QTL FOR *MELOIDOGYNE INCOGNITA* ROOT KNOT NEMATODE RESISTANCE AND OTHER TRAITS OF INTEREST IN CARROT (*DAUCUS CAROTA*)

By

Joshua D. Parsons

A dissertation submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

(Plant Breeding and Plant Genetics)

At the

UNIVERSITY OF WISCONSIN-MADISON

2014

Date of final oral examination: 04/30/2014

The dissertation is approved by the following members of the Final Oral Committee:

Philipp Simon, Professor, Horticulture
Irwin Goldman, Professor, Horticulture
Michael Havey, Professor, Horticulture
Brian Yandell, Professor, Statistics
Juan Zalapa, Professor, Horticulture

QTL FOR *MELOIDOGYNE INCOGNITA* ROOT KNOT NEMATODE RESISTANCE AND OTHER TRAITS OF INTEREST IN CARROT (*DAUCUS CAROTA*)

Joshua D. Parsons

Under the supervision of Professor Philipp W. Simon

At the University of Wisconsin-Madison

Abstract

Root-knot nematodes (RKN, *Meloidogyne* spp.) are major pests attacking carrots (*Daucus carota*) worldwide, causing galling and forking of the storage roots, rendering them unacceptable for market. Genetic resistance could significantly reduce the need for broad-spectrum soil fumigants in carrot production. In this study genetic resistance to *M. incognita* was mapped. Three diverse sources of resistance, from Syria (HM), Europe (SFF) and South America (Br1091) were identified. Two F_2 mapping populations were developed using these parents, (Br1091xHM1) and (SFFxHM2), as well as a segregating population derived from the self-pollination of a HM plant (HM3). Analysis revealed four QTLs conditioning resistance in Br1091xHM1, three in SFFxHM2, and three in HM3. A consensus genetic map of the three populations revealed five non-overlapping QTLs for *M. incognita* resistance, one each on carrot chromosomes 1, 2, 4, 8, and 9. One QTL was present in all 3 populations, in the same region of chromosome 8 as *Mj-1* which imparts resistance to *M. javanica*.

Along with *M. incognita* RKN resistance, the Br1091xHM1 and SFFxHM2 populations were segregating for several other traits of interest. Carrot root and shoulder color have important implications for nutritional value and marketable yield. Segregation patterns for orange versus yellow exterior root color revealed one QTL in both populations, on chromosome 3, along with two additional QTL detected in the SFFxHM2 population. Segregation for purple shoulder color revealed one QTL also on chromosome 3 in both populations with two additional QTL identified in SFFxHM2. An additional QTL for green shoulder color in Br1091xHM1 co-localized with the purple shoulder color QTL. Annual flowering and vernalization are important traits that have implications for root quality since, as flowering progresses, the root quickly lignifies. Evaluating early flowering in the field, early flowering in the greenhouse, and annual growth habit allowed mapping additional QTL in the Br1091xHM1 population that behave in a manner similar to days to flowering QTL identified in other crops. These additional studies lay the foundation for future research to validate and refine the QTL.

Acknowledgements

I would like to thank Dr. Philipp Simon for giving me the opportunity to work in his lab. When I applied for the program I knew nothing about carrots or nematode resistance, but really liked talking with Dr. Simon and I appreciate the wealth of knowledge I have learned under his guidance.

I would like to thank Monsanto for providing funding and professional development opportunities through their Monsanto Fellows in Plant Breeding Program. Being able to see the business side of plant breeding was very useful and a great augment to my academic education.

I would also like to thank the entire Simon lab for their help, guidance, and friendship. Mr. Robert Kane provided practical knowledge about carrot breeding and field management and many conversations while working. Dr. Doug Senalik provided technical support and lab guidance. Fellow graduate students and post-doctoral researchers made coming to work fun and provided a wealth of information on a wide variety of topics. Thanks to Pimchanok Satapoomin, Megan Bowman, Sarah Turner, Pamela Fife, Shelby Ellison, Tom Horejsi, and Massimo Iorizzo. Massimo in particular helped me in many projects and guided me through lab protocols and scientific thinking. Visiting scientists were too numerous to mention, but provided diversity to the lab and were part of a great, multi-cultural environment.

Additional thanks to Philip Roberts and William Matthews from the Department of Nematology at the University of California, Riverside, for their help evaluating the plant materials for nematode resistance and input in thinking about nematode resistance in plants. They performed all of the nematode resistance screening used in this work and for that I am thankful.

I would also like to thank Dr. Irwin Goldman, Dr. Michael Havey, Dr. Brian Yandell, and Dr. Juan Zalapa for serving on my preliminary exam and doctoral committee.

I would finally like to thank my wife, Megan Parsons, who has provided endless support, encouragement, and prodding to be more organized and procrastinate less. She makes me a better researcher and person and I thank her for that.

TABLE OF CONTENTS

List of Figures

Figure 2.7 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 8 in the (**A**) Br1091xHM1 F2, (**B**) SFFxHM2, and (**C**) HM3 populations. Significant QTL were identified in all three populations. The horizontal lines represent the population mean and the bars are ± 1 standard error.

Figure 2.8 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 9 in the (**A**) Br1091xHM1 F2, (**B**) SFFxHM2, and (**C**) HM3 populations. The QTL in Br1091xHM1 and HM3 are significant and HM3 displays over-dominance for resistance. The horizontal lines represent the population mean and the bars are ± 1 standard error.

Figure 2.9 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 4 in the (**A**) Br1091xHM1 F2, (**B**) SFFxHM2, and (**C**) HM3 populations. A significant QTL was only detected in the SFFxHM2 population. The horizontal lines represent the population mean and the bars are ± 1 standard error.

Figure 2.10 Parent-offspring regressions for the F_2:F_3 generations in the (**A**) Br1091xHM1 and (**B**) SFFxHM2 mapping populations. The regression line represents broad sense heritability, H^2.

Figure 3.1 A Br1091xHM1 F_3 family that displays the range of root and shoulder color phenotypes. Using the 0-3 phenotyping scale for Br1091xHM1, roots 2, 3, and 4 (left to right) are orange (3), roots 8 and 11 are pale orange (2), roots 5 and 9 are very pale orange (1), and roots 1, 6, 7, and 10 are yellow (0, no visible orange pigment). Shoulder color is not readily

support intervals and the numbers in parenthesis indicate the largest LOD score followed by the percent phenotypic variation explained by that QTL.

Figure 4.5 Phenotypic scores for root cracking as a binary trait (0 or 1 with 0 being no roots with cracks in the F_3 family and 1 being at least one root with a crack in the F_3 family) of AA, AB, and BB individuals at *RC-BrHM1-C5-Q1*. The bars represent the means of each genotypic class.

Figure 4.6 Phenotypic scores for annual growth habit as a binary trait (0 or 1 with 0 being no roots with annual habit in the F_3 family and 1 being at least one root with annual habit in the F_3 family) of AA, AB, and BB individuals at *AH-BrHM1-C5-Q1*. The bars represent the means of each genotypic class.

Figure 4.7 Phenotypic scores for early flowering in the field as a percentage of roots in the F_3 family that flowered in the field without requiring vernalization of AA, AB, and BB individuals at the three significant QTL on chromosomes 1, 4, and 2 (in order of % variation explained). The bars represent the means of each genotypic class.

Figure 4.8 Phenotypic scores for early flowering in the greenhouse as a percentage of roots in the F_3 family that flowered in the greenhouse without requiring vernalization of AA, AB, and BB individuals at the two significant QTL on chromosomes 7 and 5 (in order of % variation explained). The bars represent the means of each genotypic class.

Figure 4.9 Germplasm related to Br1091 segregating for root cracking. From left to right, the first root is not cracked while the second and third roots have extreme root cracking. In some Br1091xHM1 F_4 families, all plants are completely split open as in the third carrot above.

Figure 4.10 Plot of the Br1091xHM1 F_2 population with the percent of plants with annual growth habit in each F_3 family plotted against the percent of plants displaying root cracking in each F_3 family. The black circles represent Br1091 alleles, the red circles HM1 alleles, and the green circles are heterozygous.

List of Tables

Chapter 1

General introduction: root-knot nematodes and plant disease resistance

Root-knot nematodes (*Meloidogyne* spp.)

Root-knot nematodes (RKN, *Meloidogyne* spp.) are a polyphagous group of obligate plant parasites of major economic importance. RKN are soil borne pathogens that have wide geographic dispersal and cause damage to many crops, limiting biomass production through diversion of nutrients and energy normally used for plant growth to the developing nematode, or through direct loss of marketable yield in crops where the underground portion is consumed. While soil borne pathogens have usually been confined to limited areas, in modern agriculture with large monoculture fields and machinery that travels from farm to farm, RKN are spreading and becoming an increasingly important plant pathogen.

RKN taxonomy

RKN are divided into temperate and tropical groups, and traditionally four "major" species were thought to be the most widespread and damaging (Taylor et al. 1982). The "major" species are the tropical RKNs *M. incognita*, *M. javanica*, and *M. arenarea* and the temperate RKN *M. hapla*. This view of "major" and "minor" nematode species is being challenged as more research is being done in diverse geographic locations and as better differentiation of the RKN species through molecular analysis becomes routine (Elling 2013). Historically, visible differences between the RKN species were used for taxonomic classification and given the diversity of *Meloidogyne* spp. correctly classifying the nematodes visually can be difficult. With the use of molecular characterization, several RKN species have been separated from what was once thought to be one species, and some species were reclassified as the same species after extensive molecular characterization (Hunt and Handoo 2010). Elling (2013) hypothesized that these difficulties in relying on visual taxonomy to identify nematode species by Taylor et al. (1982)

may have lead to overrepresentation of the "major" RKN species when many "minor" species may in fact have been present. Whether they were present in the study by Taylor et al. (1982) or not, there are many more nematodes of significance beyond the four "major" nematode species. Some species of rising importance are the temperate *M. chitwoodi*, *M. fallax*, *M. minor*, and the tropical or sub-tropic *M. enterolobii*, *M. exigua*, and *M. paranaensis* (Elling 2013). There is a lot more diversity than originally thought, and species identification is very important for implementing control measures.

RKN life cycle

The life cycle of RKNs occurs with migratory stages in the soil and sedentary stages in susceptible host root tissue (summarized by Moens et al. 2009). Mature, sedentary females lay egg masses on the surface of or within infected roots. The eggs are protected by a gelatinous mass that protects them from adverse environmental conditions and hardens with age to provide further protection. Within each egg, the first-stage juvenile develops and moults to give rise to the second-stage juvenile (J2) when conditions are ready. The egg senses when conditions are ideal through environmental cues that indicate a susceptible host will be present, including temperature and adequate moisture for migration, and root exudates from the potential host. The J2 hatch from the eggs and migrate to a susceptible root host using the water membrane adhering to soil particles.

When the J2 reaches a susceptible host, it migrates to the end of the root and enters the root just behind the root tip. Traveling through intercellular spaces up the root, a feeding site is initiated and the sedentary stage of the RKN life cycle begins. A feeding site consists of several modified

cells, known as giant cells that feed the nematode at the expense of the plant. These giant cells give the root-knot nematodes their name as giant cells are often visible on the root as galls, giving the appearance the root has a "knot" in it. Nematodes induce changes in the giant cells allowing the cells to undergo mitosis without cell division, accumulating up to 80 nuclei, and also undergo chromosome duplication within each nucleus, giving rise to giant cells with up to 600 fold increase in DNA copy number and increase in size up to 100 fold. This increase in copy number along with reprogramming of plant metabolism and transport proteins induced by the nematode, allows the plant to feed the nematode as it develops.

After feeding for about 14 days, depending on species and conditions, the J2 moults to the third-stage juvenile (J3), then the fourth-stage juvenile (J4), and finally adulthood. The J3 and J4 do not feed, and are only developmental stages lasting a combined 4-6 days. In RKN species with male and female sexes, sexual differentiation occurs during the sedentary phase with mature male nematodes migrating out of the root, looking for a mate. Female nematodes remain sedentary and lay eggs within or on the root surface, starting the cycle again. Different nematode species can reproduce sexually or asexually with many species able to do both. In species that require sexual fertilization, the migrating mature male must find a mature female for reproduction. *M. incognita* nematodes have both male and female sexes with sexual and asexual reproduction possible.

RKN management

Given the wide host range of RKN, including many weed species, managing RKN is difficult, but chemical applications, cultural management, biological controls, and host plant resistance

can be used together to manage RKN. Some practices may not provide enough RKN control by itself, but when combined with other practices, an effective control of RKN is possible. For all control strategies, it is important to properly identify the RKN species present in the field as most control practices are species specific.

Chemical control methods have relied on broad spectrum soil fumigants that would leave a sterile seedbed after use, but due to environmental concerns and increasing restrictions on use, RKN-specific nematicides are becoming more common (Nyczepir and Thomas 2010). The nematode specific nematicides are generally viewed as more environmentally friendly and do not harm the overall soil biology as much as the broad spectrum fumigants. Many nematicides are species specific and must be applied before each crop. Higher value crops often are protected with broad spectrum pesticides because they are more expensive, but the high value of the crop can justify the costs. Lower value crops, or situations where nematodes are the only problem, are more likely to use RKN specific nematicides (Nyczepir and Thomas 2010).

Cultural methods of control include well-planned crop rotations, cover crops, and sanitation. Crop rotations and cover crops provide little protection against some RKN species due to large host ranges, but for other species they are an effective control measure. Because many weed species are also hosts for RKN species, controlling weeds in rotations is important. Planting non-host crops will reduce populations of residual nematodes in the field for future crops as nematodes hatch and then have nowhere to feed or reproduce (Bélair and Parent 1996). Non-host cover crops that are plowed-down have additional benefits of increasing soil biological activity through the addition of organic matter (Widmer et al. 2002). Soil biological activity

helps keep nematode populations in balance and less of a problem for cash crops. Because each RKN species has a different host range, selecting proper crop rotations or cover crops will be species specific and evaluating what species are present in a field is crucial to success. Sanitation is also an important practice and helps limit the spread of RKN. With crops that are transplanted, making sure planting media is RKN free is important. Because RKN are soil borne pathogens, washing soil off machinery between fields will limit the spread of RKN. Without washing, as machines travel to different fields they may inoculate the next field with RKN from the previous field. With proper species identification and management practices designed to limit the spread of RKN, management is possible.

Biological control methods include bacteria and fungi that prey upon RKN species. While several biological controls have been explored and tested in the laboratory, few products perform effectively under field conditions (summarized by Hallmann et al. 2009). Ideal conditions for the biological control agent are necessary to reduce the population of nematodes effectively and also the biological control agent must come in contact with the nematode to be effective. Given the three dimensional nature of RKN distribution in the soil, this is difficult. Biological controls are a potentially useful option, but more research is needed to effectively use them under field conditions.

Given the difficulty of other control methods, host plant resistance represents an ideal solution to RKN control. Strong plant resistance will limit the effect RKN have on the yield of the crop. Plant resistance genes also protect the next crop by decreasing the RKN population in the field, limiting the population of RKN available to infect the next crop. With plants that are grafted,

particularly perennial trees, using RKN resistant rootstock allows growing any scion as resistant. Host plant resistance is available in many crops, or crop wild relatives, and represents a resource for breeders to integrate into commercial cultivars. While plant resistance is an ideal solution, many resistance genes are species specific, and identification of RKN species in the field is important to use in selecting for resistance. To promote plant resistance durability, other IPM control measures in addition to plant disease resistance will help limit the possibility of RKN gaining virulence to specific resistance genes. Maintaining weed free fields in resistant crops is also important as the RKN could reproduce on weeds. While the resistant crop will still be protected, there will be less benefit to the next crop if RKN are allowed to reproduce on the weeds.

Plant disease resistance

Disease resistance in plants can take several forms but can broadly be divided into resistance that activate the plant immune response and genes that hinder the pathogen in other ways. Many of the genes that activate the plant immune response are single, dominant genes that follow the nucleotide binding site – leucing rich repeat motif (NBS-LRR). Many of the other genes work in a quantitative matter with resistance to some pathogens conditioned by several genes, each with a small benefit to the plant, or hindrance to the pathogen. Plant breeders have used both types of resistance effectively and have studied the genetic mechanisms behind resistance and the implications of each for long-term durability of resistance.

Nucleotide binding site – leucine rich repeat major resistance genes

NBS-LRR genes function by recognizing a pathogen and activating the plant's immune response, typically leading to rapid cell death, known as the hypersensitive response (HR). Plants rely on a signaling cascade from recognizing the presence of a pathogen to production of reactive oxygen molecules and other destructive compounds that kill the cells surrounding the pathogen and also can hinder the pathogen itself (Jones and Dangl 2006). If the pathogen survives the destructive compounds but the host cell (the pathogen relied on to grow and develop) dies, then the pathogen can no longer be sustained by the plant and the plant is protected.

To recognize a pathogen, plants rely on detecting proteins on the pathogen itself (eg. flagellin or chitin) or pathogen effectors (Jones and Dangl 2006). When pathogens attack plants, they emit virulence effectors to help them infect the plant. Effectors can down-regulate the plant immune response and promote structural or gene expression changes within the cell to make it a more suitable host for the pathogen. RKN induce changes in the cell to lead to the formation of giant cells that sustain the sedentary stages of RKN development. Plant resistance genes can be embedded in the cell wall or occupy the cytoplasm, and recognize pathogens or effectors outside or inside the cell, respectively. After the plant detects a pathogen, the resistance gene initiates a signal cascade that triggers the plant immune system leading to the HR response (Bernoux et al. 2011). The *Mi* gene product in tomato is most likely localized in the cytoplasm of the cell and the plant only initiates the HR response when the nematode stylet punctures the cell wall (Williamson and Hussey 1996). RKN are able to enter the plant and migrate through the intercellular spaces without initiating the HR response. This observation and the sequence of the *Mi* gene indicate the site of action is likely the cytoplasm and not the cell wall.

NBS-LRR resistance genes are typically effective as long as the protein or effector from the pathogen remains the same (Dangl et al. 2013). If the protein detected from the pathogen can change and still be functional or be lost and the pathogen still effective, then the resistance gene will lose effectiveness and the pathogen has gained "virulence" to the resistance gene. Because of the specific protein target, most resistance genes also have a very limited range of effectiveness, and many species of pathogens are sub-divided into races based on what plant resistance genes are effective against them. This is known as gene-for-gene resistance with one gene in the plant identifying the protein product of one gene in a pathogen. *Mi* in tomato is effective against *M. incognita* RKN, but there are some races that have developed virulence and *Mi* is no longer effective against them (Riggs and Winstead 1959). There are also exceptions to the specific nature of NBS-LRR genes as *Mi* in tomato confers resistance to several RKN species as well as potato aphids (*Macrosiphum euphorbiae*) and whiteflies (*Bemisia tabaci*) (summarized by Williamson 1998). NBS-LRR resistance genes are useful tools for breeders because they are effective and there is only one locus to introgress into commercial cultivars. But there are instances where pathogens have adapted to major resistance genes very quickly because of the intense selection pressure discussed below.

