACCTC	NP
solcap_snp_c2_3021-2	ACAGAACCCACAATCCAAACAG	GTTTCGAAGTCTTGGCTTGGT	NP
solcap_snp_c2_3007-2	TATTGTCCCTCACCGATGCA	TGCCCATTCAACATCACCTTC	NP
PotVar0105170-2	ATTCGTAACGTCCGGGAAGA	TCGATTCAACCGACTATCCCA	NP
PotVar0105222-2	TCAATCGGAACCATATGAGGACA	GTCTGCACTTGGTTATCCCTTTT	NP

PotVar0105228-2	TCAATCGGAACCATATGAGGACA	GTCTGCACTTGGTTATCCCTTTT	NP
PotVar0105349-2	ACAGTTCGTTAAGGACTAGGACA	CCTACTTACGTTGTGCTTCATGT	NP
solcap_snp_c2_3073-2	TCAAGGCTCTCATCACCAGC	GGCTTGTTTCCACTCAATTTGTT	NP
solcap_snp_c2_2992-2	CCAGTACCCTAAGATGGCGT	CCACAACATGATGGCTATGCT	NP
PotVar0108720-2	TCTCCTCTTTACCGTCTTGTGT	GCGAAGTGTTTTATCCGGGAA	NP
PotVar0108623-2	GCTTTCTTCGTTGCAGCGTA	ACAAGCAGGTGTTCGTTACG	NP
PotVar0108448-2	TTGGTGCAGCAATCAAGTAACA	CGCATGATACTTCTCCGTGTG	P
solcap_snp_c1_12229-2	GGGGATTCGTCAAAGTTTGGA	TTGTAGCATGCCATAGTTTGACT	NP
solcap_snp_c1_12236-2	CTTGGCGTGCTGTTCTTCAT	CCCGTCCACAACCAAAACTC	PNR
PotVar0122870-2	GGTTTCAACATCAACAACATACCC	TGCTAGACAGTTCATTGAGTCCT	NP
PotVar0122753-2	TGGCTGCCATTTTCTTTCTTTAGT	GGGCCTTAAAATTGAACCATTGG	NP
PotVar0122751-2	TGTGTGGTTGGTTAAATGTACATGT	GCCTAGAAGAAGAAACTTAGACCAA	NP
PotVar0122709-2	ACAGAGACATTCCTACCAAGCA	GCTATATATGTTCTCTTTGGTCCCC	NP
PotVar0122699-2	GGGACCAAAGAGAACATATATAGCA	TCCTCGCTTCTCTTTTCTCCT	NP

*For polymorphic on agarose gel, “P” as polymorphic between resistant and susceptible progenies, “NP” as no polymorphic between resistant and susceptible progenies, “NA” as no amplification production, “PNR” as polymorphic that not associate with CRS resistance.

Supplementary table 3.2. List of SSR primers on chromosome 9.