Quantitative resistance

Quantitative resistance is not as well characterized as major resistance genes in terms of gene action, but can be just as effective. Quantitative resistance relies upon several genes to impart resistance, each gene contributing to the overall resistance. Each gene for quantitative resistance may have a different mode of action and can influence functions like cell wall fortification,

membrane transporters, general plant health, trichome or wax on leaves, and symbiosis with beneficial fungi that out-compete pathogenic species. Many quantitative resistance genes are difficult to characterize because of the small effect of the individual genes, but work discerning the mode of action and genes underlying quantitative resistance is underway in several crops (see RKN resistance in cotton and RKN resistance in soybean, this work). In plants with strong quantitative resistance, several genes with different modes of action work together to hinder the pathogen attack and reproduction.

While quantitative resistance is more difficult to introgress into commercial cultivars due to the quantitative nature of the trait, there are benefits to quantitative resistance. Because quantitative resistance often does not rely on the host plant detecting specific proteins from a specific race of pathogen, most quantitative resistance is effective against the entire species, or even multiple species of pathogens (Parlevliet 1989). Quantitative resistance is typically more difficult for pathogens to gain virulence to, compared to NBS-LRR resistance genes, due to the diverse modes of action and the less intense selection of each individual gene as discussed below.

Virulence and durability

When pathogens overcome a resistance gene, they have gained virulence to it. The duration and durability of plant resistance to pathogens varies largely on the pathogen and how effective the resistance is (McDonald and Linde 2002). Pathogens that have high gene flow and wide geographical dispersal each season, or that utilize a mixture of asexual and sexual reproduction are more likely to overcome resistance genes than pathogens that are limited in geographical distribution with limited gene flow. Pathogens that can sexually reproduce have a greater chance

of recombining virulence genes to overcome host plant resistance genes, and pathogens with wide geographic dispersal can draw from a larger gene pool, making breeding for durable resistance more difficult. With completely sexually reproduced pathogens, two pathogens must overcome resistance in close proximity to each other and intermate to produce virulent offspring.

Pathogens that have mixed asexual and sexual reproduction are viewed as the greatest threat to plant resistance because there is the opportunity for gene flow through sexual reproduction and only one resistance breaking event is needed to give rise to virulent pathogens. Pathogens that have a wide geographical distribution every season (through insect vectors or wind) have a chance to recombine genes with a larger gene pool and are viewed as more likely to break resistance than localized pathogens with limited movement (like soil-bound RKN). When resistance is broken, asexual reproduction can reproduce alone while sexually reproducing pathogens would require two resistance breaking events in close proximity. Generation time also influences pathogen virulence. Polycyclic pathogens are more likely to give rise to virulence than monocyclic pathogens because there are more recombination events and more pathogens available among which to develop virulence.

The effectiveness of the resistance gene also affects the durability of resistance. Major resistance genes are effective, but also exert a huge selection pressure on the pathogen populations, so any pathogen that does survive to reproduce will reproduce virulent progeny. Since only virulent pathogens survive the plant resistance gene, the only pathogens surviving are virulent to the resistance gene and can reproduce. With quantitative resistance, the selection pressure of each gene is not as high and the mode of action of each gene contributing to resistance is unique. This

diversity makes it more difficult for pathogens to overcome all the resistance genes, and when a pathogen does overcome resistance to one gene, because of the quantitative nature, many susceptible alleles are still present in the pathogen population (Parlevliet 1989). The chance of a pathogen developing virulence to each quantitative resistance gene at once is very slim. This keeps the resistance alleles in low frequency because the selection intensity of each gene is relatively low. Quantitative resistance is consequently seen as more durable than major gene resistance, but both modes of resistance can both be managed along with other practices to provide effective, long-term resistance.

M. incognita RKN can reproduce sexually or asexually, so if one female is virulent, it can produce offspring that will be virulent and the possibility of sexual reproduction allows greater gene flow when it is used. Also, several generations are possible in a given year, so the polycyclic nature makes it more likely virulence will develop. Since *M. incognita* is a soil borne pathogen, gene flow is very low and if resistance develops, it should be isolated. To breed for resistance in *M. incognita*, quantitative resistance or major resistance genes deployed in rotation are predicted to be most effective (McDonald and Linde 2002). If resistance develops in a field, other control measures, or other resistance genes, can be deployed to control the virulent RKN population.

RKN resistance in carrot

RKN cause yield loss in many crops, but in carrot, the yield loss is not due to harvest weight yield reduction, but through galling and forking of the carrot root, rendering an infected carrot unmarketable (Roberts 1987). Root galling at sufficient levels to cause marketable yield loss can

occur at very low nematode densities, making control through resistant varieties desirable (Vrain 1982; Huang and Charchar 1982) but challenging. Research on RKN resistance in carrots has focused on *M. javanica* resistance, with some work describing *M. hapla* resistance.

M. javanica resistance

Early evaluations of germplasm for *M. javanica* resistance revealed resistance in two cultivars from Brazil, 'Kuronan' and 'Brasilia' (Huang et al. 1986). Plants were grown in pots in the greenhouse and inoculated with *M. javanica* eggs to evaluate root galling and nematode egg production. Narrow sense heritability was also calculated using half-sibs from each cultivar. Brasilia strongly suppressed galling and egg production with heritabilities of 0.48 and 0.35, respectively, while Kuronan was slightly less resistant with heritabilities of 0.16 and 0.31, respectively. Kuronan also had larger variation for each phenotype. Kuronan had a mean of 6.53 *M. javanica* egg masses per plant with a standard error of 5.11 while Brasilia had a mean of 0.54 *M. javanica* egg masses per plant and a standard error of 0.49 (Huang et al. 1986). This lower heritability and wide variation may reflect quantitative resistance in Kuronan in contrast to the single major gene later observed in Brasilia. Huang (1986) investigated these two cultivars in more detail and confirmed that Kuronan is less resistant than Brasilia, with evidence that Brasilia suppresses both gall number and egg production while Kuronan primarily suppresses the gall number, but not nematode reproduction. Because the resistance in the Brasilia background was more effective than the resistance in Kuronan, Brasilia became the primary cultivar for future studies.

Further research and characterization in the Brasilia background led to the identification of the dominant resistance locus *Mj-1*. Simon et al. (2000) used 'Brasilia 1252' as the source of resistance in an intercross to a susceptible inbred and evaluated F_2 progeny as well as, F_3 and F_4 families. Of 470 F_2 plants, 294 demonstrated no gall production. When evaluating F_3 and F_4 families, those derived from susceptible plants were uniformly susceptible. Families derived from resistant plants exhibited some dosage effect for resistance such that plants scored as 1 generally produced segregating progeny while plants scored 0 generally produced completely resistant progeny. Considering various inheritance models, it was concluded that resistance was attributable to a single, dominant locus, or possibly two closely linked loci.

To further characterize the genetics of resistance in Brasilia 1252, bulked segregant analysis (BSA) was used to map randomly amplified polymorphic DNA (RAPD) markers linked to the trait (Boiteux et al. 2000). The same F_2 population characterized previously by Simon et al. (2000) was analyzed with RAPD markers using bulked DNA from susceptible and resistant F_2 progeny as well as each parent. Four RAPD primers produced consistent results linked to the resistance locus and were evaluated in the entire F_2 population of 412 plants to map flanking markers 1.9 cM and 0.8 cM from the resistance locus (*Mj-1*). To allow easier screening of germplasm, co-dominant sequence-tagged site (STS) markers were developed from the RAPD markers flanking *Mj-1* (Boiteux et al. 2004). Using the co-dominant nature of the STS markers, the F_2 phenotypes were re-analyzed using the marker genotypes. Root galling was completely dominant and egg production was incompletely dominant with the homozygous resistant plants allowing less egg production than heterozygous plants. The STS markers are easy to evaluate on

a simple agarose gel and could be used for marker assisted selection of the *Mj-1* locus, allowing breeders to select for the presence of nematode resistance while also selecting for quality traits in a nematode free field.

Besides *Mj-1* in the Brasilia background, *M. javanica* nematode resistance has also been characterized in PI 652188 designated 'Ping Ding', an accession from China (Ali et al. 2014). Using Ping Ding as the source of resistance in an intercross to a susceptible line, F_2 and F_3 progeny were evaluated for *M. javanica* nematode resistance. While the most resistant plants had a gall score of 2 compared to a 0 for *Mj-1* resistant plants, the population still segregated as a single, incompletely dominant locus named *Mj-2*, and was further characterized by linkage mapping. BSA was used to identify segregating RAPD, STS, and simple sequence repeat (SSR) markers to map nematode resistance in Ping Ding to chromosome 8. Ali et al. (2014) also mapped the STS markers developed by Boiteux et al. (2004) and found they were also on chromosome 8, but about 41 cM away from *Mj-2*. Future work on combining *Mj-1* and *Mj-2* might lead to more durable, longer lasting resistance to *M. javanica*, but more work is needed identifying markers closer to *Mj-2* to facilitate effective utilization by plant breeders.

M. hapla resistance

While *M. javanica* nematode resistance in carrot has been characterized as controlled by dominant or incompletely dominant loci, attempts to characterize *M. hapla* resistance have not identified major resistance genes. Using two sources of resistance previously characterized by

Wang and Gabelman (1993), Wang and Goldman (1996) used F_1, F_2, and BC_1 populations from intercrosses between both sources of resistance, as well as each source of resistance to a susceptible inbred. All of the F_1 plants were susceptible, indicating recessive genes were responsible for resistance, and the F_2 populations had non-normal distributions indicating few genes were involved. In the resistant x resistant cross, the F_2 population segregated 44:36 and 48:32 for resistant to susceptible in each reciprocal cross. These ratios did not deviate from the expected 9:7 segregation ratio for two independent recessive genes, so that hypothesis can not be ruled out. However, with a small population size it is impossible to distinguish segregation ratios between different models and epistasis is another possible explanation of the observed ratios. Compared to *M. javanica* resistance conditioned by *Mj-1* where homozygous plants often have no gall formation, the resistance to *M. hapla* does not appear as strong as both resistant parents characterized by Wang and Goldman (1996) developed some galls. No mention was made if the most resistant plants from the resistant x resistant F_2 population were more resistant than either parent (Wang and Goldman 1996).

RKN resistance in other crops

Given the broad host range of RKN and the crop losses they cause, it is no surprise that many crops have been screened for resistance. In many cases, dominant resistance genes are identified, but in some cases QTL for resistance, or recessive resistance genes are identified. Some factors distinguishing different resistance genes are mode of inheritance or expression (dominant, recessive, or quantitative), different levels of resistance to different *Meloidogyne* species, and temperature sensitivity of the gene.

RKN resistance in tomato

RKN resistance in vegetables is best characterized in tomato (*Solanum lycopersicum*). The first report of a systematic screening for RKN resistance in tomato used infected roots as inocula for individual pots and cultivated tomato and four wild related species were screened (Bailey 1941). Only *S. peruvianum* exhibited resistance, and due to incompatibility between *S. peruvianum* and *S. lycopersicum*, embryo transfer was necessary to create a resistant, interspecific hybrid to bring resistance to cultivated tomato (Bailey 1941). Three successful interspecific hybrids were recovered, all self-incompatible and not readily crossable to *S. lycopersicum*, so the hybrids were distributed to other researchers as clones (Smith 1944). Backcrossing to *S. lycopersicum* using the hybrid as the pollen donor was eventually achieved and three backcrossed seedlings were generated from the clones (Watts 1947). Two of the three were highly resistant to nematode damage, and only one of those was self-compatible. This self-compatible, resistant plant has been the source of *Mi* for all tomato cultivars even today.

Screening that backcrossed generation with *M. incognita* nematodes, an observed segregation ratio of 322 resistant: 266 susceptible plants led to the hypothesis that inheritance of resistance was conditioned by two dominant factors (Watts 1947). Further characterization confirmed dominant inheritance and also observed that while juvenile nematodes penetrated both resistant and susceptible roots, in resistant roots they did not form feeding sites or galls (Frazier and Dennett 1949). It was later observed that the resistance was effective against *M. javanica*, *M. acrita*, and *M. arenaria*, that resistance broke down above 28° C, and interestingly, it also

conveyed resistance to potato aphids (*Macrosiphum euphorbiae*) (summarized by Williamson 1998), and whiteflies (*Bemisia tabaci*) (Nombela et al. 2003).

By 1974 it was determined that the resistance locus, referred to as *Mi* for resistance to *Meloidogyne incognita* (the original test species), was linked to an allozyme of acid phosphatase, *APS-1* (Rick and Fobes 1974). Using *APS-1* to genotype all sources of RKN resistance in cultivated tomato, *APS-1* was linked to all known sources of resistance except in one pedigree that was probably a recombination event between *APS-1* and *Mi* at an early generation of introgression into tomato (Medina-Filho and Stevens 1980). Along with the presence of the resistant *APS-1* marker, the pedigree of all resistant tomato plants tested traced back to the single BC_1 plant introgressing *Mi* from *S. peruvianum* into cultivated tomato (Medina-Filho and Stevens 1980).

The *Mi* gene was cloned in 1998 after an extensive research effort (summarized by Williamson, 1998). Within the region around *Mi*, there were 2 genes with sequence motifs similar to known plant resistance genes *Mi-1.1* and *Mi-1.2*. By transforming each gene independently into susceptible tomato plants, it was determined that *Mi-1.2* was responsible for *Mi* resistance (Milligan et al. 1998). Once the gene responsible for nematode resistance was known, evaluations were made to determine if the observed resistance to potato aphids (previously named *Meu-1* (Kaloshian 1995)) was also due to *Mi-1.2* which would mean they are the same gene. *Mi-1.2* did confer resistance to both pathogens and is now recognized as *Mi-1* or simply *Mi* (Rossi et al. 1998). It was found *Mi-1* contained a nucleotide binding site and a leucine rich repeat, but lacked a signal sequence domain, indicating the protein is most likely present in the

cytoplasm and not the cell wall (Williamson and Hussey 1996). This hypothesis is consistent with the nematodes being allowed to enter plant roots without causing a plant disease resistance response. The disease response is initiated after nematodes penetrate the cell wall with their stylet to induce a feeding site.

Schaff et al. (2007) characterized the chain of events after nematode infection in *Mi-1*/*Mi-1* nematode resistant tomato plants exposed to either *M. incognita* or to *M. hapla* (for which resistance is not conferred by *Mi-1*). Evaluating transcript expression in an array of 1,547 root-expressed gene probes with RNA collected at 0, 12, 36, and 72 hours post inoculation, as well as 4 weeks post inoculation, they found over half the probes indicated differential gene expression at one time or another in the experiment across all treatments. Further analyzing the probes, they found 217 differentially regulated genes during infection while nematodes established feeding sites and 58 genes differentially regulated in comparing resistant and susceptible cultivars infected with *M. incognita*. After 4 weeks, the transcriptome of resistant plants was similar to those of uninfected plants, presumably because the nematodes had all died. One of the genes differentially regulated was a glycosyltransferase, a protein that has been associated with plant defense response. Expression of the glycosyltransferase was up regulated almost 6 fold in resistant roots than susceptible roots infected with *M. incognita* but not *M. hapla*. To confirm the importance of the glycosyltransferase in the defense response, virus induced gene silencing (VIGS) of the gene was used to modify *Mi-1* expression. Resistant and susceptible plants were agroinfected with a VIGS construct to knock down the expression of the glycosyltransferase gene. Half of the resistant agroinfected plants (4/8) displayed galling similar to susceptible

plants while the other half (4/8) had no galls. This indicates the glycosyltransferase is important to the resistance reaction (Schaff et al. 2007).

Other RKN resistance genes in tomato

Mi-1 resistance breaks down with soil temperatures above 28°C and virulent strains of nematodes that can overcome resistance have been recorded. This led to the search for new genetic sources of resistance. In tomato, nematode populations pathogenic to the *Mi-1* resistance gene were reported as early as 1959 with symptoms as severe as in traditionally susceptible varieties (Riggs and Winstead 1959).

The *Mi-3* resistance gene was identified and characterized in *S. peruvianum* (Yaghoobi et al. 1995). *Mi-3* confers a dominant resistance gene effective against nematode populations pathogenic to *Mi-1* up to 32°C. *Mi-3* has been mapped in *S. peruvanum* to the short arm of chromosome 12 and segregates as a single locus with flanking markers as close as 0.25 cM (Yaghoobi et al. 1995; Yaghoobi et al. 2005). All of this work was done in *S. peruvianum*, with the hopes of using the flanking markers to integrate *Mi-3* into *S. lycopersicon*.

Mi-9 is another RKN resistance gene from *S. peruvianum* that confers heat stability. *Mi-9* maps to the short arm of chromosome 6, near *Mi-1*, and is phenotypically distinct from *Mi-1* in that resistance is not compromised at 32°C and *Mi-9* is not overcome by nematode strains pathogenic to *Mi-1* (Ammiraju et al. 2003). The *Mi-1* region includes a cluster of resistance genes including *Mi-9* and many other homologs of *Mi-1* (Jablonska et al. 2007). By using a VIGS construct designed to repress *Mi-1* expression, *Mi-9* resistance was compromised at 32°C, indicating *Mi-1*

and *Mi-9* have very similar sequence and are homologs. The genes were silenced by the same VIGS construct, so *Mi-1* and *Mi-9* are concluded to be similar. But they are distinct genes or different alleles of the same locus as reflected by the different phenotypes they expressed (Jablonska et al. 2007). No mention of testcrosses or recombination between *Mi-1* and *Mi-9* was noted.

Several other RKN resistance genes have been reported in tomato, with many of them genetically linked. *Mi-2* and *Mi-8* are linked (position not reported), *Mi-6* and *Mi-7* are linked (position not reported), and *Mi-3* and *Mi-5* are both on chromosome 12 (Williamson 1998; Jablonska et al. 2007), as well as *Mi-1* and *Mi-9* previously mentioned on chromosome 6. Clusters of disease resistance genes are quite common in plants (Michelmore and Meyers 1998) as has been observed with RKN resistance genes in tomato. In the rest of this work (and in much of the literature), *Mi-1* is simply referred to as *Mi*.

RKN resistance in pepper

Several RKN resistance genes have also been identified in pepper (*Capsicum annum*), with histological studies to characterize the resistance gene action, and given the close phylogenetic relationship to tomato, comparative genetics to tomato RKN resistance genes. The first systematic screening of pepper identified 4 resistant, 14 moderately resistant, and 135 susceptible accessions from a total of 162 released pepper varieties or breeding strains (Hare 1956). The pepper accessions were grown in soil in a greenhouse and RKN, later confirmed to be *M. incognita*, were introduced to the soil with cowpeas, a susceptible host, cultivated to increase the inoculation level. None of the commercially available varieties were resistant and all of the

resistant peppers were small fruited hot peppers (Hare 1956). Utilizing the resistant peppers previously identified, crosses between susceptible and resistant accessions identified a dominant resistance gene, *N*, that conferred effective resistance to *M. incognita* (Hare 1957).

Since that original screening and identification of *N*, many resistance genes have been identified in pepper and they follow a similar pattern to those in other crops, effective against different strains of RKN. *Me1*, *Me3*, and *Me7* confer broad resistance to *M. incognita*, *M. arenaria*, and *M. javanica* while *Mech1* and *Mech2* confer resistance to *M. chitwoodi* (summarized by Djian-Caporalino et al. 2007). *Me2* confers resistance to *M. hispanica*, *Me5* to *M. javanica*, and *Me4* to *M. arenaria* (Hendy et al. 1985). To differentiate the resistance genes and verify that they were separate genes, pair-wise crosses were made. Independent assortment in segregating progeny indicated two different sources of resistance genes.

Another way researchers differentiated the resistance genes was through histological evaluations of infected roots. While the two resistance genes *Me1* and *Me3* confer resistance to the same major nematode species (*M. arenaria*, *M. incognita*, and *M. javanica*), the disease response under nematode challenge was quite different (Bleve-Zacheo et al. 1998). *Me3* resistance allowed significantly fewer *M. incognita* second-stage juvenile (J2) nematodes to penetrate their roots, and a hypersensitive reaction occurred soon after penetration, resulting in an area of necrotic cells at the nematode feeding site. *Me1* resistance allowed more *M. incognita* J2 nematodes to enter the roots but then had a delayed hypersensitive reaction after the nematode initiated a number of (imperfect) giant cells, resulting in necrotic cells around the putative feeding site. Both genes completely suppressed visual gall formation and egg production, in

spite of the differential response to *M. incognita* observed histologically (Bleve-Zacheo et al. 1998).

A similar study was performed evaluating the *Me7* gene action, another resistance gene effective against *M. arenaria*, *M. incognita*, and *M. javanica* (Pegard et al. 2005). Plants with *Me7* were tested with the three major nematode species to observe differences in the response from 1-5 days and then 6 weeks after inoculation. More *M. incognita* J2 nematodes penetrated the roots of the resistant plants than in attacks by *M. javanica* and *M. arenaria*; however, more *M. incognita* nematodes also penetrated the roots in the susceptible check than either *M. javanica* or *M. arenaria*. After five days, 18 *M. incognita*, seven *M. javanica*, and six *M. arenaria* J2 nematodes penetrated the roots of resistant plants compared to the susceptible check where 255 *M. incognita*, 184 *M. javanica*, and 173 *M. arenaria* J2 nematodes penetrated the roots. While more *M. incognita* nematodes penetrated the roots of the resistant plants compared to *M. javanica* or *M. arenaria*, there was still complete suppression of galling and egg production (Pegard et al. 2005).

These authors also compared *Me7*, *Me1*, *Me3*, and *Me5* gene action to a susceptible control with the same three nematode species and found differing responses with the different genes, helping differentiate the genes and characterize the gene mode of action. While they did not quantify the results, they were able to compare the resistance genes based on the plant response to nematode attack. *Me7* is resistant to all three nematodes with necrosis surrounding the J2 nematodes one day after infection with *M. arenaria* and *M. javanica*, but three days after infection with *M. incognita*. *Me1* roots were necrotic one and two days after infection with *M. arenaria* and *M.*

javanica, but not until five days after *M. incognita* infection. *Me1* also allowed the formation of incomplete giant cells with *M. incognita* before necrosis. *Me3* caused necrosis 5 days after infection with *M. arenaria* but one and two days after with *M. incognita* and *M. javanica*. *Me5* caused necrosis five days after infection with *M. javanica*, but allowed normal gall formation for *M. arenaria* and *M. incognita* (Pegard et al. 2005). These results helped researchers characterize and differentiate the resistance genes *Me1*, *Me3*, *Me5*, and *Me7* based on mode of action, and are representative of similar studies that allow researchers to differentiate resistance genes that are effective against the same pathogens.

Histological studies differentiated the various resistance genes in pepper, but as the genes were mapped, it became clear many of the genes are linked, and that the resistance gene cluster of the pepper genome on chromosome 9 compares to resistance gene clusters in tomato and potato in their homologous regions on chromosome 12. Linkage between pepper RKN resistance genes was first observed between *Me3* and *Me4* in a set of 54 double haploid lines and then mapped using AFLP fragments and found to be 9.9 cM apart (Djian-Caporalino et al. 2001). This resistance gene cluster on pepper chromosome 9 was in the homologous region to tomato chromosome 12 where *Mi-3* and *Mi-5* mapped and potato chromosome 12 where *Gpa2* mapped, effective against the potato cyst nematode *Globodera pallida* (Djian-Caporalino et al. 2001). As further resistance genes were mapped in pepper, many of them localized to this resistance gene cluster spanning 30 cM, including RKN resistance genes *Me1*, *Me3*, *Me4*, *N*, *Mech1*, and *Mech2* as well as *PVY(0)* and *PVY(1,2)* (resistance to *Phytophthora capsici*) and *Bs2* (resistance to *Xanthomonas campestris* pv. *vesicatoria*) (Djian-Caporalino 2007; Fazari et al. 2012). This resistance gene cluster in pepper supports previous findings for resistance genes commonly

occurring in clusters and with the homologous resistance clusters in tomato and potato support the observation that resistance gene clusters are evolutionarily conserved. This research in pepper demonstrates the importance of histological studies to characterize the different resistance genes based on mode of action. Because of the clustering on chromosome 9, it would be difficult to differentiate the genes with linkage mapping alone. The histological studies allowed researchers to differentiate the genes and also evaluate the effectiveness of the gene to different strains and provide a valuable tool for breeders to combine resistance genes with different modes of action for enhanced and more durable resistance.

RKN resistance in potato

Several sources of RKN resistance have been identified for potato (*Solanum tuberosum*) in wild relatives, and efforts to introgress the resistance into cultivated potato have been successful. The genes introgressed are typically single, dominant resistance genes, and at least one gene maps to a resistance gene cluster on chromosome 12 that is conserved in the homologous tomato and pepper genomes. Diverse germplasm that provides resistance to various nematodes include *S. bulbocastanum*, *S. hougasii*, *S. brachistotrichum*, *S. cardiophyllum*, *S. fendleri*, and *S. stoloniferum* (Brown et al. 1989; Brown et al. 1991; Janssen et al. 1996). Potato wild relatives have several different ploidy levels and endosperm balance numbers and while RKN resistance has been identified in several related species, the main focus of research has been on wild relatives that are most easily crossed to potato, but much of the gene characterization has been performed within the species the resistance was identified in (Janssen et al. 1997a). Intraspecific or very close crosses generated more progeny to evaluate segregation ratios.

RKN resistance in *S. fendleri*, and *S. stoloniferum* were characterized through intraspecific crosses, while RKN resistant *S. hougasii* was crossed to the closely related susceptible *S. iopetalum* because no susceptible *S. hougasii* plants were identified (Janssen et al. 1997a). Clones from each population were screened separately for *M. chitwoodi* and *M. fallax* in individual pots for the number of egg masses on the underground plant structures. RKN resistance in *S. fendleri* and *S. hougasii* segregated as single, dominant resistance genes effective against *M. chitwoodi* and *M. fallax*, with all F_1 hybrids being resistant, and all F_2 and BC_1 populations segregating 3:1 or 1:1 as expected for a single, dominant gene. RKN resistance in *S. stoloniferum* segregated quantitatively with differential responses to the two nematodes evaluated. *M. fallax* was almost completely suppressed while *M. chitwoodi* was only moderately suppressed, with different F_2 and backcross populations having different population distributions for *M. chitwoodi* egg masses per plant, supporting the quantitative inheritance model (Janssen et al. 1997a).

Building on the work just described, introgressing resistance to *M. fallax* and *M. chitwoodi* into cultivated, tetraploid potato through backcrossing was initiated with different success rates in the different wild relatives (Janssen et al. 1997b). As expected with potato and related germplasm, crosses made with matching endosperm balance numbers were much more successful than crosses between plants with different endosperm balance numbers. However, when introgressing germplasm, only a few seeds are needed for the initial cross, and some crosses with different endosperm balanced number produced hybrid seed. After the hybrid seed was produced, backcrossing to cultivated potato was generally easier, and resistant BC_1 populations

were developed from all three wild relatives, paving the way for breeders to use the resistant germplasm in the production of commercial cultivars (Janssen et al. 1997b).

Another source of a single, dominant gene effective against *M. incognita*, *M. javanica*, and *M. arenaria* was found in *S. sparsipilum* and named *Mh* (Berthou et al. 2003). *Mh* conditions a hypersensitive response to nematodes it is effective against, leading to necrotic cells where the nematode initiates a feeding site. Eleven RKN isolates were used from four species including *M. incognita*, *M. javanica*, *M. arenaria*, and *M. mayaguensis* (used as a virulent check). Root tips were stained and microscopically evaluated 10 days after infection so the nematode and necrotic cells were visible. Plants that had necrotic cells surrounding J2 nematodes were classified as resistant while plants that did not show necrotic tissue were susceptible. Using F_1 and BC_1 tetraploid progeny, segregation ratios did not deviate from the expected 1:1 ratios and along with resistance, selection was made for agronomic traits as well, providing another source of germplasm for breeders to use to introgress resistance into cultivated potato cultivars (Berthou et al. 2003).

Using the same populations just described with *Mh* introgressed from *S. sparsipilum*, some plants were grown under greenhouse conditions and evaluated at different temperatures. Similar to *Mi* of tomato, there was a loss of resistance at high temperatures (Berthou et al. 2003). There was a gradual response to temperature from 20 to 29°C with most clones susceptible at 29°C. The variation of the clones in response to temperature was used as the final selection criteria after selection during the initial resistance screening at cooler temperatures and selection for agronomic traits and only the most resistant clones at high temperatures were selected. This final

selection step allowed the development of breeding lines resistant to nematodes under most field conditions (Berthou et al. 2003).

Interestingly, when plants with resistance conditioned by *Mh* are grown in a naturally inoculated field, there were some visible egg masses on most potato clones by the end of the season unlike plants grown in the greenhouse with the same inoculum. The authors speculated this could be a response to temperature variation over the course of the growing season, but given the previously described selection criteria for resistance at high temperatures, it could also be "leaky" resistance. While the nematode damage did not affect the crop in any significant way, the present egg masses meant the nematodes were able to reproduce in the field and the overall population of nematodes after cultivating resistant varieties can still increase (Berthou et al. 2003). While this resistance is effective for RKN resistance in the current potato crop, the use of *Mh* must be evaluated on a farm-wide approach to ensure the cultivation of *Mh* potato cultivars do not inadvertently lead to cultivating nematodes for the subsequent crop, especially if the next crop is susceptible.

While many resistance genes have been identified in potato wild relatives, few genes have been mapped. One resistance gene to *M. fallax* has been mapped in *S. sparsipilum* and it maps to the same resistance gene cluster on chromosome 12 as the resistance genes previously discussed in the homologous regions on the pepper and tomato genome (RKN resistance in pepper, this work; Kouassi et al. 2006). Resistance to *M. fallax* was characterized in *S. sparsipilum* as a hypersensitive response in the cells surrounding J2 nematodes at feeding site initiation, the same phenotype as identified by Berthou et al. (2003). A population of 128 F_1 progeny from a cross

between resistant and susceptible accessions of *S. sparsipilum* was mapped with AFLP markers, and the resistance gene *NR* mapped to a single locus on Chromosome 12 of the potato map, near a cluster of resistance genes previously identified in potato. Using a single co-dominant marker, IPM4, *NR* mapped 11.7 cM from the *Gpa2*/*Rx1* 1cluster conferring resistance to potato cyst nematodes and potato virus X. This gene cluster on chromosome 12 in potato is homologous to regions on the tomato and pepper genome where *Mi3*/*Mi5* and several RKN resistance genes map, respectively. Being linked to the marker IPM4, marker assisted selection would be possible, although closer, flanking markers would be ideal (Kouassi et al. 2006). Potato wild relatives represent a useful source of RKN resistance genes, but since relatively few have been mapped, much more research is needed to identify flanking markers and develop molecular tools useful for speeding the introgression of RKN resistance into cultivated potato varieties. Potato breeding is made difficult by the many ploidy levels and endosperm balance numbers present in wild and cultivated potato, but progress has been made and many sources of resistance are available to breeders.

RKN resistance in cotton

The first publication describing the inheritance of *M. incognita* RKN resistance in cotton (*Gossypium hirsutum*) reported quantitative resistance involving at least two genes (Jones et al. 1958) and numerous other studies have reported similar results including reports of recessive resistant genes and transgressive segregation (summarized by Gutierrez et al. 2010). While dominant, major genes have been identified in several crops, for many crops of economic importance major resistance genes for RKN have not been reported, and quantitative resistance is the only source available. Unlike dominant genes previously discussed that trigger the

hypersensitive response in the host plant where J2 nematodes initiate a feeding site, QTL for RKN resistance can affect any stage of nematode interaction with the plant. In cotton, QTLs imparting RKN resistance have been characterized as contributing to either root gall formation or reproductive ability, and transgressive segregation has been reported.

Gutierrez et al. (2010) generated three sets of recombinant inbred lines (RILs) that segregated for two *M. incognita* RKN resistance genes previously identified on chromosomes 11 and 14 (Ynturi et al. 2006). The RILs were evaluated in a greenhouse test for both root gall index rating (the number and severity of root galls on the roots) and egg count (the number of eggs extracted from the infected plant). Genotyping was performed and two QTLs were identified as expected. Interestingly, the QTL on chromosome 11 affected the root galling index, and the QTL on chromosome 14 affected egg production. From the QTL analysis, one SSR marker closely associated with each QTL was identified and used in a marker assisted selection program to accelerate the incorporation of nematode resistant QTLs into cotton cultivars. The research goal was promising, but with only one marker per QTL, putative resistance needs to be validated every generation as recombination between the QTL and the marker can lead to misclassifications of resistance (Jenkins et al. 2012). The resistance genes identified had different modes of action, but both are useful for limiting the effect of RKN on the resistant cotton crop, as well as limiting the growth of the RKN population in the soil, and combining the resistance QTL is more effective than each QTL alone.

Besides the two QTL previously discussed, recessive genes and transgressive segregation for RKN resistance have been characterized in cotton. The recessive resistance gene *rkn1* was

mapped using a combination of intraspecific *G. hirsutum* crosses and interspecific *G. hirsutum* x *G. barbadense* crosses. Simple sequence repeat (SSR) markers were used for bulk segregant analysis (BSA) after exposure to *M. incognita* (Wang et al. 2006a). Screening 384 SSR markers led to the identification of two markers linked to the resistance. Mapping the linked SSRs as well as other SSRs known to be on the chromosome revealed the two previous markers flanked the recessive resistance gene, 2.6 cM and 18.4cM away from *rkn1* (Wang et al. 2006a). This resistance gene was characterized further by Wang et al. (2006b) using *M. incognita* and greenhouse screening on a set of *G. hirsutum* RILs. Evaluating both root galling and egg production, *rkn1*/*rkn1* plants were resistant, while *RKN1*/*rkn1* and *RKN1*/*RKN1* plants were as susceptible as the susceptible parent. The set of RILs that was used segregated in the expected 1:1 ratio for a single gene, but interestingly, 21 of 34 RIL lines were more resistant than the resistant parent, indicating there might be modifier loci from the susceptible parent enhancing the resistance (Wang et al. 2006b).

To explore this enhanced resistance, Wang et al. (2008) characterized and mapped *RKN2*, a resistance gene from the susceptible parent. When evaluating some of the F_1 hybrids as well as individual plants in segregating populations screened with *M. incognita*, some progeny had greater resistance than the resistant parent. *RKN2* was localized to chromosome 11 using a BSA approach and was 9 cM from *rkn1*. Looking at the genoytes and phenotypes of the populations at *RKN2* and *rkn1*, *RKN2* only enhanced the resistance in the presence of *rkn1*. *RKN2* was not effective alone. Interestingly, only one *rkn1* resistant allele was necessary to provide strong resistance combined with *RKN2*, compared to needing *rkn1*/*rkn1* genotypes for strong resistance without *RKN2*. *RKN2* and *rkn1* are linked, approximately 9 cM apart, and together represent

strong resistance that can be used for breeding, with flanking SSR markers allowing marker assisted selection (Wang et al. 2008).

Transgressive QTL for RKN resistance have also been identified in interspecific cotton populations derived from susceptible parents (Wang et al. 2012). Using 138 RILs from a cross between *G. hirsutum* and *G. barbadense*, plants were evaluated with *M. incognita*. Compared to the susceptible parents, 74 and 72 of 138 lines were more resistant than the susceptible parent when phenotyped with a gall rating and egg count, respectively, with some progeny as resistant, or more resistant, than the unrelated resistant check varieties. QTL mapping using SSR markers, revealed four QTL significant for root galling accounting for 8-12.3% of the phenotypic variation each and two QTL significant for egg number accounting for 9.7% and 10.6% of the phenotypic variation. Several QTL of minor significance were also detected. Combining the resistance alleles at each QTL identified conditioned stronger resistance than the susceptible checks, and was much more resistant than the susceptible alleles at each QTL. For the four significant QTL for root galling, the average gall rating of all resistant alleles was 1.5 compared to 6 for all susceptible alleles. The resistant checks had ratings of 2.5-3 and the susceptible parents had ratings of 5-7.5 using the same scale. These results reveal the ability to select for very resistant progeny from two susceptible parents and also identified SSR markers linked to the resistance QTL that could be useful for marker assisted selection (Wang et al. 2012). These QTL along with QTL previously identified in cotton and *rkn1* and *RKN2* provide diverse sources of resistance for breeders to use in breeding programs to develop not only stronger resistance, but also more durable resistance.

RKN resistance in soybean

When major, dominant resistance genes are characterized at the causative genetic level, they often follow the motif of nucleotide-binding-site leucine-rich-repeats (NBS-LRR). NBS-LRR domains trigger the cascade of plant defenses often leading to the hypersensitive response and cell death. In the case of QTLs for disease resistance, the causative genes are difficult to identify. In soybean (*Glycine max*) there have been several QTL identified for *M. incognita* RKN resistance from diverse genetic backgrounds and a QTL on chromosome 10 has recently been mapped and characterized at the genetic level with candidate genes identified in two separate research efforts concluding resistance is not due to NBS-LRR genes. To identify candidate genes under the RKN resistance QTL, a combination of fine mapping and gene expression data were used in two accessions, PI 96354 and PI 438489B.

To characterize the resistance in PI96354, intercrosses between the *M. incognita* resistant PI96354 and 'Bossier' (a susceptible cultivar) were evaluated under greenhouse conditions for nematode resistance at the F_1, F_2, and F_3 generations (Luzzi et al. 1994a). Evaluating the number of galls per plant, the F_2 population and F_3 families had continuous distributions of scores, indicating quantitative control, but the heritability was also high with a parent offspring regression representing broad sense heritability of 0.73. The high heritability indicates there are a few larger effect loci contributing to RKN resistance compared to many, small effect loci. PI 96354 was also intercrossed with 'Forrest', a previously characterized resistant cultivar (Luzzi, 1994b) and the progeny evaluated for *M. incognita* RKN resistance. Some transgressive segregants for susceptibility were identified with as many galls as the susceptible check indicating the resistance between PI 96354 and Forrest differ by at least one locus (Luzzi,

1994a). RKN resistance in crosses between Forrest and Bossier were previously characterized and segregated as a single, additive gene designated *Rmi1* (Luzzi, 1994b). The authors hypothesized *Rmi1* was present in both parents and PI 96354 segregated for at least one additional resistance locus (Luzzi, 1994b).