	Motif	Forward primer	Reverse primer	Polymorphic on agarose gel*
SSR571A	TTC	CCCCAAATTCGCTACACG	GAAGAGGAAGAGGAGAAGGAGAAT	NA
SSR571B	TCC	CCTCCTCTTCCTACTTCTCCTTCT	CCGACCAACTCAAAATATCCTCTA	P
SSR573	TTG	TAGGGATAAGGTCTGGGTACACTC	TCCTACAACCTACTTCACTATGCAA	NP
SSR574	TC	GTTTAAGAAATGCTCCTTCGAGAC	CCGAATGCAGAAAAGTTCAGTT	NP
SSR575A	ACA	TTCTTAGGAGTTCTTGAGGTCACA	TAGTGGAAATACCCTTTCTTGAGC	NP
SSR575B	AG	GGTCCTGCACAAGTATAAGTTTGA	CTCATTTGGTTAGGGCTTTTGT	NP
SSR576	TGT	CTGAGATAGGGGTAAGGTTTGAGT	TGACCTGTTAATCCAACTATGTGTC	PNR
SSR579	TG	TTCCGTTGATACTGCCTGAATA	ATAAAAGCACGAAAGTCCTCCA	NA
SSR580	GT	ATCTTGTCCTCTCAAGGTGCAT	GGAGTAACATCAACACATACCCAC	NP
SSR581	GA	GTAGAAACTCAAACCGCCAATC	GCCTGGTATGCTCTATTTTGCT	NP
SSR582	CCT	GGGAAACTAGACAAAACAGGCA	GTCATCTTATCCCCTTGGAGTG	P
SSR584	TGG	GTGAGGGGTTTGATAGTGGTTATG	GCAACAAGCAACTAAACATCCA	NP
SSR586A	ACT	CCCCTTTTGTACCACCACC	CCCCTCACTCTCTGACTTGTAAA	NP
SSR586B	GA	GTGATAAAACCCAACTCTCTCACA	TGCTGTGTACTTCAATCCCTTCT	NP
SSR588	AG	GCACTGAGAACCTGTTATTTGAAG	TTGTTGTCGTCTTTTCCTTGTG	NP
SSR591A	CCA	CTTTTCTATAATGTACGGACGGC	TGATTAACATGAGGGCTTTGG	NP
SSR591B	AG	GGGTTGTGTGTGTGGTGATT	TTATTGAGAGAGAGAGAGGGGAAA	NP
SSR591C	TC	TCAACGTCAGATCCAATTTCC	CTAGCAAAGAAGAAAGCGAGAGAG	NP
SSR595	GA	TGCCACATCACCTTCTCTACAT	GCCACATCAGCAATCTTATATCC	NP
SSR596	CT	CCCTGCTCTATCAATTCCATCTA	ACTGTAGGCATAGTCAAACGCATA	NP
SSR597	GA	AAAATTACGTGTCTACAGCTTGCC	GAACTACACCTGACCTGATTCCTT	NP
SSR600	GGA	TGAGCCCCTGATTTAGTTCATT	GAAGTCCTCCTACGATTCCTCC	PNR
SSR601A	GTG	GAAGTACATGAAGCCGAATATGG	CGCCTGAGTATCTACCACCAC	NP
SSR601B	AGA	TGAGGAAGAAGATGCAGTGTAGAG	GTAGCAAATCACCCCAAAACAT	PNR
SSR602	TC	GAGTAATGACACACACGCCTTAGT	AACTTGAGCTTTAACCACTGCAC	NP
SSR604	GT	CAGAATGATGCAATCGCTTAAC	GTAGCACGATAGACTGAAATCGAA	NA
SSR605	GAA	AGGAGGAGAGAAGAAGAGGAGAAG	CGACTTAAACGAAGAGTTGCG	NP
SSR606	CT	GAATGTTTGAAGGAAGAAGGAAGAG	ACAGATACCACCAAAGGCAACT	NA
SSR607A	CCG	TGAAAACTTACCAGTATCACCTGC	GGAACAATCGAATTTACAGAGGTC	NP
SSR607B	TGT	TCTCCACTTACATCTTCATAGCTCA	CCTACAGCAACAAGAAGCAGTTTA	NP
SSR608A	TGT	TTCTGTCCGCTAACAAGTAACATC	TCCAGCAACAAGAAGCAGTAAA	NP
SSR608B	GT	ATAGTTGAGGTGTGGGCAAGTTA	AAGTAAAAGTGAACGGAGGGAGTA	NP
SSR610	CCA	GAATACATGGGTTTGGCATCTT	CACACAAGTGGTAAGGGGAAA	P
SSR611A	AGT	ATATCAGTTCAGTCTCACGCCTTT	GTCTGTTTGATGATGGGGTTTT	NP
SSR611B	TC	CAGTGTGCTGTTGGATGATGT	CAAGGAATATGGCTGTATGTACCA	NP
SSR612	TG	AACGGTGGAGATTGTTTCTGAT	AATGTGTTGGGAAAGAGGAAGA	NP

*For polymorphic on agarose gel, “P” as polymorphic between resistant and susceptible progenies, “NP” as no polymorphic between resistant and susceptible

progenies, “NA” as no amplification production, “PNR” as polymorphic that not associate with CRS resistance.

Supplementary table 3.3. List of INDEL primers with short insertion and deletions on chromosome 9.