To further characterize the resistance in PI 96354, RKN QTL were mapped in PI 96354 x Bossier progeny with RFLP markers (Tamulonis et al. 1997). Two QTL were identified using 110 F_2 plants and 121 restriction fragment length polymorphism (RFLP) markers explaining 31% and 14% of the phenotypic variation for gall number. The larger QTL was proposed to be closely linked to the *Rmi1* locus, on what is now known to be chromosome 10 (verified by Li et al. 2001) and the minor QTL is on chromosome 18. With two QTL identified in PI 96354 and only one locus characterized in Forrest, this supported the hypothesis of the previous work that there was at least one locus different between the accessions. The QTL support interval for the QTL explaining the most phenotypic variation spanned 13.1 cM, and given the large support interval and no sequenced genome at the time, identifying candidate genes was not feasible (Tamulonis et al. 1997).

To help refine the QTL support interval and develop markers for marker assisted selection, additional SSR markers were evaluated in the QTL regions of the same population of 110 F_2 plants characterized previously (Li et al. 2001). With higher marker density, more accurate QTL localization and estimates of percent variation explained were obtained. The major QTL on chromosome 10 was refined to a region of 9.1 cM and explained 56% of the variation in gall number and the minor QTL spanned 8.2 cM and explained 18% of the variation. To verify the

QTL model and aid selection in a breeding program, one marker from each QTL region was used in a marker assisted selection program on 96 previously unevaluated F_2 plants from the same cross. Five families homozygous for resistant alleles at both markers representing the QTL and one family homozygous for susceptible alleles were selected for comparison. Of the five families homozygous resistant for the two markers, 4 families were as resistant as PI 96354 (1.1±0.3 galls per plant) and one family was significantly more susceptible (3 galls per plant). The more susceptible family may have a crossover between the marker and the minor QTL, showing the importance of flanking markers in a marker assisted selection program. With the limitation of only one marker per QTL, the overall results are promising for selecting nematode resistance in unevaluated germplasm (Li et al. 2001).

To further refine the QTL interval on chromosome 10 and use the now sequenced soybean genome to identify candidate genes, 188 recombinant inbred lines (RILs) from the cross of PI 96354 and Bossier were screened with *M. incognita* RKN for gall number under greenhouse conditions (Pham et al. 2013). Two markers flanking each QTL previously identified were used to screen the RILs for recombination events between the flanking markers, identifying 17 RILs with recombination events. Those 17 RILs were then screened with five more SSRs in the region to refine the QTL region to a 235 kb region of the genome containing 30 annotated genes. None of the candidate genes fit the classic NBS-LRR motif common for disease resistance genes. These 30 genes were refined to 13 candidates by looking at expression data available from the published soybean genome (Severin et al. 2010). Genes that were expressed in roots or that had altered expression levels in previous studies when infected with nematodes were selected for further analysis. To further refine the candidates, the 13 genes were sequenced in

the parents of the cross and aligned to the 'Williams 82' sequenced genome for variation analysis. Of the 13 genes sequenced, four genes related to cell-wall function emerged as the best candidates for causing the *M. incognita* RKN resistance.

Two of the candidate genes were in the extensin gene family that play pivotal roles in cell wall structure, shape, and self-defense (Hall and Cannon 2002; Lamport et al. 2011). Using qPCR, these two extensin genes were up-regulated in PI 96354 after infection with *M. incognita* RKN compared to Bossier and to the un-inoculated controls. The other two candidate genes were in the pectin esterase family that are involved in the process of cell wall break-down (Cosgrove 1997) and are also up-regulated in the presence of nematodes (Barcala et al. 2010). These two genes were down regulated in PI 96354 under *M. incognita* RKN infection compared to Bossier and the un-inoculated controls. Pham et al. (2013) hypothesize the over-expression of the extension genes allows cell-wall fortification and the down-regulation of pectin esterase genes limit the ability of the nematode to penetrate the cell walls. This combined mode of action is quite different than traditional NBS-LRR disease resistance genes that trigger a hypersensitive response to hinder an invading pathogen as seen in tomato and many other crops and represents one of the few genetic characterizations of genes underlying QTL for resistance genes.

In a different genetic background and a larger mapping population, the resistance QTL on chromosome 10 was further refined. 246 RILs from a cross between Magellan (susceptible) and PI 438489B (resistant) were shotgun sequenced to a depth of 0.2x and the parents were sequenced to more than 10x coverage (Xua et al. 2013). The deeper sequencing of the parents was used to impute the genotypes of the RILs and call SNPs. A genetic bin map was constructed

representing all of the recombination events in the population and QTL mapping detected three QTL accounting for 23.6%, 7.4%, and 5.6% of the variation in gall number. The major QTL was located on chromosome 10 in the same region as the QTL previously discussed on chromosome 10 and the region was refined to 29.7 kb containing five genes when looking at the soybean sequenced genome. Using sequence variation between the parents and gene expression data for the possible genes, two genes were identified as good candidates. Glyma10g02150 and Glyma10g02160 are the genes identified here and are the same two pectin esterase genes identified by Pham et al. (2013), with the same conclusion about the down-regulation of pectin esterase genes hindering the ability of the attacking nematode to penetrate the cell. No comment about the nearby extension genes or other comparison was made to the work by Pham et al. (2013), but this paper was submitted in January, 2013 and Pham et al. (2013) was published in April. These two papers independently identified similar findings and represent characterization of genes underlying QTL for disease resistance and offer alternatives to the traditional NBS-LRR motif. With the genetic mapping used in each population, molecular markers can be easily identified to aid the introgression of resistance into cultivated soybean cultivars.

This work

This research was undertaken to determine inheritance patterns and map chromosomal regions responsible for resistance to *M. incognita* in cultivars from Brazil, the Middle East, and Europe. Segregating populations were evaluated for the response to *M. incognita* and analyzed for resistance QTL. One major goal was to discern the relationship between resistance to *M. incognita* and the previously identified resistance to *M. javanica* conditioned by *Mj-1* and *Mj-2*.

Chapter 2 discusses *M. incognita* RKN resistance in each population and compares the QTL for resistance to a consensus map of all three populations. While evaluating the populations for RKN resistance, other segregating traits of interest were observed and QTL analysis was performed on these traits.

Chapters 3 and 4 present evaluations of inheritance of traits that were not planned at the onset of this work and as a consequence were not consistently scored for the traits reported, nor evaluated adequately to draw firm conclusions about inheritance patterns. But since these traits are of interest to growers, preliminary data are presented. Chapter 3 covers QTL mapping for exterior root color and shoulder color in Br1091xHM1 and SFFxHM2. Root color has important nutritional implications and shoulder color can influence marketable yield. Chapter 4 covers QTL mapping for early flowering and root cracking in Br1091xHM1. Both of these traits influence marketable yield and overall quality and have not been extensively studied in carrot. For these latter traits, comparisons are made to *M. incognita* RKN resistance. These QTL mapping efforts integrate important traits to carrot linkage maps and lay the foundation for future research in these areas.

Chapter 2

QTL for *Meloidogyne incognita* nematode resistance in carrot

Introduction

Carrot (*Daucus carota*) is a major crop worldwide, providing an economically important crop for farmers and important nutritional benefits to consumers. Root-knot nematodes (*Meloidogyne* spp., RKN) are major pests in many carrot production regions causing direct economic loss of the crop due to galling and forking of the carrot roots, rendering an infected carrot unmarketable (Roberts 1987). *M. hapla* is the predominant RKN species in cooler production areas while *M. javanica* and *M. incognita* are major pests in warmer areas. Limited control of RKN through crop rotations and non-chemical management is possible, but difficult due to wide host ranges, including many weed species (Hunt and Handoo 2010). Current control measures rely on soil-applied nematicides that are effective, but expensive and harmful to the environment. This has led to the conclusion that genetic resistance provides an ideal solution to RKN control (e.g. Nyczepir and Thomas 2010).

RKN resistance has been characterized in many species including alfalfa (Potenza et al. 2001), common bean (Omwega and Roberts 1992), cowpea (Roberts et al. 1996; Ehlers et al. 2000), grape (Cousins et al. 2003), lima bean (Roberts et al. 2008), pepper (Djian-Caporalino et al. 2007), soybean (Li et al. 2001), sugar beet (Yu et al. 1999), sweet potato (Jones and Dukes 1980), tobacco (Yi et al. 1998), and most extensively in tomato (*Solanum lycopersicum*). The *Mi-1* locus in tomato confers resistance to *M. incognita, M. javanica,* and *M. arenaria* (Dropkin 1969), as well as conferring resistance to potato aphids (Rossi et al. 1998, Vos et al. 1998) and whiteflies (Nombela et al. 2003). *Mi-1* was mapped to the short arm of chromosome 6 and since then several other genomic regions of resistance have also been identified (Williamson 1998).

At least one of these (*Mi-9*) is homologous to *Mi-1*, and mapped to the same genomic region (Jablonska et al. 2007). The mode of action of *Mi-1* allows the second-stage juvenile nematodes to penetrate the root and migrate through intercellular space, but when the nematode attempts to establish a feeding site, a hypersensitive plant cell response is activated and infection, feeding, and root-galling is prevented (Paulson and Webster 1972). Other modes of action conferring genetic resistance to RKN have been described in cotton (*Gossypium hirsutum*) where two major QTLs were identified, one on chromosome 11 influencing gall production, and the other on chromosome 14 allowing galling but limiting egg production (Gutiérrez et al. 2010).

The *Mj-1* locus of carrot was discovered in a 'Brasilia' cultivar, line 'Br1252' and it imparts resistance to *M. javanica* as a monogenic dominant trait (Boiteux et al. 2000; Simon et al. 2000). Co-dominant STS flanking markers were developed from dominant AFLP and RAPD flanking markers to facilitate selection (Boiteux et al. 2004). Simon et al. (2000) also observed partial resistance to *M. incognita* in Br1252 and derivatives, in which determination of resistance could not be explained by *Mj-1* alone. Recently, a second *M. javanica* resistance locus, *Mj-2*, was mapped in PI 652188 from China, and found to be distantly linked to *Mj-1*, on chromosome 8 (Ali et al. 2014).

The widespread occurrence of *M. incognita* in carrot production regions and partial resistance observed in Br1252 has led to a wider search for genetic resistance to *M. incognita*. This research was undertaken to determine inheritance patterns and map chromosomal regions responsible for resistance to *M. incognita* identified in Brazilian, Middle Eastern, and European

carrot cultivars, to gain a broader picture of *M. incognita* resistance in these diverse germplasm resources.

Materials and methods

Plant materials

Preliminary screening of several hundred cultivated and wild carrot populations led to the discovery of *M. incognita* resistance in "Brasilia" seed lot 1091 (Br1091) (Matthews et al. 1999), the Syrian cultivar "Homs" (HM), and in a population derived from an cross between the European cultivars "Scarlet Fancy" and "Favourite" (SFF). A Br1091 plant was crossed with a HM plant to generate the Br1091xHM1 F_2 population from a single F_1 plant. A SFF plant was intercrossed to a second HM plant to generate the SFFxHM2 F_2 population from a single F_1 plant. HM1 and HM2 were siblings derived from a self-pollinated HM selection. A third HM plant was self-pollinated to generate the HM3 population. This population had undergone five generations of selfing before the final self-pollination to produce the population HM3, and it was still segregating for resistance. All parent plants (Br1091, SFF, HM1, HM2, and HM3) had been previously evaluated in a greenhouse screen as described below (Br1091) or evaluated in a *M. incognita* infested field and identified as resistant to *M. incognita* with limited gall formation on the carrot root. F_3 families were derived from 95 Br1091xHM1 F_2 and 34 SFFxHM2 F_2 plants.

Nematode screening

All RKN screening was performed under greenhouse conditions at the University of California, Riverside, California. The Br1091xHM1 F_2, and SFFxHM2 F_2 populations were evaluated in the same trial in 2009, the HM3 population was evaluated in 2010, and 11 plants from each Br1091xHM1 and SFFxHM2 F_3 family were evaluated in the same trial in 2012. All evaluations also included susceptible checks of the same variety, "Imperator 58". Individual plants were grown in 10cm pots filled with fine blow sand and the resistance screening was carried out according to the methods described by Simon et al. (2000). Inoculations were made using *M. incognita* race 1 isolate "Beltran" cultured on susceptible tomato plants. Based on estimates of hatching rate of extracted eggs in hatch tests, the egg inoculum density was adjusted to ensure 8,000 - 10,000 second-stage juveniles per plant were inoculated into the root zone using a syringe with three holes along the length of the needle. One month old plants were inoculated and evaluated approximately 60 days after inoculation. Fibrous roots of individual plants were evaluated on a 0 (no galls) to 8 (severely galled) point scale modified from Bridge and Page (1980), and the resulting scores used for the QTL analysis and heritability estimation. Screened plants were shipped to Wisconsin for vernalization and planting in the University of Wisconsin, Department of Horticulture greenhouse at Arlington, WI.

DNA extraction and marker evaluations

Leaves were sampled lyophilized from the greenhouse in Arlington, WI for the Br1091xHM1 F_2, Br1091xHM1 F_3, and SFFxHM2 F_2 populations. Leaves from the HM3 population were harvested in the greenhouse at the University of California, Riverside, California, and placed in

plastic bags with silica gel to desiccate the leaves. DNA for all populations was extracted according to Murray and Thompson (1980), and quantified on 1% agarose gel electrophoresis.

AFLP reactions were performed according to Vivek and Simon (1999). DNA was digested with a combination of EcoRI and MseI restriction enzymes and for amplification the EcoRI primer had a HEX fluorochrome tag for fluorescent evaluation. Fluorescent SSR primers and PCR procedures were performed according to Cavagnaro et al. (2011) and Iorizzo et al. (2011). AFLP and SSR markers were evaluated at the University of Wisconsin Biotechnology Center using an ABI 3730xl capillary sequencer. Polymorphic SSRs were identified by screening a subset of 10 individuals from each population. GeneMarker version 1.5 was used to score alleles (SoftGenetics, State College, Pennsylvania).

SNPs were evaluated using the KASPar system (http://www.KBioscience.co.uk). The Br1091xHM1 F_2 population (138 individuals), 12 individuals from SFFxHM2 F_2 population, and 6 bulk samples of 8 individuals each from HM3 population were evaluated on a panel of 4,000 SNPs previously developed by Iorizzo et al. (2013a). Selected polymorphic SNPs in the SFFxHM2 F_2 and HM3 populations were evaluated in the full population (Table 2.1). To validate the QTLs identified in the Br1091xHM1 F_2 population, SSR markers within the QTL support intervals were evaluated in 507 of the Br1091xHM1 F_3 individuals as described above.

Genetic map construction

Linkage maps were constructed with JoinMap 3.0 software (Van Ooijen 2001). Markers and genotypes with more than 10% missing data and markers that significantly deviated from expected segregation ratios using a Chi-square test ($P<0.01$) were removed. For linkage groups with significant segregation distortion, all markers were used to generate the linkage map for that distorted linkage group. Linkage groups were obtained at a LOD threshold >3.0. The regression mapping algorithm was used with Haldane's mapping function to calculate distances between markers. Haldane's mapping function provides more accurate marker placement according to the carrot physical map than the Kosambi's mapping function (data not shown). Each marker was coded twice, once for each parental phase. The linkage groups were properly phased by using marker scores for individuals related to the parents (Gomez et al. 1996; Vivek and Simon 1999). The marker order was further examined using CheckMatrix (http://www.atgc.org/XLinkage, Truco et al. 2013) for inconsistencies and markers with more than one inconsistent score were removed. To remove redundant markers in the Br1091xHM1 population, a genetic bin map was developed. For each linkage group pair-wise recombination values among all markers were calculated. Adjacent markers with zero recombination among them were assigned to the same genetic bin. In addition, adjacent markers with "false" recombination due to missing data were considered to belong to the same genetic bin. The marker with the least missing data was chosen to represent each genetic bin.

SNPs and SSRs with known chromosome locations were used to anchor the linkage groups (Iorizzo et al. 2013b; Cavagnaro et al. 2011; Iovene et al. 2011; Yildiz et al. 2013). After being

assigned to chromosomes, linkage groups were oriented and numbered following the chromosome orientation and classification of Iovene et al. (2011).

Map merging

JoinMap version 3.0 was used to merge the maps. For each pair-wise comparison, common co-linear markers were identified as anchoring markers, and used to develop the consensus map. QTL coordinates were transferred from the individual maps to the merged maps according to the location of the nearest flanking markers mapped in each specific linkage map. MapChart version 2.2 was used to draw all chromosome diagrams (Voorrips 2002).

QTL mapping

QTL analysis was performed in all three populations using R/qtl with the multiple imputations method (Broman and Sen 2009). QTL detection for each population included preliminary QTL identification using scanone followed by QTL modeling. The largest LOD peak from the analysis was added to the QTL model and if the QTL model was significant, it was retained. This process was then repeated using addqtl, instead of scanone, followed by QTL modeling and testing for interactions until adding additional QTL to the model was no longer significant. The final step used addpair to add a pair of interacting QTL or the interaction between a QTL in the model and a newly identified QTL. The support intervals were calculated using a 1.5 LOD drop (Broman and Sen 2009). QTL were named *Mi-population-C_-Q_* where "*population*" is the population in which the QTL was identified, "*C_*" is the chromosome on which the QTL was

identified and "*Q_*" is the QTL identifier from the QTL model. For example, *Mi-BrHM1-C2-Q1* was mapped in the Br1091xHM1 population, is on chromosome 2, and is QTL that explains the most variation in the model.

Heritability estimation and QTL validation

Parent offspring regression was used to estimate broad sense heritability using F_3 family averages in the Br1091xHM1 and SFFxHM2 populations (Nyquist 1991) calculated with the statistical program R. For the Br1091xHM1 F_3 QTL validation, the marker that best approximated the F_2 QTL was used to fit a QTL model in the F_3 population. To better customize the model to the F_3 population, refineqtl was used and the refined qtl positions used in the final fitqtl analysis.

Results

Genetic linkage maps

The Br1091xHM1 linkage map was generated from the evaluation of 138 individuals and included 389 SNP markers (Table 2.1, Figure 2.1). The total genetic distance covered 563.3 cM with an average marker spacing of 1.5 cM. Markers on chromosome 9 displayed significant segregation distortion from the 1:2:1 expected ratio (data not shown).

The SFFxHM2 linkage map was generated from the evaluation of 113 individuals and included 138 AFLP, SSR, and SNP markers (Table 2.1, Figure 2.2). The total genetic distance covered 520.1 cM with an average marker spacing of 4.0cM.

The HM3 linkage map was generated from the evaluation of 281 individuals and included 70 SSR and SNP markers (Table 2.1, Figure 2.3). The total genetic distance covered 219.2 cM with an average marker spacing of 3.4 cM. HM3 had no segregating markers on 3 chromosomes and relatively few segregating markers on the 6 other chromosomes. Out of 3,636 SNPs screened, only 226 were polymorphic, and many of those SNPs were clustered together in a few chromosomal regions.

The merged linkage map for all three populations included 445 markers covering a total genetic distance of 556.9 cM and an average marker spacing of 1.3 cM (Table 2.1, Figure 2.4). There were 13 markers shared by all 3 maps, and the order of markers was conserved in the merged map, relative to each individual map (data not shown).