	Forward primer	Reverse primer	Polymorphic on agarose gel*
INDEL1	TACTATCGAAATAACTCCGTCCATC	AATTTGCAATATTTTGGGTGGTAT	NA
INDEL2	AATCTCCTTACATGCCAACCTAAC	TCTCTTCGAGAGGTTTCAGACTTT	NP
INDEL3	TAGGTGCTATTGAACAATCCAGAG	TCTCTTCGAGAGGTTTCAGACTTT	NP
INDEL4	TACCAATGCTTCTTGATTTCTTCA	AAACGGGTCTGTAACATGATTTTT	NP
INDEL5	GGACTTTCCAAAAATGCACTACTT	CGGATAACTCTGTCTACCAAGGTT	NP
INDEL6	CATTCCTCCACAACTGTACAAAAG	CTGCTCCTTTAATTGTATGTGTCG	NA
INDEL7	AAAAGGAAGGTTCTTGATTTAGCA	TCATCAATGTCCCAAAAATATGAC	NP
INDEL8	TCCTTCAGAAATAACCTCTCAACC	GAGGAAAACAAATAGATGGGAGAA	NP
INDEL9	CTCTTCACATGTACGAACCATCTC	AGCTTATGATTGTCACAAAGTCCA	P
INDEL10	TGTGATCAGACAACTAAAAGTCCAA	GTCAATCAAATAATCTGCCATATCC	PNR
INDEL11	GGAGGATAGAGTATAGGCAGACCA	GCGGAAAGGAAAGTGATAGAAATA	NP
INDEL12	TGGTAAAATCTCTCGTGTGCTAGA	AAAAATTTGTAGCCCAAGTGACTC	NA
INDEL13	CTAGTCTGAGTGAATCCCACTTGA	TTTTTGGGATGGAGAAAACTCTAC	NP
INDEL14	TACTCGCCAAGTTGTTGTCACTAT	AAGATGTCGATCACCTTTTCCTAC	PNR
INDEL15	CTTGTTCTGCTTACCAGTTTGAGA	GTGAAATGAGAGATTCAAATGACG	PNR
INDEL16	CTTGTTCTGCTTACCAGTTTGAGA	GATTCAAATGACGAGAGAAGGACT	NP
INDEL17	ACCCTTTTGAAAAAGAATTCACAG	ATGGTTTTGGAAATATTGGTCATT	NP
INDEL18	AAATGTGGAAAATTAGAGGAGCAG	TTCAACCTTTGCATAGACGATTTA	NP
INDEL19	CATACATCTTTTTGGTGGTGAGAG	CATGTAAGCGCACCACTAATTCTA	NP
INDEL20	CCTTCTACAAATGTGTGAAACCTG	GTTGTTGAGTCCGACAACAAAATA	P
INDEL21	AAGGTAGGGAGAAATTGTGTGTGT	ACACTCGCGATGACGTATAAAGTA	NA
INDEL22	TTGCTGTGCAAGATAAATACCAGT	GGAAAACAGCAAGGAATAAAAGAA	NP
INDEL23	AGTAACGATCTCAAAACAATGCAG	GCAACACACTTAGAAACCATGAAC	NP
INDEL24	ACCAACTTCAATCCACTCTTCTTC	GATCCTTTTTCGTAGTTGAGAAGG	NP
INDEL25	TTTGCATTTGTGAAATATCCTCAT	ACGTCCTTATCAACCATCTCATTT	NP
INDEL26	CTTTGTATATCACTTGACGCCTTC	AGTTGGACTTGGTAAAGTTTGAGG	PNR
INDEL27	GGTTCAAACGAACGAGTAACTTTT	GTGGATCCAAGATTAGGAGTTTTG	PNR
INDEL28	ACAATTTTAACCAACGATCCAAGT	GTGACACGATTCTTGAGGTGATAG	NP
INDEL29	CTCAATAAAGCGGGTAAAAACTGT	CACCAAAGTACCCTTCCTAAAAGA	NP
INDEL30	TCGAGATGCTTGACGTAATTTTTA	AGGGAGAGTATTCTTTCCTCCAGT	PNR
INDEL31	AAACTCATTTTGCTTGATTTAGCC	AAGTTAGCTAATGCCCGATTTATG	PNR
INDEL32	AAGAAGATGGCATGTAATTGTTCA	CACCTCAAGTAAAGCATAGCAAGA	NP
INDEL33	ATTTCGCAATGACAAATCATAACT	TTCCCATTTTGTTGTCTTTTCATA	NP
INDEL34	GGAGCCTATAAAGGGTGTGTTAGA	GGCTCTGATACCATGTGAAAAATA	PNR
INDEL35	GTTACCGGCTTATCACATTCTCTT	CACCTTCTAGGGTTTTAGGGTTTT	NP

INDEL36	ATCCAAAGTTTTCCTATTCCTTCC	CAGACTAGAGCTCAAACCAGTGAA	NP
INDEL37	CTCTACTCACATGGATGCTAATGG	TTTAGGAAAGTTGCCCTTTGTAAG	NP
INDEL38	GGCTTCGATTAGAATTAGCTCAAC	CGGATGACAATGAAACAATATCTC	NP
INDEL39	ATAACCTTCTTGTGAACCTGATCC	AACTAGGGACTTCTCTCCCCTTTA	NP
INDEL40	TCTGGATAGTGTTTTGAGCTTGAG	AATAACACGACGGTTACAGTCAAA	NA
INDEL41	CCTTTAACATGTCATTAGGCATGA	TTCGAAAACAGTCTCTCTTCCTTT	PNR
INDEL42	TCGTTAACCTATTGGCTCCATAAT	TGGCATTGAATCAGTGTTTATTCT	NP
INDEL43	CATCGAAAAATTTGGTTGTCATAA	CACTCCGTTAATTAAACACGACAC	NP
INDEL44	TGTCCAAGAGTTGTTATTCAAGGA	TCGAACTCTTACATGTCCTCAAAA	NP
INDEL45	ATAGGATGGGTCTGACTATGCTTC	CATTACAACCTGCAAAAATCAAAC	NP
INDEL46	TCACCTTTGAGATGTGTTTCAACT	CCTATTATCTCACAAGCTTCAGCA	PNR
INDEL47	TAGCTACATGAAGGAGAAGCAATG	ATTCGAAGGAGAAAATGATCAAAA	NP
INDEL48	TCTTAGATGCATTTGTTTTTGCAT	ATTGCATTGGGAAACTGATTTATT	NP
INDEL49	AAATGTACGACCACTCTAAGCACA	ATGAAACAGAGATGATGATGATGG	NP
INDEL50	GAGTCTGCAAGTGTAACTGGATTG	CTGTTTTATGAGCTTGTCATCACC	NP
INDEL51	CTTGTCTGATTAACATGCAACTCC	AGTCTCAAGGGTTCAGATCTATGG	NP
INDEL52	TCAACAATGGAATATTAACACCCTAA	GCCTCAATTAATTTGGATTGTGTA	PNR
INDEL53	CTGTCCTCTCTGTGAAGGTACTGA	AATGATGAGAGAACTTGTGACTGC	NA
INDEL54	GACTCTCCAAAATTGTTGTCACAC	ATAATGGGCATGCAAAGTAAAAGT	NA
INDEL55	ACTTAGGATGAAAACCACCAGAAG	ATGCAAGTGAGAAACTTGATTCAT	P
INDEL56	AAAAGAAGCAGCCAAACATATACC	GAAGTTTGGGAAATTGGTTGTTAC	NP
INDEL57	GTACCATCATCAATCACACCATTT	AAATTGATCCTCGGTCATAAGAAA	NA
INDEL58	TTTGCTGTTAGAAAGAACATCGAC	ATTCAACAATCGCAAGTCAAAGTA	NP
INDEL59	TGACCTTTGTTGAATGAGTGAAAT	ATTCACTTGAATTGCACTTTCGTA	NP
INDEL60	CTGCAACAAAATTCATATCACACA	TATTGGGGGTTAAGTTGTATTGCT	NA
INDEL61	CAGGAAAATGATACAACTTTGTGC	TCTATTTCCAAGCTCTACGTTTGA	P
INDEL62	CATTTGGTTGGAAAGGAATAAGAG	CCAATAAGCAAGTACCAAGAAGGT	NP
INDEL63	TTCACTGTTTGTGCTAGCTTTTTC	GGAAGAGAGTGCTGATAGAAGTCC	NP
INDEL64	CAGCCTCTGTAATGTTTCTTCTGA	AGTTAAAAGTGAGATGGGTTACGC	NP
INDEL65	TAGATTATGCTATCTCGGCCTTGT	ACGAACATACAGTTGGGAATCTCT	NP
INDEL66	AAGGTCTGCTAGAAGAAAGGTCAA	CTGCATTGTTTTGAGATCGTTACT	NP
INDEL67	AAGAATGGCAACATCCTCTTAAAC	GACTCAATGAACAACATCATCTCC	NP
INDEL68	TTCTATTTTCTCATGTTCGGTTCA	CAAAAGTCTTTGCACTGATAGAGG	NP
INDEL69	GATTACCCCATGACCTAAGATTTG	ACACATGAATGTATCAGCCTCCTA	NP
INDEL70	TTCTACTCAGTTTGGTGGAGATGA	ATGCGTCTCCAAGTTTAGAGCTAC	NA
INDEL71	ATTTTTCACTTTTCCTTTTTCGTG	GCAGAAAAGATAAATCCATGACCT	NP
INDEL72	TCCCATAACCAAAAATTAGACGAT	GCATATTCAAGATTCGAAAGAGGT	NP

*For polymorphic on agarose gel, “P” as polymorphic between resistant and susceptible progenies, “NP” as no polymorphic between resistant and susceptible progenies, “NA” as no amplification production, “PNR” as polymorphic that not associate with CRS resistance.