QTL mapping

QTL for *M. incognita* resistance in the Br1091xHM1 population were located on chromosomes 1, 2, 8, and 9 and they accounted for 55.5% of the phenotypic variation of the resistance reaction (Table 2.2, Figure 2.4, Figure 2.5, Figure 2.6, Figure 2.7, Figure 2.8). QTL attributable to the

Br1091 parent included those on chromosomes 1 and 8. The QTL on chromosomes 2 and 9 were derived from the HM1 parent. All four QTL displayed additive effects ranging from 0.6 to 1.4. The additive effect is half the difference between the susceptible and resistant homozygous means.

QTL for *M. incognita* resistance in the SFFxHM2 population were on chromosomes 2, 4, and 8 and they accounted for 34.0% of the phenotypic variation in the resistance reaction (Table 2.2, Figure 2.4, Figure 2.6, Figure 2.9, Figure 2.7). The QTL on chromosomes 4 and 8 were derived from the SFF parent while the QTL on chromosome 2 was derived from the HM2 parent. The QTL displayed additive effects ranging from 0.8 to 1.1.

QTL for *M. incognita* resistance in the HM3 population were on chromosomes 1, 8, and 9 and they accounted for 35.7% of the phenotypic variation (Table 2.2, Figure 2.4, Figure 2.5, Figure 2.6, Figure 2.8). The QTL on chromosomes 1 and 8 had additive effects of 0.4 and 0.9, respectively, while the QTL on chromosome 9 displayed over-dominance with the heterozygous genotype more resistant than either homozygous genotype (Figure 2.8).

Heritability and QTL validation

Broad sense heritability for resistance to *M. incognita* was 0.33 and 0.25 in Br1091xHM1 and SFFxHM2, respectively (Figure 2.10). For validating the QTL in Br1091xHM1, the full model

including the QTL on chromosomes 1, 2, 8, and 9, accounted for 23.7% of the variation with individual QTL effects ranging from 3.0% to 12.5% (Table 2.3). All QTL effects were additive (data not shown) as in the Br1091xHM1 F_2 population.

Discussion

A significant QTL for resistance to *M. incognita* was detected on chromosome 8 in each of the 3 populations evaluated and those QTL support intervals spanned the same region as *Mj-1*, which confers resistance to *M. javanica* and co-segregates with GSSR-044 (Ali et al. 2014). While the QTL on chromosome 8 co-localize to *Mj-1*, there is no evidence the QTL identified this work and *Mj-1* are the same. The additive effects of this QTL in each population were also similar with values of 0.8, 0.9, and 1.0 for SFFxHM2, HM3, and Br1091xHM1, respectively (Table 2.2, Figure 2.7). Since *M. incognita* resistance was noted in *M. javanica* resistant segregants derived from a different 'Brasilia' cross (Boiteux et al. 2000; Simon et al. 2000), it was not surprising to observe *Mi-BrHM1-C8-Q2* in the Br1091xHM1 cross, but the discovery of similar QTL in the unrelated SFFxHM2 and HM3 populations was unexpected. It remains to be determined if the same alleles are responsible for resistance to *M. incognita* in each population and also how the *M. incognita* resistance relates to alleles at the *Mj-1* locus, but their co-segregation is worth noting. Resistance genes can occur in tandem clusters. In fact many of the tomato resistance genes are clustered with *Mi-1* and *Mi-9* on chromosome 6, linkage between *Mi-2* and *Mi-8* (position not reported), linkage between *Mi-6* and *Mi-7* (position not reported), and linkage between *Mi-3* and *Mi-5* on chromosome 12 (Williamson 1998; Jablonska et al. 2007). Clusters of resistance genes can have greater numbers of non-synonymous vs. synonymous mutations and

it has been hypothesized that this allows for plasticity in the sequence to adapt to emerging pathogens (Michelmore and Meyers 1998). In fact, variation in copy numbers of tandem repeated nematode resistance genes (*Rhg1*) has been observed in soybean (Cook et al. 2012). Simon et al. (2000) also presented evidence for the scenario that two fairly closely linked genes in coupling phase explain resistance to *M. javanica* at the *Mj-1* locus, so there is some evidence for more than one gene affecting nematode resistance in the region. Fine mapping and testcrosses between the different sources of resistance, coupled with extensive molecular evaluation of the carrot genome, will be necessary to validate a common allelic basis for the QTL conferring resistance to *M. incognita* and *M. javanica*.

In addition to the common QTL on chromosome 8, a QTL in both the Br1091xHM1 and HM3 populations mapped near the end of the short arm of chromosome 9. The resistance in Br1091xHM1 is derived from the HM1 parent and given the shared ancestry of HM1 and HM3, it is possible that the QTL on chromosome 9 could be identical by descent between the two populations. However, the QTL effects of the two populations differ, with *Mi-HM3-C9-Q1* displaying over-dominance and *Mi-BrHM1-C9-Q4* being additive. The average score of each homozygous genotype at *Mi-HM3-C9-Q1* is 5.4 while the average score of the heterozygous genotype is 4.4 (Figure 2.8). It is possible the QTL represent different alleles of the same locus in each population, or two closely linked genomic regions each coming from a different resistant parent.

Br1091xHM1 and HM3 also share a QTL on chromosome 1, but in this case the resistance comes from Br1091 and HM3, respectively (Figure 2.5). Both of these QTL have relatively minor effects (6.1% and 4.3% of the variation explained for *Mi-BrHM1-C1-Q3* and *Mi-HM3-C1-Q3*, respectively) and the QTL support intervals are quite large (23 cM and 54 cM, respectively, Figure 2.4) so it is difficult to draw firm conclusions about the map locations of these two QTL.

QTL for *M. incognita* resistance in the SFFxHM2 population had large support intervals (60 cM, 33 cM, and 31 cM for the QTL on chromosomes 2, 4, and 8, respectively) and small QTL effects (8-13%). The large support intervals may be due to a relatively small number of individuals (N=113), small individual QTL effects, low marker density (average spacing of 4 cM but not evenly distributed throughout the linkage map, Figure 2.2), or some combination of these factors. Other researchers have shown each of these constraints can limit the detection of QTL and the refinement of QTL support intervals (e.g. Darvasi 1993; Li 2010; Stange 2013). *Mi-SFFH2-C2-Q3* and *Mi-BrHM1-C2-Q1* both mapped to chromosome 2, but given the very large support interval of *Mi-SFFH2-C2-Q3*, they may not be allelic even though the resistance comes from the HM parent in each cross. Furthermore, because *Mi-BrHM1-C2-Q1* accounted for 34% of the variation, compared to only 8% by *Mi-SFFH2-C2-Q3*, it might be conjectured that these QTL are either at different loci or are different alleles of the same locus (Table 2.2, Figure 2.6).

The full QTL model for each population accounted for 55.5%, 34.8%, and 35.7% of the variation in Br1091xHM1, SFFxHM2, and HM3, respectively (Table 2.2). There were no significant interaction effects among QTL for a given cross and with the exception of over-dominance noted

for *Mi-HM3-C9-Q1*, all QTL effects were additive. Many major RKN resistance genes have been identified in other crops, but RKN QTLs are more difficult to characterize. Tamulonis et al. (1997) identified 2 QTL in soybean that accounted for 54% of the variation in *M. javanica* root-galling, and Gutierrez et al. (2010) identified 2 QTL in cotton accounting for 41% of the variation for *M. Incognita* root-gall index for *M. incognita*. The QTL detected in this work demonstrate similar values for the percent variation explained.

There were 3 QTL identified in the HM3 population, but with 6 generations of selection for RKN resistance, limited polymorphism was observed. Only 6 chromosomes had segregating markers, and only chromosome 1 had extensive marker coverage, compared to the merged map (Figure 2.3). When screening this population for segregating markers, only 226 SNPs were polymorphic out of 3,636 SNPs screened. This reduced polymorphism means only a small part of the genome was actually analyzed in the QTL analysis and other, homozygous regions of the genome might contain nematode resistance genes fixed for resistance or susceptibility. Even so, the HM3 population segregated widely for nematode resistance (Table 2.1). A cross using resistant individuals from the HM3 population to a susceptible cultivar would help detect any fixed QTL as well as those QTL segregating in HM3.

The Br1091xHM1 and SFFxHM2 populations were both derived from intercrosses between unrelated sources of genetic resistance with the presumption that at least one resistance locus was derived from each parent. That expectation was validated in the QTL analysis and the low broad sense heritability estimates of 0.33 and 0.25 in the Br1091xHM1 and SFFxHM2 populations,

respectively. The validation of the F_2 QTL in the F_3 generation of Br1091xHM1 confirms that the QTL are significant. Because the QTL detected displayed additive effects, the percent of the phenotypic variation explained estimates narrow-sense heritability (Broman and Sen 2009). In the F3 generation, the percent of the phenotypic variation explained was 23.7% and represents a narrow sense heritability of 0.237. This compares to the broad sense heritability from the parent-offspring regression of 0.33 in the same population, indicating resistance in the Br1091xHM1 population are mostly additive. The finding of quantitative inheritance for *M. incognita* resistance in these populations make the development of uniformly resistant lines difficult, but with recurrent selection and molecular markers, progress to develop resistant germplasm is ongoing.

The nematode screening for this work was carried out on an individual plant basis with each population evaluated at one time. While there was no replication of the individual populations, the combining of the populations and the detection of co-localized QTL provide a "biological replication" that adds validity to the results. Also, the Br1091xHM1 QTLs were validated as significant in the F_3 population. The evaluations for RKN screening in this work were carried out in different greenhouse trials but the same susceptible check, 'Imperator 58', was used in all trials. The gall-rating of Imperator 58 varied from 5 to 8 with an average rating of 6.5 and a standard deviation of 0.80 (data not shown), so there is variation in the plant response to the RKN attack. While the plant response to RKN attack is difficult to evaluate on an individual plant basis, the results obtained here are encouraging, particularly because individual plants of a

single F_2 population are more economical to produce, evaluate, and genotype than F_3 families or recombinant inbred lines.

The parents in all three populations exhibited unique resistance QTL that are being introgressed into susceptible germplasm and combined to develop what may be a more durable resistance effective against both *M. incognita* (from the QTLs detected here) and *M. javanica* (from the *Mj-1* locus). The development of markers flanking the resistance regions will be useful to select for the larger effect QTLs with smaller support intervals for use in marker-assisted selection. The sequencing of the carrot genome will provide a rich resource for not only identifying markers to select for resistance but also assist the identification of candidate resistance genes. Further research is needed to determine whether all QTLs are required to provide adequate, durable resistance to commercial three-way hybrids under field conditions and whether certain combinations of resistance alleles confer broader resistance. These studies will also provide further insights into the question of whether QTL from different genetic backgrounds are allelic or different, closely linked genes and whether *M. javanica* resistance imparted by the *Mj-1* locus is a pleiotropic function of a *M. incognita* QTL or a separate gene. With fine mapping of resistance alleles and genes, research is needed to elucidate the biological mechanism of individual alleles and genes that underlie the mode of gene action in impeding nematode infection. This research lays a foundation for future studies in these areas and provides useful information to the carrot breeding community.

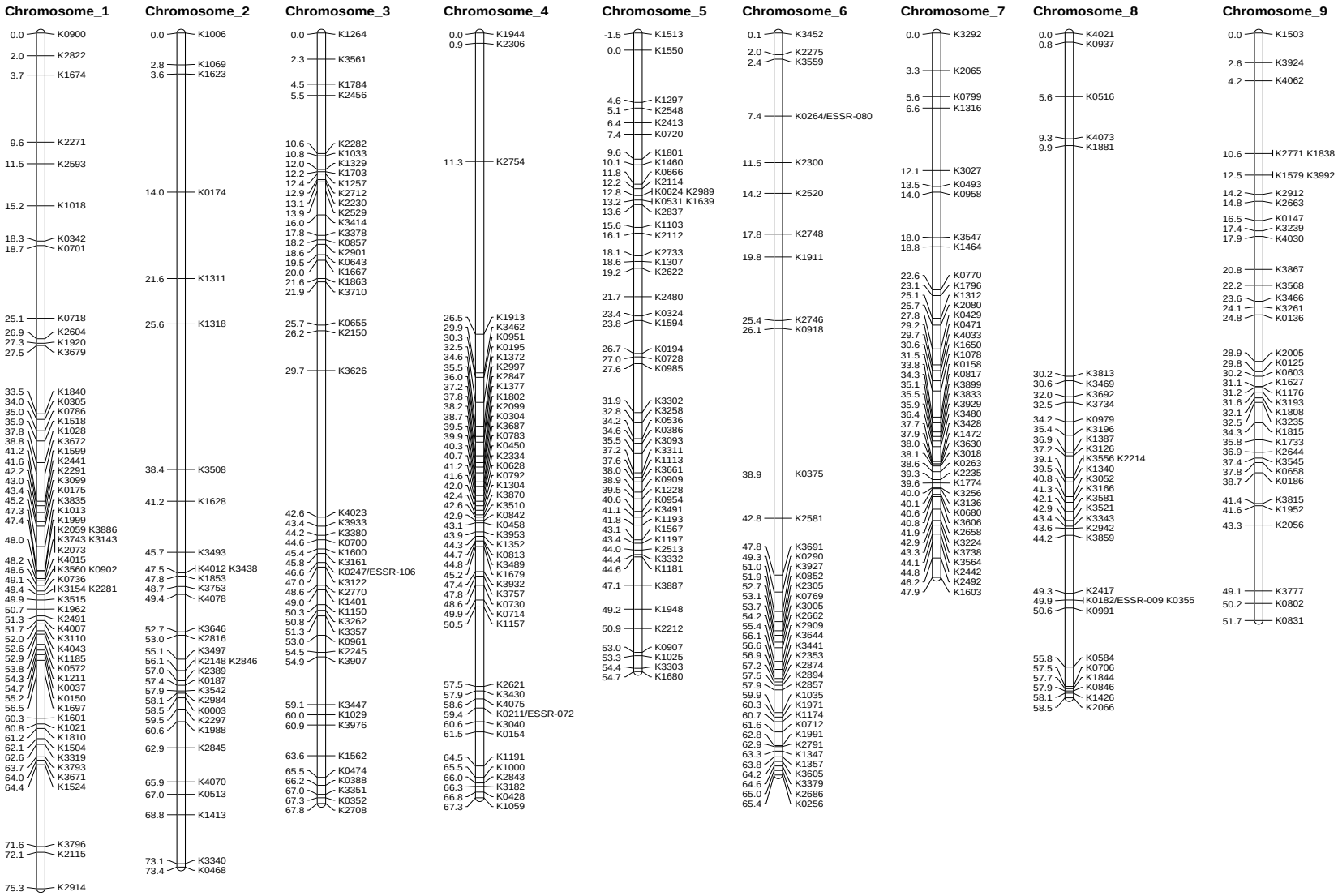

Figure 2.1 Linkage map for the F_2 mapping population Br1091xHM1.

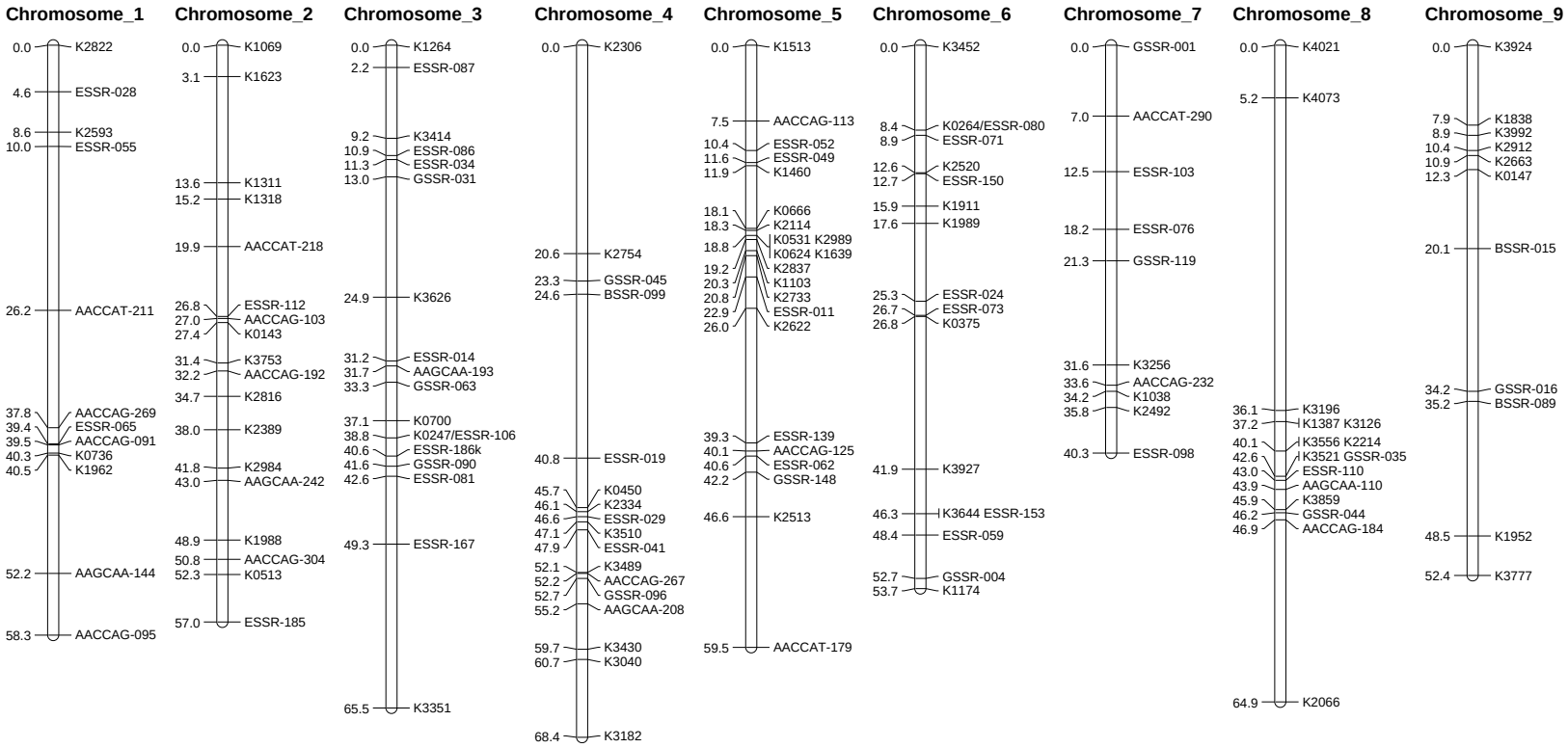

Figure 2.2 Linkage map for the F_2 mapping population SFFxHM2.