Supplementary table 3.4. List of INDEL primers with long insertion and deletions on chromosome 9.

	Forward primer	Reverse primer	Polymorphic on agarose gel*
INDEL490-1	TTTTGTCCAATTATTTCTGCTTCA	TTGTGAATGAATTTTGATTTTGCT	P
INDEL490-2	TGGGTCAAAATAAGTAGATGCAAA	AGAATAATCTTGCCCTTTGTCTTG	NP
INDEL490-3	GGGATCCATCTGTTATACTTGGAC	GGCCTATTTCTATGGAACACATTC	NP
INDEL490-4	TGAGTTTCCAGGAACAAACAATTA	CTTCACCTCTGAAACTGAGAAACA	NP
INDEL490-5	TTTTTACCAAAATTAACCGAAGGA	GGCCATTACTATTGCTCAAAGATT	PNR
INDEL490-6	AATGCAAGGATGTAACCAAAATCT	AATTGTGTGATCATTTGGTAATGG	NP
INDEL490-7	ACTTCATAAAATGCGGAAAACAAT	TATTCCCCAAAATCAATGATAACC	P
INDEL490-8	ACAATACAAGACAAAGGGCAAGAT	GATTTGTGGGTTCATGTGATTTTA	PNR
INDEL490-9	TGTATCTCGTGGACATCCTACATT	TTTTCCGCATTTTATGAAGTGTTA	NA
INDEL490-10	AGCAAAATCAAAATTCATTCACAA	TGTAGGATGTCCACGAGATACATT	P
INDEL490-11	AGTGACTGAAAATGAATCCATCAA	AATGTAGGATGTCCACGAGATACA	P
INDEL490-12	CAAGGATGTAACCAAAATCTAGGC	GGCCATTACTATTGCTCAAAGATT	NA
INDEL490-13	ATGAGCAAAATCAAAATTCATTCA	AATGTAGGATGTCCACGAGATACA	P
INDEL490-14	GGTTAGGAGATTTAGAGGGAAACC	AGATTTTGGTTACATCCTTGCATT	P
INDEL490-15	ACTCATCCACCGTGTATAGGATCT	TTTGTGAATGAATTTTGATTTTGC	P
INDEL490-16	ACTAGATGCAATTTATGCAGAGCA	GAATAATCTTGCCCTTTGTCTTGT	NA
INDEL490-17	TTAGGAATGGTTCTCTGGTTTGTT	TACTGTTATCTGTTCCCCACCTTT	NP
INDEL490-18	TAAAATCACATGAACCCACAAATC	TTCTCCACTTTTGAATTGACACAC	NP
INDEL490-19	ATTGGAACTGAAAGAATGGAAAAG	ATTACTCCCTCGTTCCAATTTATG	NP
INDEL490-20	TTCCTCTAGTTGGTGTCTTGATGA	TTTCCACTTTTCCATTCTTTCAGT	NP
INDEL490-21	GAGGGAAACCTACATGCTTTACAC	CCTAGATTTTGGTTACATCCTTGC	P
INDEL490-22	CATCCACGCTATCTCATTATCATC	CCCTAAAGAAAGTTAATCGAAACG	NA
INDEL490-23	TGGCCTTTTGATAGACCTAACAGT	TGTTGGTCCAAGTATAACAGATGG	NP
INDEL490-24	GGAGAATCATCTTCTTCTTCCAAC	GTTCAATTGCTTTCTCAAGTTCAA	NP
INDEL920-1	CATCACTAGAGAATGAGCCGAGTA	TAATTCGATGAGAATGGGGTCTAT	PNR
INDEL920-2	TAGATCCTTTAATCCATTCGAAGC	TGCATGGTTTATGTCTGGAATATC	PNR
INDEL920-3	AACATTGCGATAGATATGGAGACA	GTCCTTGCTTTGGTCTGTTAAGAT	PNR
INDEL920-4	ATAGACCCCATTCTCATCGAATTA	TGTCTCCATATCTATCGCAATGTT	PNR
INDEL920-5	TTGCTAAACAGTTGAAGGATCAAA	GCTTCGAATGGATTAAAGGATCTA	P
INDEL920-6	GGCTTAGGCCCAAAATAATAAAAT	TTCTTCATGAACTCCTATCCCATT	NA
INDEL7200-1	TTGTGGTGGAGAAAAACTGTTAAA	CTTGATGATGTGGATCATAGAAGC	NP
INDEL7200-2	AATTTGATATTGACGGGAGTGTTT	TAGGGATATTGGGAGTTTATCCAA	NP
INDEL7200-3	GACTTCTTGAGGGTTGAACAATCT	CGTGTGGTTTTTATTCTGAAAGTG	NP
INDEL7220-1	TGGTAGCCCACCTGAATATTTTAT	TGGTTTGGCTCTGGTAGTGTAATA	NP
INDEL7220-2	GACTTCTTGAGGGTTGAACAATCT	CGTGTGGTTTTTATTCTGAAAGTG	NP
INDEL7220-3	TGGTTGGCAAACAACTAGAGATAA	GAGACTGTGGAAAACGGAGTAGAT	NP