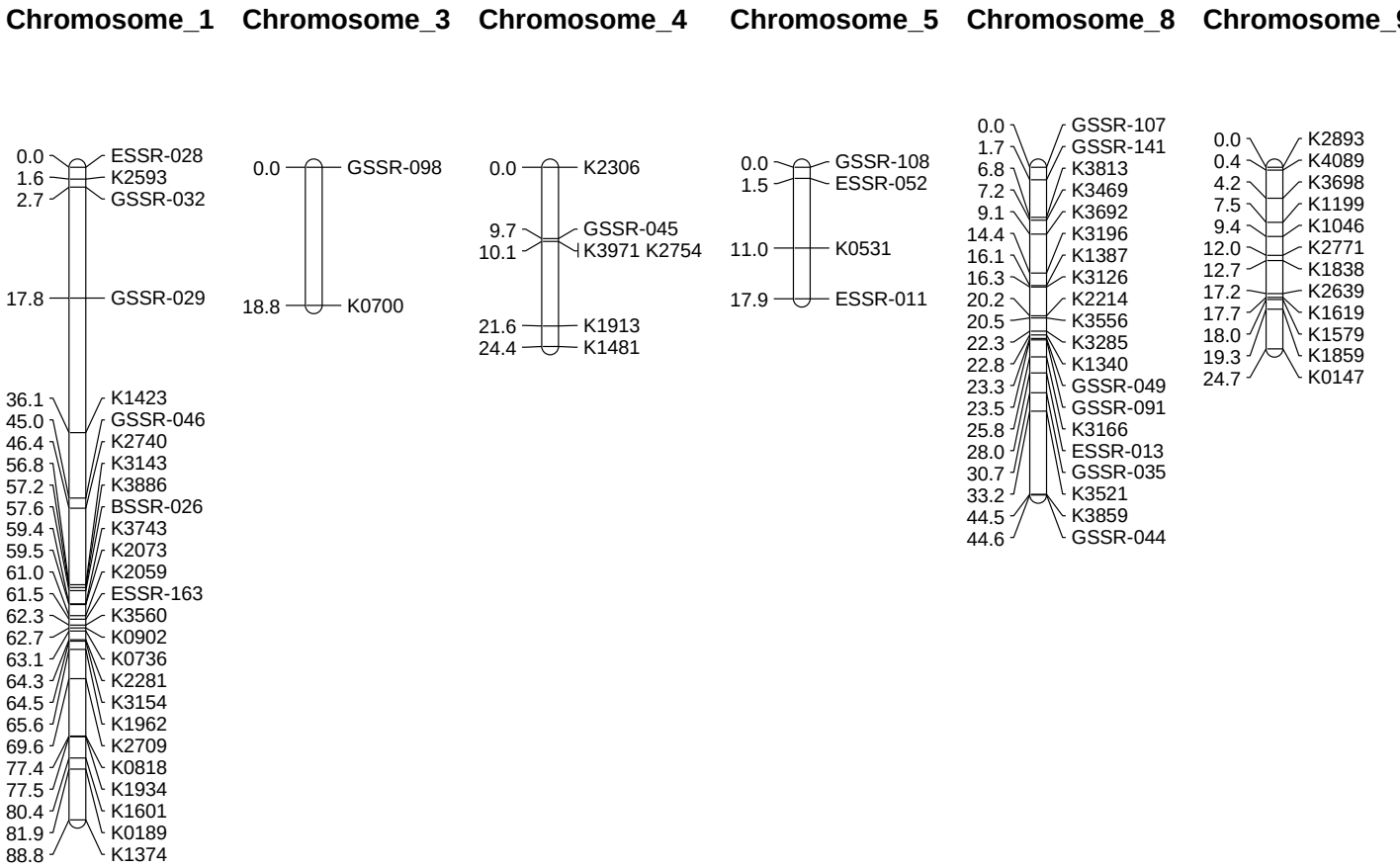

Figure 2.3 Linkage map for the mapping population HM3.

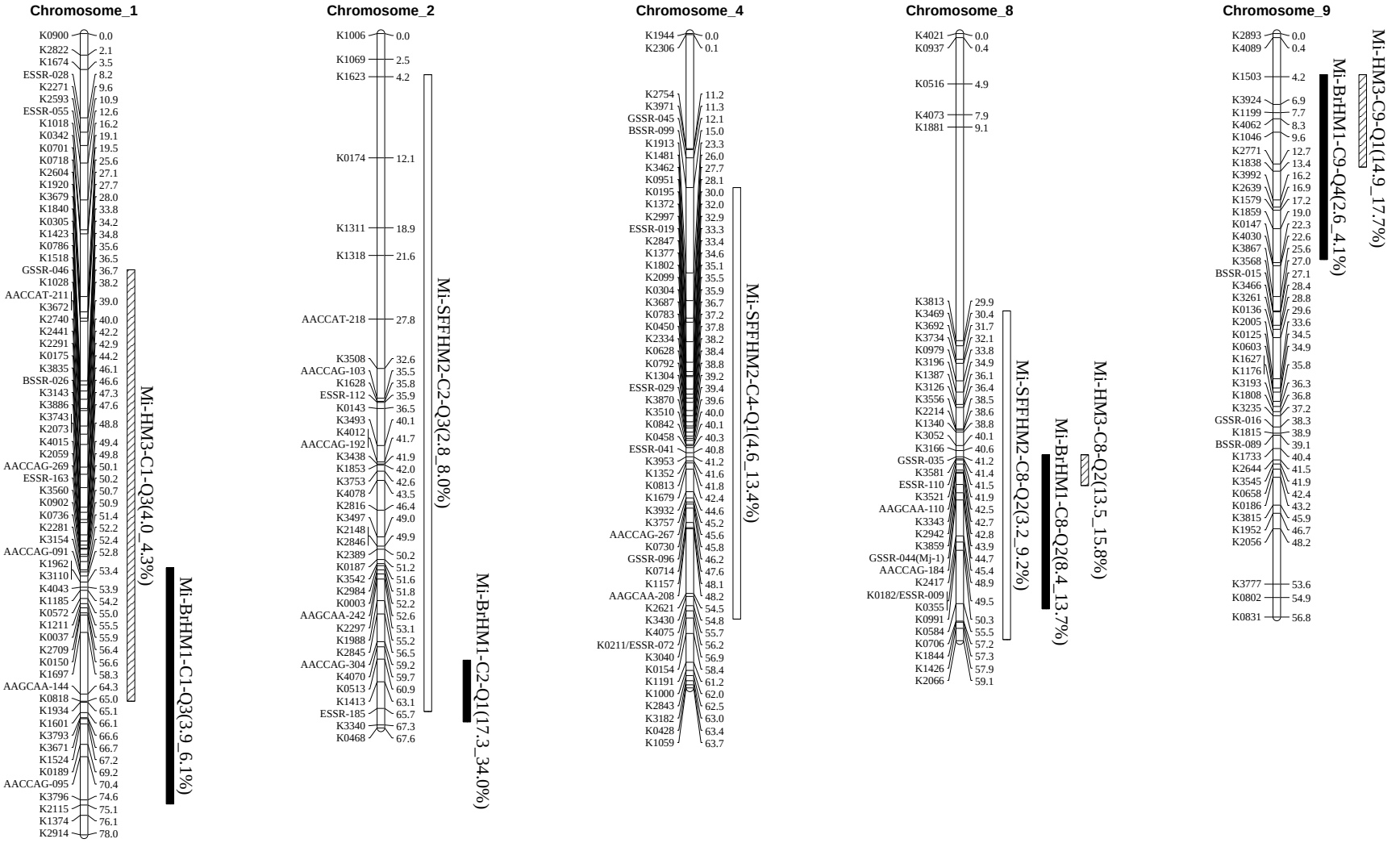

Figure 2.4 Merged linkage map of carrot chromosomes that incorporates significant QTL for *M. incognita* nematode resistance from three populations (Br1091xHM1, SFFxHM2, HM3). The bars represent 1.5 LOD support intervals and the populations are coded with Br1091xHM1 as solid bars, SFFxHM2 as open bars, and HM3 as cross hashed bars. Numbers in parentheses indicate the largest LOD score followed by the percent phenotypic variation explained by that QTL.

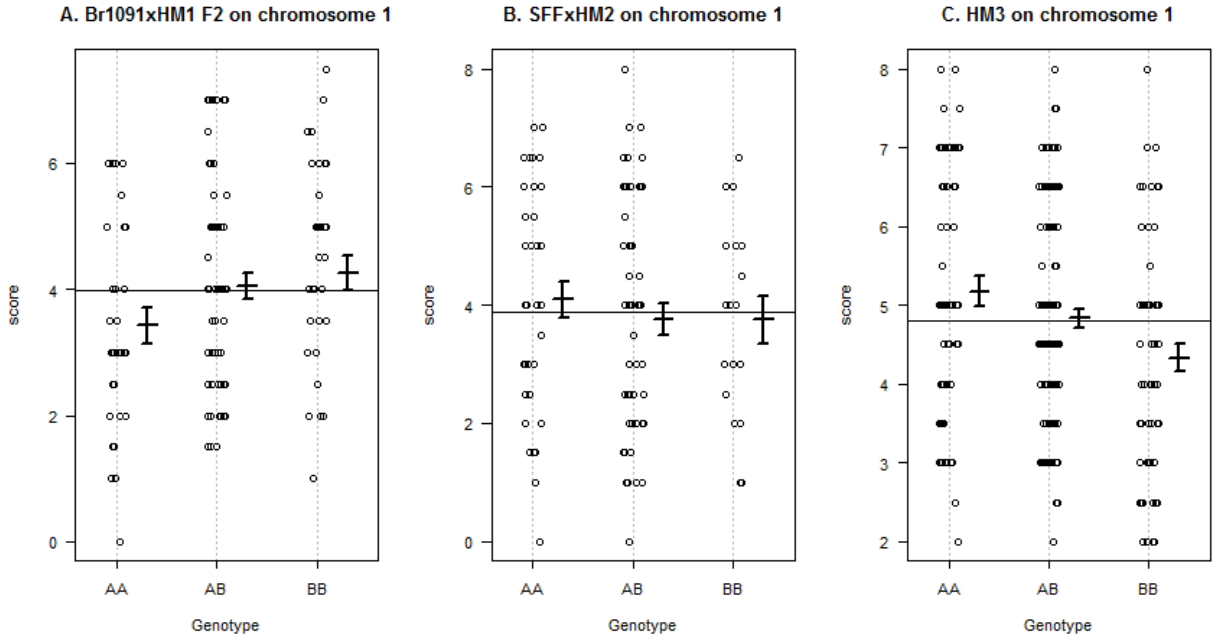

Figure 2.5 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 1 in the (**A**) Br1091xHM1 F2, (**B**) SFFxHM2, and (**C**) HM3 populations. Significant QTL were identified in the Br1091xHM1 and HM3 populations. The horizontal lines represent the population mean and the bars are ± 1 standard error.

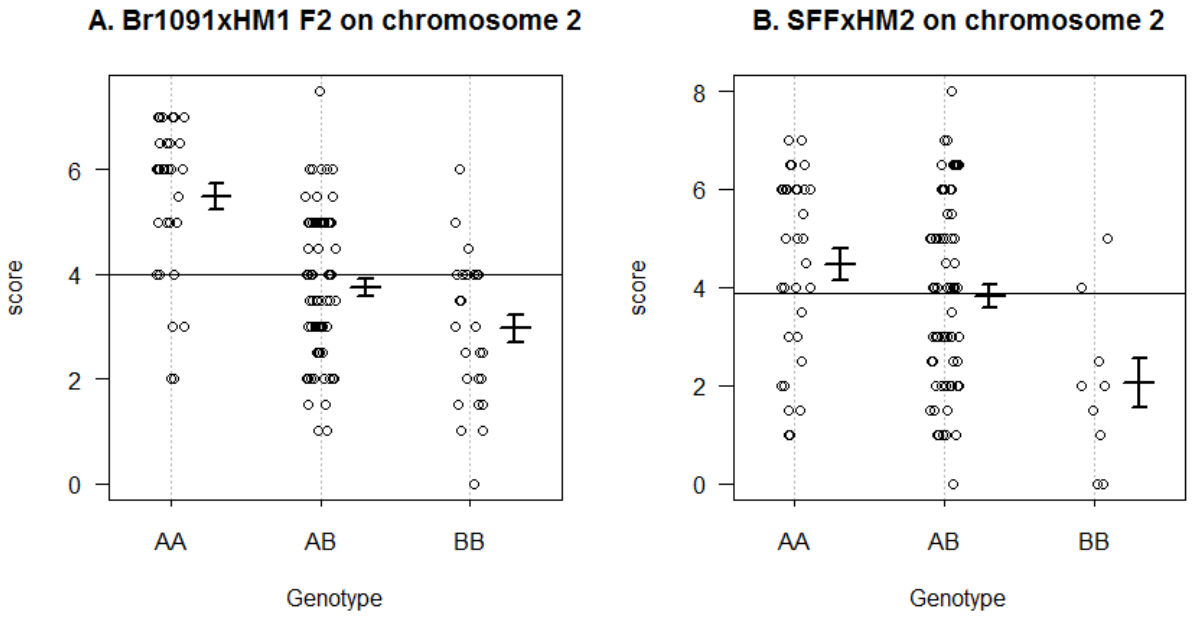

Figure 2.6 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 2 in the (**A**) Br1091xHM1 F2 and (**B**) SFFxHM2 populations. Significant QTL were identified in both populations. The horizontal lines represent the population mean and the bars are ± 1 standard error. No segregating markers were identified for HM3 on chromosome 2.

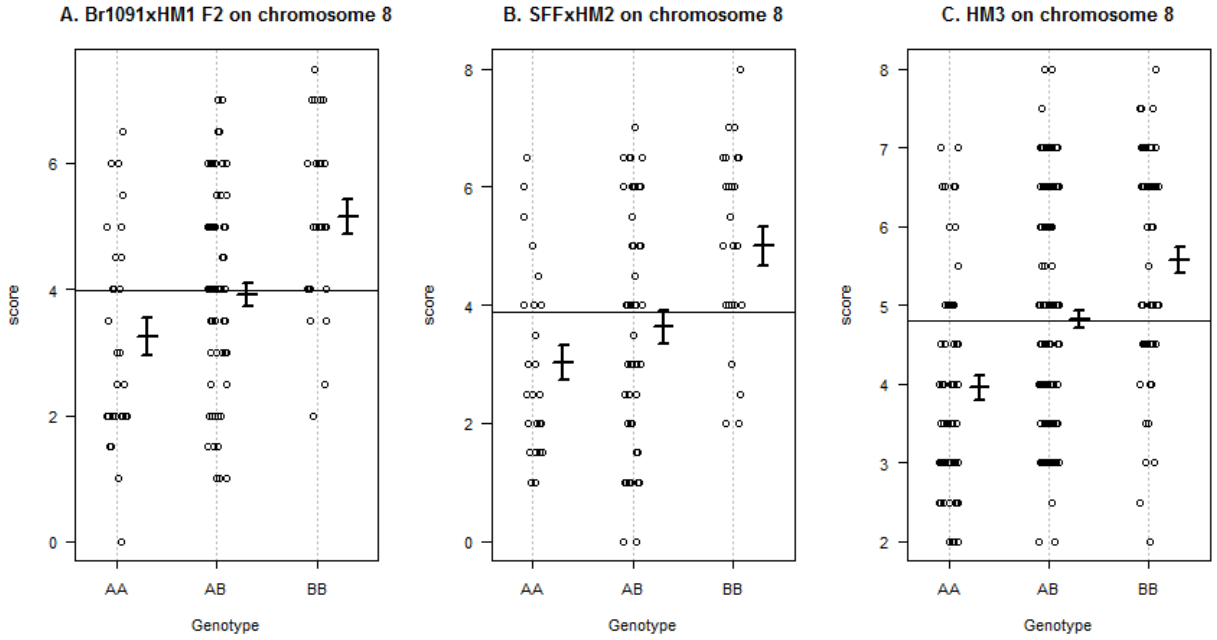

Figure 2.7 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 8 in the (**A**) Br1091xHM1 F2, (**B**) SFFxHM2, and (**C**) HM3 populations. Significant QTL were identified in all three populations. The horizontal lines represent the population mean and the bars are ± 1 standard error.

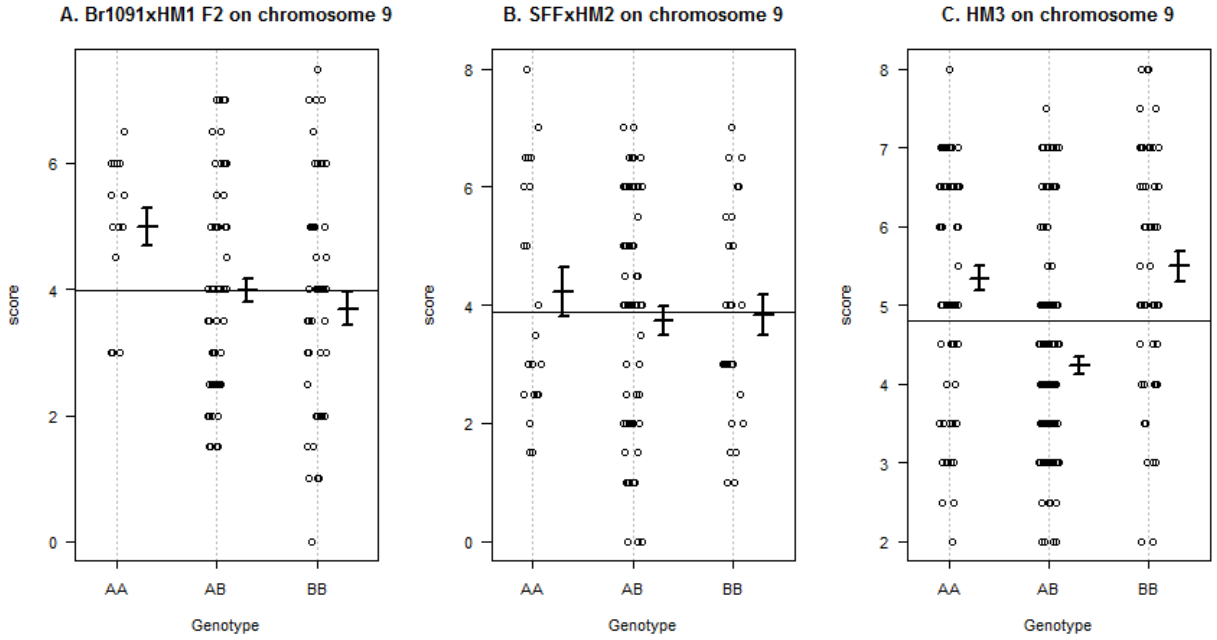

Figure 2.8 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 9 in the (**A**) Br1091xHM1 F2, (**B**) SFFxHM2, and (**C**) HM3 populations. The QTL in Br1091xHM1 and HM3 are significant and HM3 displays over-dominance for resistance. The horizontal lines represent the population mean and the bars are ± 1 standard error.