*For polymorphic on agarose gel, “P” as polymorphic between resistant and susceptible progenies, “NP” as no polymorphic between resistant and susceptible progenies, “NA” as no amplification production, “PNR” as polymorphic that not associate with CRS resistance.

Supplementary table 3.5. Table of CRS disease evaluation in 2018, 2019 and 2020 for progeny POR16V001.

	2020		2019	2018		Phenotype
	Avg DSI	RT-PCR Result	Avg DSI	Avg DSI	RT-PCR result	
POR16V1-1	15	1	4.545454545	5	1	susceptible
POR16V1-2	NA	NA	59.82142857	41.80555556	1	susceptible
POR16V1-3	NA	NA	NA	0.453431373	1	susceptible
POR16V1-4	28.64583333	1	19.94047619	10	1	susceptible
POR16V1-5	NA	NA	0	0.625	0	resistant
POR16V1-7	58.03571429	1	68.75	5.178571429	1	susceptible
POR16V1-8	13.19444444	1	0	5	1	susceptible
POR16V1-9	0	0	0	0	0	resistant
POR16V1-11	25	1	0	20.73529412	1	susceptible
POR16V1-12	42.85714286	1	24.30555556	15.71412506	1	susceptible
POR16V1-14	18.84137427	1	7.575757576	1.041666667	1	susceptible
POR16V1-15	0	0	0	NA	NA	resistant
POR16V1-17	0	0	8.333333333	0.462962963	0	resistant
POR16V1-18	60.3219697	1	4.166666667	1.215277778	1	susceptible
POR16V1-19	38.75	1	9.722222222	10.18518519	1	susceptible
POR16V1-20	NA	NA	NA	12.08333333	1	susceptible
POR16V1-21	5.384615385	1	10.95238095	20.65527066	1	susceptible
POR16V1-22	43.95833333	1	10.26785714	7.824074074	1	susceptible
POR16V1-23	43.99305556	1	23.66071429	10.97222222	1	susceptible
POR16V1-24	19.09722222	1	0	0	1	susceptible
POR16V1-25	NA	NA	NA	0	0	resistant
POR16V1-26	63.88888889	1	NA	5.147058824	0	susceptible
POR16V1-27	15.625	1	7.986111111	15.41666667	1	susceptible
POR16V1-28	0	0	0	3.621794872	0	resistant
POR16V1-29	6.25	1	NA	11.93885449	1	susceptible
POR16V1-30	0	0	0	0	0	resistant
POR16V1-32	NA	NA	20	0	0	resistant
POR16V1-33	0	0	0	6.578947368	1	resistant
POR16V1-34	43.75	1	NA	9.558823529	1	susceptible
POR16V1-35	0	0	0	NA	NA	resistant
POR16V1-36	NA	NA	NA	0	1	resistant
POR16V1-37	NA	NA	NA	2.708333333	0	resistant
POR16V1-38	35.69444444	1	20	17.01388889	1	susceptible
POR16V1-39	0	0	0	0	0	resistant
POR16V1-40	0	0	0	1.388888889	0	resistant
POR16V1-41	NA	NA	NA	0.297619048	0	resistant
POR16V1-42	0	0	NA	2.34375	0	resistant