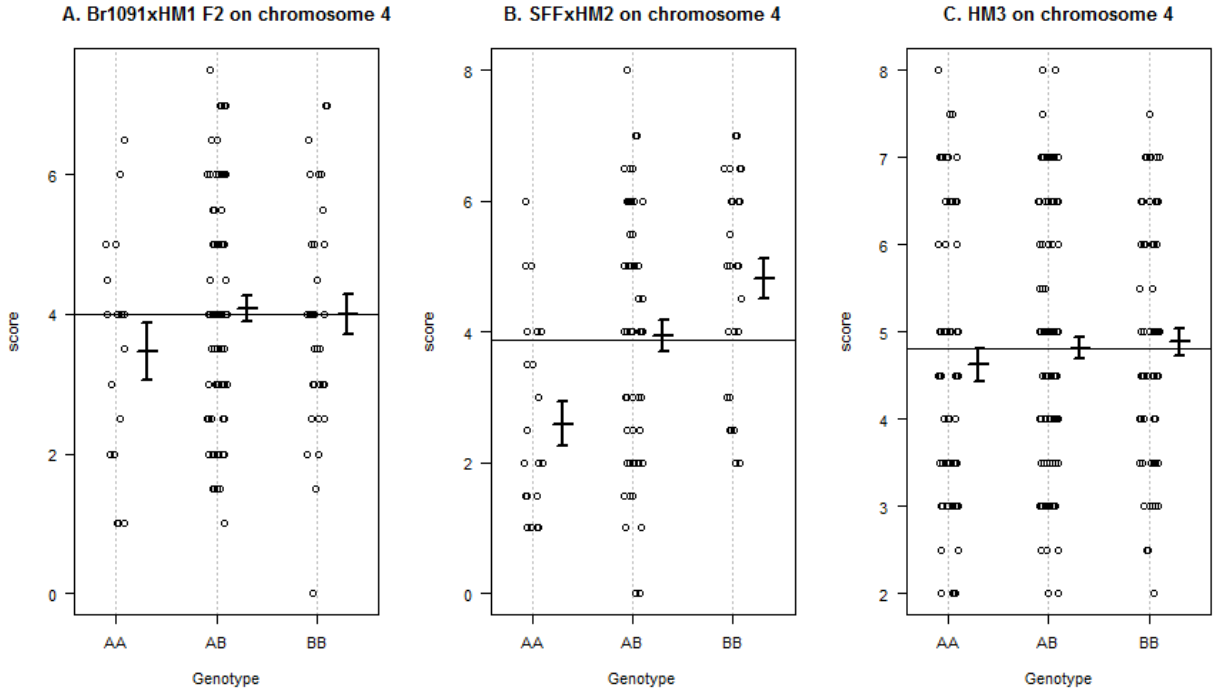

Figure 2.9 Phenotypic scores for root-galling (0 to 8 scale with 0 being resistant and 8 being susceptible) of AA, AB, and BB individuals at the QTL region on chromosome 4 in the (**A**) Br1091xHM1 F2, (**B**) SFFxHM2, and (**C**) HM3 populations. A significant QTL was only detected in the SFFxHM2 population. The horizontal lines represent the population mean and the bars are ± 1 standard error.

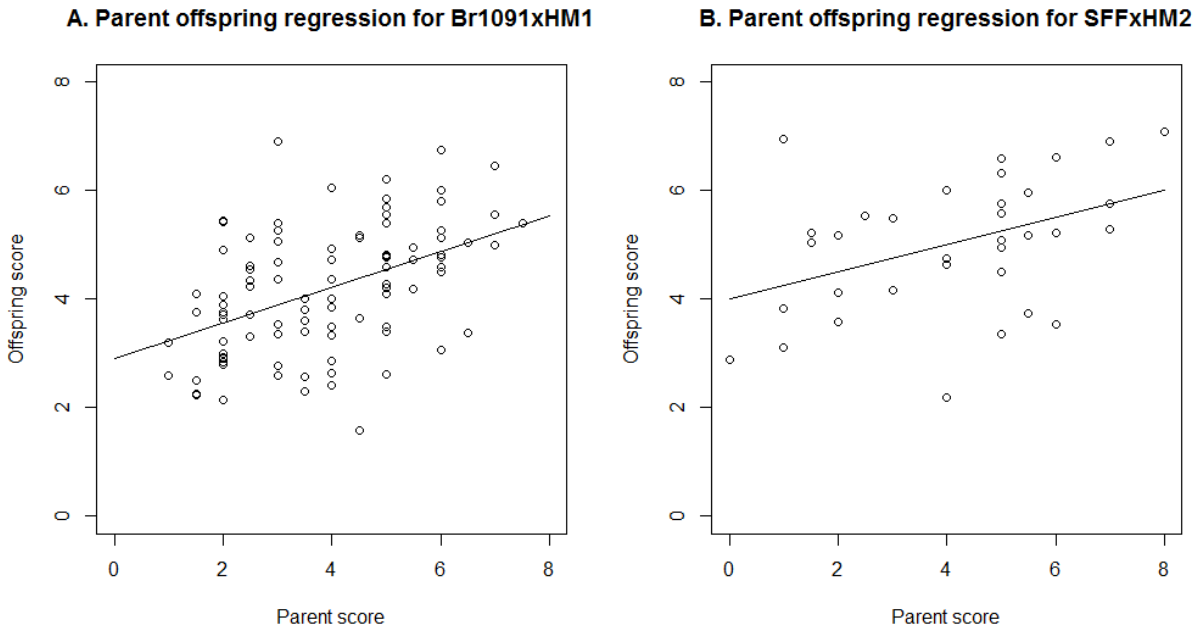

Figure 2.10 Parent-offspring regressions for the F_2:F_3 generations in the (**A**) Br1091xHM1 and (**B**) SFFxHM2 mapping populations. The regression line represents broad sense heritability, H^2.

Table 2.1 Summaries of the carrot nematode resistance maps for populations Br1091xHM1, SFFxHM2, and HM3.

	Br1091xHM1	SFFxHM2	HM3	Merged Map
Number of individuals	138	113	281	-
Range of nematode scores[a]	0-7.5	0-8	2-8	-
Number of polymorphic markers	389	138	70	445
AFLPs	0	20	0	17
SSRs	0	47	18	44
SNPs	389	71	52	384
Total distance (cM)	563.3	520.1	219.2	556.9
Average inter-marker spacing (cM)	1.5	4.0	3.4	1.3
Maximum marker spacing (cM)	20.3	30.9	18.8	20.8

[a]Root-galling index (0 to 8 scale)

Table 2.2 Chromosomal location of QTL conferring *M. incognita* nematode resistance in the three mapping populations Br1091xHM1, SFFxHM2, and HM3.

(Mapping population) Chromosome	QTL	Position (cM)	LOD	% VE[a]	Resistance source	Closest marker	Marker interval	1.5 LOD[b]	Additive effect[c]
(Br1091xHM)									
1	*Mi-BrHM1-C1-Q3*	67.2	3.9	6.1	B1091	K1524	K2281 – K2115	52 - 75	0.6
2	*Mi-BrHM1-C2-Q1*	63.1	17.3	34.0	HM1	K1413	K0513 – K3340	61 - 67	1.4
8	*Mi-BrHM1-C8-Q2*	41.9	8.4	13.7	B1091	K3521	K3166 – K0584	41 - 56	1.0
9	*Mi-BrHM1-C9-Q4*	4.2	2.6	4.1	HM1	K1503	K1503 - K0147	4 - 22	0.6
Summed % variance explained by multi-QTL model = 55.5%									
(SFFxHM2)									
2	*Mi-SFFHM2-C2-Q3*	42.6	2.8	8.0	HM2	K3753	K1623 - ESSR-185	4 - 66	1.1
4	*Mi-SFFHM2-C4-Q1*	33.3	4.6	13.4	SFF	ESSR-019	BSSR-099 – K3040	15 - 57	1.0
8	*Mi-SFFHM2-C8-Q2*	41.5	3.2	9.2	SFF	ESSR-110	K4073 – K2066	27 - 59	0.8
Summed % variance explained by multi-QTL model = 34.8%									
(HM3)									
1	*Mi-HM3-C1-Q3*	34.8	4.0	4.3	HM3	K1423	K2593 - K0818	23 - 65	0.4
8	*Mi-HM3-C8-Q2*	41.9	13.5	15.8	HM3	K3521	GSSR-035 – K3859	41 - 44	0.9
9	*Mi-HM3-C9-Q1*	9.6	14.9	17.7	HM3	K1046	K4089 - K2771	4 - 13	0.1
Summed % variance explained by multi-QTL model =35.7%									

[a]Percentage of the variation explained

[b]1.5 LOD support interval (cM) (Broman and Sen 2009)

[c]Half the phenotypic difference between the means of the resistant and susceptible homozygous genotypes

Table 2.3 LOD scores and % variation explained at the QTLs identified in the Br1091xHM1 F_2 population in the F_3 progeny.

Chromosome	QTL	LOD	% VE[a]
1	*Mi-BrHM1-C1-Q3*	5.0	3.7
2	*Mi-BrHM1-C2-Q1*	12.5	12.5
8	*Mi-BrHM1-C8-Q2*	7.1	5.3
9	*Mi-BrHM1-C9-Q4*	4.1	3.0
Summed % variance explained by multi-QTL model = 23.7%			

[a]Percentage of the variation explained

Chapter 3

QTL for root and shoulder color in carrot

Introduction

Carrot (*Daucus carota*) is a major crop worldwide and is an important source of nutrients, especially pro-vitamin A carotenes. 34,000 hectares of carrots are produced in the US (USDA, National Agricultural Statistics Service, 2011) and many of these carrots are consumed as "baby" or "cut-and-peel" carrots providing a convenient source of fresh nutrients to consumers (Simon et al. 2008). In addition to orange carrots that provide the most carotenes so familiar to consumers today, purple carrots, popular 500 years ago are experiencing a renewed popularity. Purple carrots provide anti-oxidant anthocyanins.

Y, Y_1, and Y_2 are major genes influencing root accumulation of carotenes (Buishand and Gabelman 1979; Simon 1996). *Y* blocks the synthesis of carotenes and xanthophyls leading to white roots whereas Y_1 and Y_2 block carotenes but not xanthophylls leading to yellow roots. Y_2 specifically affects the core color of carrot roots. Along with these major carotenoid genes, QTLs have been identified for the different carotenoids as well as xanthophyll. Pro-vitamin A carotenes and lycopene QTLs were mapped in a cultivated orange x wild white F_2 population (B493 x QAL) and an orange x very dark orange F_2 population (Brasilia x HCM) identifying several QTLs on different linkage groups in each population (Santos and Simon 2002). Just et al. (2007) used the same B493 x QAL population to map carotenoid biosynthesis structural genes and perform additional QTL mapping (2009). They found two major, interacting QTL and correlated the QTL to *Y* and Y_2 from previous studies. These major genes were mapped to chromosome 5 and 7 respectively (Cavagnaro et al. 2011).

Purple root color was reported and characterized on a chemical level by Harborne (1976) and one gene controlling anthocyanin accumulation in storage roots (P_1) was genetically characterized by Simon (1996). P_1 is a dominant gene that was later mapped to chromosome 3 in a cross between B1896 and B7262 (a yellow rooted selection and a purple exterior and orange interior selection) (Yildiz et al. 2013). Several other genes in the anthocyanin biosynthesis pathway were also analyzed for expression differences between purple and orange roots, and the genes mapped, with no candidate gene identified for P_1. The expression pattern of genes in the pathway indicated P_1 is probably a transcription factor that activates many genes (Yildiz et al. 2013). Besides purple root color, purple petiole was characterized as a single dominant locus (*G*) by Angell and Gabelman (1970) and P_2 was another locus characterized by Simon (1996) that conditions purple node pigmentation that can also spread from the node into the leaf, bract, or petiole. It is not clear if P_2 was conditioned by the *G* locus or if the phenotypes are under separate genetic control. P_1 and P_2 are linked approximately 36 cM apart and P_2 was mapped to chromosome 3 by Cavagnaro and Iorizzo (unpublished data). Along with P_2 they phenotyped the coverage of purple on the exterior of the root as a percentage of the whole and identified a QTL on chromosome 1 and 3. The QTL on chromosome 3 was in the region of P_2 but the heterozygous individuals had more purple than either homozygous class. The expression was quantitative and explained about 50% of the variation (Cavagnaro and Iorizzo, unpublished data). Other QTL for different anthocyanins were detected on several other chromosomes as well. Root color can vary widely in diverse genetic backgrounds from the exterior to the phloem to the xylem of the root, and can be under separate genetic control. Root color in carrot has been characterized for major, dominant genes phenotyped visually, or QTLs have been identified using HPLC analysis for both carotenoids and anthocyanins.

In the course of evaluating nematode resistance in progeny of diverse breeding stocks of carrots that differed for root color, clear patterns of inheritance were observed for root and shoulder color. This work uses visual phenotyping of two segregating populations to map both root pigmentation and shoulder pigmentation in carrot in genetic backgrounds previously not characterized. The traits do not segregate as dominant, major genes and leads to the hypothesis the traits are under quantitative control in these populations.

Materials and methods

Plant materials

Two F_2 populations originally studied for *Meloidogyne incognita* nematode resistance were also segregating for root and shoulder color (Chapter 2). The same plants were evaluated for root-knot nematode resistance and the color traits described here. Br1091xHM1 is an F_2 population from the cross between the Brasilian cultivar 'Brasilia 1091' (Br1091) and the Syrian cultivar 'Homs' (HM1). SFFxHM2 is an F_2 population between a selection from the intercross of European cultivars 'Scarlet Fancy' and 'Favourite' (SFF) and another 'Homs' plant (HM2). HM1 and HM2 are sibling plants from the same self-pollinated parent. The roots of Br1091 and SFF are orange and the roots of HM1 and HM2 have purple exteriors and yellow interiors. Both populations were developed from the self-pollination of a single F_1 plant of each respective cross and the F_2 population was genotyped and phenotyped. For the Br1091xHM1 population, 84 F_3 families were generated and 11 individuals from each family grown out, phenotyped, and genotyped for QTL validation and support interval refinement. All plants were pot grown in the

greenhouse and screened for nematode resistance according to chapter 2 and also evaluated for color as outlined below.

Root and shoulder color phenotyping

Exterior root color in both F_2 populations was phenotyped as a binary trait with orange pigmentation on the exterior of the root coded "1" and no orange pigmentation (yellow roots) as "0". In the Br1091xHM1 F_3 population, orange pigmentation on the root was evaluated on a 0-3 scale: plants with no orange pigmentation were coded "0", plants with slight orange pigmentation were coded "1", plants that were pale orange were coded "2", and plants that were dark orange were coded "3" (Figure 3.1).

In the SFFxHM2 population purple shoulder color was evaluated on a 0-3 scale as follows: 0 with no purple shoulder, 1 with minimal or trace purple shoulder, 2 with obvious purple shoulder covering the whole circumference of the shoulder, and 3 with spreading purple beyond just the shoulder of the root. No shoulder color was evaluated in the Br1091xHM1 F_2 population, but F_3 individuals were evaluated on a binary scale. Purple shoulder and green shoulder were both independently phenotyped on individual roots as present or absent with no effort made to quantify the expression (Figure 3.1). If purple shoulder was present but green shoulder was not visible, the purple shoulder was slightly scratched to verify there was no green underneath the purple. For preliminary analysis in the Br1091xHM1 F_2 population, the percentage of F_3 individuals in each F_3 family with more than 3 roots was used as a phenotype in the F_2 map.

DNA extractions

DNA extractions, marker evaluations, and genetic map construction for both F_2 populations was performed as described in chapter 2. Phenotyped, vernalized roots were planted in pots in the University of Wisconsin, Department of Horticulture greenhouse at Arlington, Wisconsin. Leaf samples from plants that survived the vernalizing and transplanting process were harvested and lyophilized. DNA was extracted according to Murray and Thompson (1980), and quantified on 1% agarose gel electrophoresis.

Marker evaluations

AFLP reactions were performed according to Vivek and Simon (1999) with slight modifications to allow fluorescent evaluation described in chapter 2. Fluorescent SSR primers and PCR procedures were performed according to Cavagnaro et al. (2011) and Iorizzo et al. (2011). Additional SSR markers were developed in regions of interest using the assembled carrot genome and MISA 1.0 (MIcroSAtellite; http://pgrc.ipk-gatersleben.de/misa/) to identify SSRs and design primers (Table 3.1). AFLP and SSR markers were evaluated at the University of Wisconsin Biotechnology Center using an ABI 3730xl capillary sequencer. Polymorphic SSRs were identified by screening a subset of 10 individuals from each F_2 population. Select SSRs from the QTL regions of several traits identified in the Br1091xHM1 F_2 population were selected for evaluation on the entire F_3 population. GeneMarker version 1.5 was used to score alleles (SoftGenetics, State College, Pennsylvania).

SNPs were evaluated using the KASPar system (http://www.KBioscience.co.uk). The entire Br1091xHM1 F_2 population (138 individuals) and 12 individuals from SFFxHM2 population were evaluated on a panel of 4,000 SNPs previously developed by Iorizzo et al. (2013a). Selected polymorphic SNPs in the SFFxHM2 population were evaluated in the full population (113 individuals).

Genetic map construction

Linkage maps were constructed with JoinMap 3.0 software (Van Ooijen 2001) as previously described in chapter 2. Briefly, linkage groups were obtained at a LOD threshold >3.0. The regression mapping algorithm was used with Haldane's mapping function to calculate distances between markers and individuals related to the parents of each population were used to phase the markers. For the Br1091xHM1 F_3 population, the F_2 parents were used to phase the map. A bin map was developed with the Br1091xHM1 F_2 population. SNPs and SSRs with known chromosome locations were used to anchor the linkage groups (Iorizzo et al. 2013b; Cavagnaro et al. 2011; Iovene et al. 2011;Yildiz et al. 2013). After being assigned to chromosomes, linkage groups were oriented and numbered following the chromosome orientation and classification of Iovene et al. (2011).

Map merging

JoinMap version 3.0 was used to merge the maps. The F_2 consensus map was reported in chapter 2. QTL coordinates were transferred from the individual maps to the merged maps

according to the location of the nearest flanking markers mapped in each specific linkage map. To compare the Br1091xHM1 F_3 map to the merged F_2 map, SNPs or SSRs from the F_2 merged map closest to the SSRs analyzed in the F_3 according to the assembled carrot genome were used to link the maps (data not shown). MapChart version 2.2 was used to draw all chromosome diagrams (Voorrips 2002).

QTL mapping

QTL analysis was performed using R/qtl (Broman and Sen 2009) with interval mapping (using the multiple imputations method) or a binary model depending on the phenotype and population. QTL detection for each phenotype included preliminary QTL identification using scanone followed by QTL modeling. The largest LOD peak from the analysis was added to the QTL model and if the QTL model was significant, it was retained. This process was then repeated using addqtl, instead of scanone, followed by QTL modeling and testing for interactions until adding an additional QTL to the model was no longer significant. The support intervals were calculated using a 1.5 LOD drop.

QTL were named *trait-population-C_-Q_* where "*trait*" is the phenotype being analyzed, "*population*" is the population the QTL was identified in, "*C_*" is the chromosome on which the QTL was identified and "*Q_*" is the QTL identifier from the QTL model in the order of percent variation explained. For example, *Or-SFFHM2-C3-Q1* is a QTL for orange pigment accumulation, mapped in the SFFxHM2 population, is on chromosome 3, and explains the most variation in the model. "*F_2*" or "*F_3*" was added after "BrHM1" to differentiate the F_2 or F_3 population.

Results

Genetic linkage maps

The Br1091xHM1 linkage map, as reported in chapter 2, was generated from the evaluation of 138 individuals and included 389 SNP markers (Table 3.2, Figure 3.2). The total genetic distance covered was 563.3 cM with an average marker spacing of 1.5 cM. Markers on chromosome 9 displayed significant segregation distortion from the 1:2:1 expected ratio (data not shown).

The SFFxHM2 linkage map, as reported in chapter 2, was generated from the evaluation of 113 individuals and included 138 AFLP, SSR, and SNP markers (Table 3.2, Figure 3.3). The total genetic distance covered was 520.1 cM with an average marker spacing of 4.0 cM.