POR16V1-43	0	0	NA	1.041666667	0	resistant
POR16V1-44	12.5	1	NA	0.555555556	1	susceptible
POR16V1-45	0	0	0	34.375	0	resistant
POR16V1-46	NA	NA	NA	0	0	resistant
POR16V1-47	0	0	0	0.625	1	resistant
POR16V1-48	0	0	0	0	0	resistant
POR16V1-49	NA	NA	NA	21.04166667	1	susceptible
POR16V1-50	45.53571429	1	3.125	7.916666667	1	susceptible
POR16V1-51	4.464285714	1	0	6.359649123	1	susceptible
POR16V1-52	NA	NA	NA	17.70833333	1	susceptible
POR16V1-54	NA	NA	NA	0	0	resistant
POR16V1-55	26.94444444	1	3.125	40.27777778	1	susceptible
POR16V1-56	NA	NA	NA	9.375	1	susceptible
POR16V1-57	53.125	1	6.818181818	0	1	susceptible
POR16V1-58	36.50793651	1	0	3.333333333	0	susceptible
POR16V1-59	26.5625	1	0	5.902777778	1	susceptible
POR16V1-60	NA	NA	NA	19.89583333	1	susceptible
POR16V1-62	0	0	NA	1.875	0	resistant
POR16V1-63	39.60784314	1	26.83150183	9.010416667	1	susceptible
POR16V1-64	NA	NA	NA	4.642857143	1	unknown
POR16V1-66	NA	NA	NA	0	0	resistant
POR16V1-67	NA	NA	0	25.69444444	0	resistant
POR16V1-68	0	0	2.5	0	0	resistant
POR16V1-69	0	0	NA	4.166666667	0	resistant
POR16V1-70	42.24431818	1	0	13.75	1	susceptible
POR16V1-71	9.868421053	1	0	2.083333333	0	susceptible
POR16V1-72	0	0	7.5	0.277777778	0	resistant
POR16V1-73	18.53693182	1	NA	0	1	susceptible
POR16V1-74	NA	NA	NA	13.33333333	1	susceptible
POR16V1-75	NA	NA	NA	6.875	1	susceptible
POR16V1-76	35.17857143	1	3.571428571	5.625	1	susceptible
POR16V1-77	NA	NA	NA	0	0	resistant
POR16V1-78	0	0	0	0	0	resistant
POR16V1-79	NA	NA	NA	17.1875	1	susceptible
POR16V1-80	NA	NA	0	0	0	resistant
POR16V1-81	26.35416667	1	0	3.538441692	1	susceptible
POR16V1-82	28.98755656	1	NA	15.85648148	1	susceptible
POR16V1-83	NA	NA	NA	13.54166667	1	susceptible
POR16V1-84	0	0	0	0	0	resistant
POR16V1-85	37.5	1	25	6.944444444	1	susceptible
POR16V1-86	8.958333333	1	0	17.8125	1	susceptible
POR16V1-87	18.75	1	2.573529412	0.625	1	susceptible
POR16V1-88	9.375	1	0	0	0	susceptible
POR16V1-89	52.70833333	1	26.11111111	8.75	1	susceptible

POR16V1-90	0	0	NA	0	0	resistant
POR16V1-91	0.3125	0	6.730769231	0	0	resistant
POR16V1-92	29.375	1	NA	0	0	susceptible
POR16V1-93	0	0	12.5	0	0	resistant
POR16V1-94	NA	NA	NA	12.5	1	susceptible
POR16V1-95	7.5	1	0	0	0	susceptible
POR16V1-98	0	1	0	0	0	resistant
POR16V1-99	NA	0	NA	17.01388889	1	susceptible
POR16V1-100	0	0	0	0.833333333	0	resistant
POR16V1-101	25	0	NA	1.666666667	1	susceptible
POR16V1-102	NA	NA	NA	60.625	1	susceptible
POR16V1-103	0	0	NA	3.539230019	0	resistant
POR16V1-104	61.9047619	1	0	3.75	1	susceptible
POR16V1-105	60.97222222	1	30.35714286	12.96052632	1	susceptible
POR16V1-106	0	0	0	0.833333333	0	resistant
POR16V1-107	0	0	0	1.960784314	0	resistant
POR16V1-108	0	0	0	0	0	resistant
POR16V1-109	0	1	0	0	0	resistant
POR16V1-110	NA	NA	NA	0	0	resistant
POR16V1-112	NA	NA	NA	9.523809524	1	susceptible
POR16V1-115	NA	NA	NA	0	0	resistant
POR16V1-117	0	0	NA	0	0	resistant
POR16V1-118	0	0	NA	0.595238095	0	resistant
POR16V1-119	0	0	0	3.28125	0	resistant
POR16V1-120	NA	NA	NA	10	1	susceptible
POR16V1-121	0	0	NA	8.553921569	0	resistant
POR16V1-125	NA	NA	NA	4.513888889	1	susceptible
POR16V1-127	NA	NA	NA	12.84722222	1	susceptible
POR16V1-128	60.71428571	1	NA	8.078703704	1	susceptible
POR16V1-129	0	0	0	0	0	resistant
POR16V1-130	34.23295455	1	32.8125	6.875	1	susceptible
POR16V1-131	NA	NA	14.28571429	9.259259259	1	susceptible
POR16V1-132	6.25	1	11.45833333	13.28431373	1	susceptible
POR16V1-133	NA	NA	NA	41.875	1	susceptible
POR16V1-134	NA	NA	NA	0	0	resistant
POR16V1-136	0	0	NA	0.641025641	0	resistant
POR16V1-137	NA	NA	47.91666667	19.07679739	1	susceptible
POR16V1-138	NA	NA	NA	16.45833333	1	susceptible
POR16V1-139	0	0	0	0	0	resistant
POR16V1-140	NA	NA	NA	10.68181818	1	susceptible
POR16V1-142	0	0	NA	0	0	resistant
POR16V1-143	0	1	NA	0	0	resistant
POR16V1-144	27.63157895	1	NA	25.20833333	1	susceptible
POR16V1-145	0	0	NA	0	0	resistant

POR16V1-146	NA	NA	NA	0.416666667	0	resistant
POR16V1-148	0	1	8.333333333	13.7377451	1	susceptible
POR16V1-149	0	0	0	NA	NA	resistant
POR16V1-150	14.0625	1	NA	2.5	1	susceptible
POR16V1-151	0	0	NA	0	0	resistant
POR16V1-152	0	0	0	0	0	resistant
POR16V1-153	0	1	0	0.416666667	0	resistant
POR16V1-154	NA	NA	NA	10.47697368	1	susceptible
POR16V1-155	0	0	0	0.657894737	0	resistant
POR16V1-156	24.26470588	1	NA	0.694444444	1	susceptible
POR16V1-157	0	0	0	0	0	resistant
POR16V1-159	8.333333333	1	0	1.547619048	0	susceptible
POR16V1-160	26.57563025	1	NA	1.666666667	1	susceptible
POR16V1-161	22.5	1	NA	4.375	1	susceptible
POR16V1-162	NA	NA	NA	0	0	resistant
POR16V1-163	NA	NA	NA	0	0	resistant
POR16V1-164	21.42857143	1	0	2.1875	1	susceptible
POR16V1-165	NA	NA	NA	1.5625	0	resistant
POR16V1-167	NA	NA	NA	0	0	resistant
POR16V1-168	7.8125	1	41.66666667	40.625	1	susceptible
POR16V1-169	NA	NA	3.571428571	2.631578947	1	susceptible
POR16V1-170	0	0	NA	4.166666667	0	resistant
POR16V1-171	54.21875	1	NA	8.767361111	1	susceptible
POR16V1-172	NA	NA	NA	8.125	1	susceptible
POR16V1-173	0	0	NA	0	0	resistant
POR16V1-174	18.42532468	1	17.36111111	14.45868946	1	susceptible
POR16V1-175	41.34615385	1	19.44444444	17.11538462	1	susceptible
POR16V1-176	54.79910714	1	NA	3.703703704	0	susceptible
POR16V1-178	NA	NA	NA	18.19444444	1	susceptible
POR16V1-179	8.333333333	1	32.5	43.05555556	1	susceptible
POR16V1-180	NA	NA	NA	0	0	resistant
POR16V1-182	36.71875	1	33.33333333	4.861111111	1	susceptible
POR16V1-183	7.03125	1	1.388888889	6.219362745	1	susceptible
POR16V1-184	31.81818182	1	NA	20.52083333	1	susceptible
POR16V1-185	NA	NA	NA	18.51190476	1	susceptible
POR16V1-187	12.5	1	0	0	1	susceptible
POR16V1-188	20	1	2.777777778	6.805555556	1	susceptible
POR16V1-190	34.79166667	1	0	13.65740741	1	susceptible
POR16V1-191	22.72727273	1	12.5	21.2962963	1	susceptible
POR16V1-192	NA	NA	NA	13.42592593	1	susceptible
POR16V1-193	0	0	0	0	0	resistant
POR16V1-194	NA	NA	NA	3.333333333	1	resistant
POR16V1-195	46.875	1	NA	12.92293233	1	susceptible
POR16V1-196	NA	NA	NA	1.041666667	1	susceptible

POR16V1-198	0	0	5	0.277777778	0	resistant