The Br1091xHM1 F_3 linkage map was generated from the evaluation of 497 individuals and included 48 SSR markers (Table 3.2, Figure 3.4). The total genetic distance covered 387.1 cM with an average marker spacing of 9.7 cM representing 8 chromosomes (chromosome 6 not represented).

Phenotypic ratios

Phenotypic counts and ratios for all traits are reported in table 3.3. While some of the ratios do not deviate significantly from expected segregation ratios for a single locus, coding the phenotypes for mapping as dominant markers did not result in successful mapping with JoinMap (data not shown).

QTL mapping

One QTL for orange versus yellow root color in the Br1091xHM1 F_2 population was located on chromosome 3 accounting for 50.6% of the variation (Table 3.4, Figure 3.5, Figure 3.6) with the orange pigmentation attributed to the Br1091 parent. QTL for orange versus yellow root color in the SFFxHM2 population were located on chromosomes 3, 5, and 7, with significant interaction between the QTL on chromosomes 3 and 5 with the multi-QTL model accounting for 61% of the variation (Table 3.4, Figure 3.5, Figure 3.7). The QTL on chromosomes 3 and 5 attributed orange pigmentation to the SFF parent while the QTL on chromosome 7 derived orange pigmentation from the HM2 parent. In the Br1091xHM1 F_3 population, one QTL for orange versus yellow root color was identified on chromosome 3, accounting for 75.5% of the variation with the same parental contributions as the QTL identified in the F_2 population (Table 3.4, Figure 3.8, Figure 3.7).

For purple shoulder pigmentation, one QTL was identified in the Br1091xHM1 F_2 population using F_3 family averages on chromosome 3, accounting for 42.4% of the variation (Table 3.4, Figure 3.5, Figure 3.6) with purple shoulder pigmentation attributed to the HM1 parent. QTL for

purple shoulder pigmentation in the SFFxHM2 population were located on chromosomes 3, 2, and 1 with the multi-QTL model accounting for 45.2% of the variation (Table 3.4, Figure 3.5, Figure 3.9). The QTL on chromosomes 2 and 3 attributed purple shoulder pigmentation to the HM2 parent while the QTL on chromosome 1 attributed purple pigmentation to the SFF parent. In the Br1091xHM1 F_3 population, one QTL for purple shoulder pigmentation was identified on chromosome 3, accounting for 30.9% of the variation with the same parental contributions as the QTL identified in the F_2 population (Table 3.4, Figure 3.8, Figure 3.6).

One QTL for green shoulder pigmentation was identified in the Br1091xHM1 F_2 population using F_3 family averages as the phenotype on chromosome 3, accounting for 23.9% of the variation (Table 3.4, Figure 3.5, Figure 3.6) with the green shoulder pigmentation attributed to the Br1091 parent. In the Br1091xHM1 F_3 population, one QTL was identified on chromosome 3, accounting for 20.2% of the variation with the same parental contributions as the QTL identified in the F_2 population (Table 3.4, Figure 3.8, Figure 3.6).

Discussion

Root color

Both F_2 populations identified a QTL at the top of chromosome 3 (Table 3.4, Figure 3.5) so SSRs on chromosome 3 were evaluated in the Br1091xHM1 F_3 population to validate this QTL. All three populations had significant QTL for orange versus yellow root color in this region (Figure 3.5, Figure 3.8). These QTL account for 50.6% and 43.1% of the variation for

OrBrHM1F$_2$-C3-Q1 and *Or-SFFHM2-C3-Q1* respectively while *Or-BrHM1F$_3$-C3-Q1* accounts for 75.5% of the variation with the orange pigmentation attributable to the non-HM parent in each population (Table 3.4). While the support intervals for the QTL identified in Br1091xHM1 and SFFxHM2 do not overlap, they are adjacent to each other with similar QTL effects attributed to the non-HM parent. These results could represent the same QTL with some phenotyping or mapping errors or small population sizes.

This QTL for orange versus yellow root color on chromosome 3 does not co-localize with the previously well characterized loci controlling orange and yellow pigmentation previously mapped (*Y* and *Y$_2$*, Just et al. 2007, Cavagnaro et al. 2011) but is near a QTL significant for phytoene, zeta carotene, lycopene, and beta carotene accumulation that co-localized near a carotenoid biosynthesis pathway gene *PSY2* (Santos and Simon 2002; Just et al. 2007). The orange pigmentation visually phenotyped here is associated with alpha and beta carotene accumulation, so the QTL causing the same phenotype previously identified at this chromosomal region could be the same QTL identified here. Given the lack of markers present in both maps and the many dominant AFLP markers that were used for the maps developed by Santos and Simon (2002) and Just et al. (2007) it is difficult to say if the QTL co-localize or not. The QTL previously identified was also a minor QTL after *Y* and *Y$_2$*, while the QTL identified here is the major QTL in each population. Given these constraints, it is still possible the QTL are the same and further analysis in each population, or crosses made between the populations would be needed to determine if they are in fact the same QTL. While *PSY2* is near the QTL region, it is beyond the support intervals for both Br1091xHM1 populations and is upstream in the

carotenoid biosynthesis pathway so while it could affect the total amount of pigmentation, it is not clear how it would affect the differential accumulation of carotenoids (Figure 3.10).

Besides the QTL identified on chromosome 3, two other QTL for orange versus yellow root color were identified in the SFFxHM2 population. *Or-SFFHM2-C5-Q2* accounts for 12.9% of the variation for orange pigmentation on chromosome 5 and the support intervals overlap a QTL previously identified (Santos and Simon 2002) and later determined to be the *Y* gene (Just et al. 2004; Cavagnaro et al. 2011) (Table 3.4, Figure 3.5). The QTL characterized previously by Santos and Simon (2002) affected total carotenes, alpha and beta carotenes and xanthophyll and this current work would represent a QTL affecting alpha and/or beta carotenes to xanthophyll accumulation. While it is difficult to compare the QTL effects in the two populations due to different phenotyping methods, the *Y* locus was identified as a major QTL interacting with Y_2 in the B492 x QAL population studied previously and that is not the case in this work because there was no significant QTL near Y_2, but it does interact with *Or-SFFHM2-C3-Q1*. The support interval for *Or-SFFHM2-C5-Q2* is also rather large, covering from 11-37 cM on the map, making it difficult to confirm if the QTL previously identified is the same as the one in this current work (Table 3.4).

Or-SFFHM2-C7-Q3 accounts for 7.9% of the variation for orange versus yellow root color and is on the same chromosome as Y_2, but not near it (Table 3.4, Figure 3.5, Just et al. 2004; Cavagnaro et al. 2011). For *Or-SFFHM2-C7-Q3*, the HM2 parent allele contributed to orange root color while the other two QTLs for orange pigmentation were attributed to the SFF parent.

The HM2 parent in this cross had purple exterior and yellow interior root color, so this QTL for orange pigmentation attributed to the HM2 parent might be related to overall carotenoid accumulation.

Shoulder color

Both F_2 populations identified a QTL at the bottom of chromosome 3 for purple pigment accumulation on the shoulder (Figure 3.5, Table 3.4). SSRs in the region on chromosome 3 were evaluated in the Br1091xHM1 F_3 population to validate this QTL. *PS-SFFHM2-C3-Q1* accounted for 22.1% of the variation, *PS-BrHM1F$_2$-C3-Q1* accounted for 42.5% of the variation and *PS-BrHM1F$_3$-C3-Q2* accounted for 30.9% of the variation for purple shoulder with the purple pigmentation attributed to the HM parent in each population (Figure 3.5, Figure 3.8, Table 3.4). 'Homs' cultivars typically have a thin purple layer of pigment on the root surface, and in these populations the purple pigment expression is mostly localized to the shoulder (except for a few individuals in each population with purple spreading down much of the root). The QTL in this region co-localizes to *P$_2$*, and a QTL detected for the percent purple on the exterior of the root characterized by Cavagnaro and Iorizzo (unpublished data). There are two *Myb* transcription factors in the region that activate genes in the anthocyanin pathway. With the phenotyping in this current work, the QTL that controls percent purple previously characterized by Cavagnaro and Iorizzo (unpublished data) is possibly the same QTL. If that were the case, *Myb* transcription factors are again possible candidate genes.

Besides the QTL on chromosome 3, two other QTL influencing purple pigmentation were identified in the SFFxHM2 population. *PS-SFFHM2-C1-Q2* accounted for 14.6% of the variation and *PS-SFFHM2-C2-Q3* accounted for 13.4% of the variation (Figure 3.5, Table 3.4). While *PS-SFFHM2-C2-Q3* attributed the purple pigmentation to the HM2 parent, *PS-SFFHM2-C1-Q2* attributed the purple pigmentation to the SFF parent. SFF is typically pale orange with no noticeable purple pigmentation. Upon further analysis, the QTL on chromosomes 2 and 3 appear to affect the presence or absence of purple pigmentation while the QTL on chromosome 1 affects the amount of purple pigmentation. When using a binary analysis with phenotypic scores of 1-3 all coded as 1, only the QTL on chromosomes 2 and 3 are detected (data not shown). It should also be noted that when looking at purple shoulder as a 2 part model, the LOD peak at the QTL on chromosome 3 shows significance for the presence or absence of purple pigmentation while the LOD peak at the QTL on chromosome 1 shows significance for the amount of purple pigmentation (Figure 3.11). *PS-SFFHM2-C1-Q2* could affect the amount or extent of shoulder gene expression and that would account for the observed gene action.

PS-SFFHM2-C1-Q2 is in the same region as a QTL for the percent of purple covering the exterior of the root identified by Cavagnaro and Iorizzo (unpublished data) and given the similar phenotyping methods, could be the same QTL. No previously mapped anthocyanin biosynthesis genes are in this region. *PS-SFFHM2-C2-Q3* on chromosome 2 has a wide support interval, but the support intervals cover *LDOX2* as mapped by Yildiz et al. (2013).

A QTL for green versus non-green shoulder was also detected in the Br1091xHM1 populations in the same region as the QTL for purple shoulder accounting for 23.9% and 20.2% of the variation in the F_2 and F_3 populations, respectively, with the green shoulder coming from the Br1091 parent (Figure 3.8, Table 3.4). Both purple shoulder and green shoulder can be present on the same root and the QTL for the two traits co-localized in the Br1091xHM1 populations (Figure 3.12). The purple shoulder pigmentation can cover the green shoulder making phenotyping difficult (data not shown) so care was taken to scratch any roots with purple shoulders to determine if green color is also present. Often the green color was observed to spread outside the region of the purple shoulder, or if a purple shoulder was scratched a green layer would be visible between the purple layer and the underlying root color. Because the anthocyanin and chlorophyll pathways are not closely related, the QTL are likely two separate, closely linked genomic regions. Shoulder trait phenotypes are difficult to evaluate and expression of shoulder pigmentation can also be related to sun exposure (Simon 1996). QTL for shoulder pigmentation expression might be related to carrot growth habit and the pigmentation due to sun exposure resulting in the same phenotype, but a very different mechanism than pigment biosynthesis candidate genes. The roots evaluated here were all evaluated after harvest, so exposure above the soil line was not noted.

Because the Br1091xHM1 F_3 families were used to provide an F_2 phenotype, it was not surprising the QTLs for purple and green pigmentation were validated in the F_3 individuals, but the QTL position for purple shoulder was found at a different location and the support intervals for purple shoulder and green shoulder were refined. The large QTL support intervals in the F_2

population and the altered position of the purple shoulder QTL from the F_2 to the F_3 generation are likely the result of a small population size in the F_2 (N=83). This effect of population size was reported by other researchers (e.g. Stange et al. 2013). Based upon this rationale, increasing the population size from N=83 in the F_2 population to N=471 in the F_3 population would be predicted to provide map locations and QTL support intervals that are more reliable, so the map position of *PS-BrHM1F$_3$-C3-Q1* is likely more reliable than *PS-BrHM1F$_2$-C3-Q1* (Table 3.4). The F_3 families were used to approximate the F_2 individual phenotype, but the increased resolution of evaluating the F_3 individuals is probably more reliable than the F_2 individuals. Given the large population size in the F_3 population, further genotyping in the QTL regions could help refine the support intervals further (Stange et al. 2013).

Comparison to Meloidogyne incognita resistance QTL

Both populations were previously characterized for QTL conferring resistance to *Meloidogyne incognita* root-knot nematode resistance, and while several QTL were identified in that study as well as this current work, only one region has linked QTL within a population. In the SFFxHM2 population *PS-SFFHM2-C2-Q3* overlaps with *Mi-SFFHM2-C2-Q3* (the support interval for *Mi-SFFHM2-C2-Q3* covers from 4 to 66 cM on the merged map). The nematode resistance QTL has a very large support interval and is relatively weak compared to other QTL identified, but that QTL is linked to the purple shoulder QTL identified here in the same population (Chapter 2). In this chromosomal region, the HM2 parent contributes both to purple shoulder and nematode resistance, so while purple shoulder could be used to contribute to the selection of more nematode resistant carrots, given the quantitative nature of both traits, little progress may

be made. The average nematode score for roots in the SFFxHM2 population with purple shoulders compared to those without are both 3.9 (data not shown). Br1091xHM1 also has a QTL in this region, but comparing QTL for different traits in different populations is difficult and often QTL are population specific. There are also linked QTL on chromosome 1 with support intervals for *PS-SFFHM2-C1-Q2* and *Mi-BrHM1-C1-Q3* overlapping, but the QTL were identified in different populations, again making any extrapolation of selecting for purple shoulder being linked to *M. incognita* resistance tenuous.

Conclusions

Two F_2 populations segregated for root color and shoulder color with only one QTL identified for each trait in the Br1091xHM1 populations. The populations were originally evaluated for *M. incognita* root-knot nematode resistance and observations were also made for these other segregating traits. Because the populations were not designed for studying color traits, binary analysis was used for several of the traits that might reduce the complexity of the trait and not accurately represent the phenotypes in the populations. Despite these challenges, this work lays a foundation for future work. For orange versus yellow pigmentation, the QTLs identified on chromosome 3 in each population represent major QTL accounting for a majority of the phenotypic variation in the population and the only previously identified QTL in the region had a minor effect. To better delineate the genetic control of purple shoulder color, more work will be needed to discern if the QTLs identified here are the same as P_2 previously characterized. The Br1091xHM1 population represents an ideal population to study these QTL because each trait had only one significant QTL so there are fewer confounding factors than other populations in which *Y* and Y_2 segregate, dominating the expression of root color. To further study both traits,

evaluations should be pursued in F_3 or F_4 families that are segregating for the QTL regions on chromosome 3, and homozygous for as much of the rest of the genome as possible. Fine mapping and further QTL characterization should include both visual phenotypes as well as HPLC analysis for carotenes and shoulder expressed anthocyanins. The complete sequencing of the carrot genome will provide a resource to develop markers for fine mapping as well as aid identification of candidate genes. This research lays a foundation for future studies in these areas and validates the use of simple visual color phenotyping for QTL analysis in carrot.

Figure 3.1 A Br1091xHM1 F_3 family that displays the range of root and shoulder color phenotypes. Using the 0-3 phenotyping scale for Br1091xHM1, roots 2, 3, and 4 (left to right) are orange (3), roots 8 and 11 are pale orange (2), roots 5 and 9 are very pale orange (1), and roots 1, 6, 7, and 10 are yellow (0, no visible orange pigment). Shoulder color is not readily discernable but root 4 only has a purple shoulder, roots 3 and 11 only have green shoulders, and the rest have both green and purple shoulder.

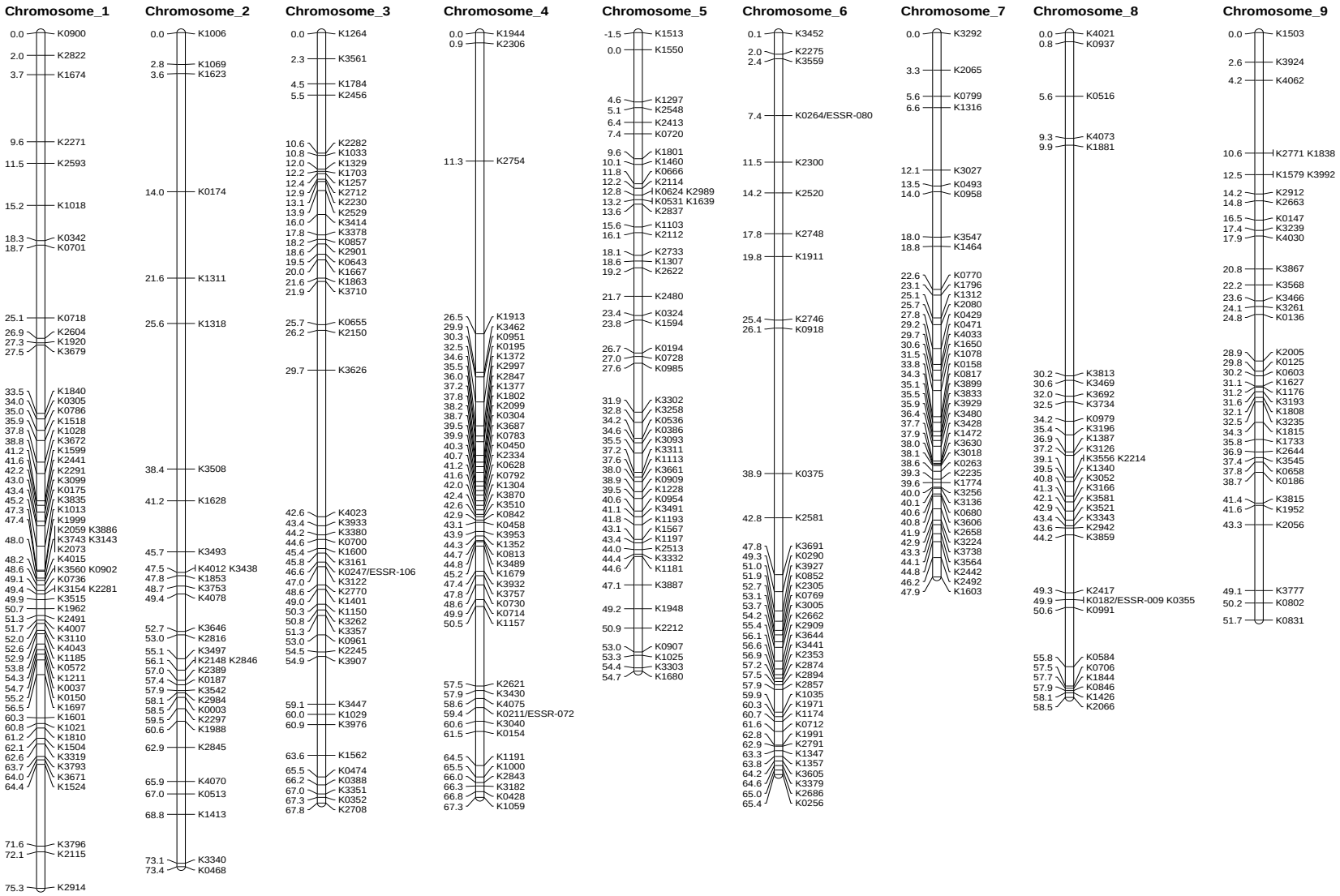

Figure 3.2 Linkage map for the F_2 mapping population Br1091xHM1 (Chapter 2, this work).

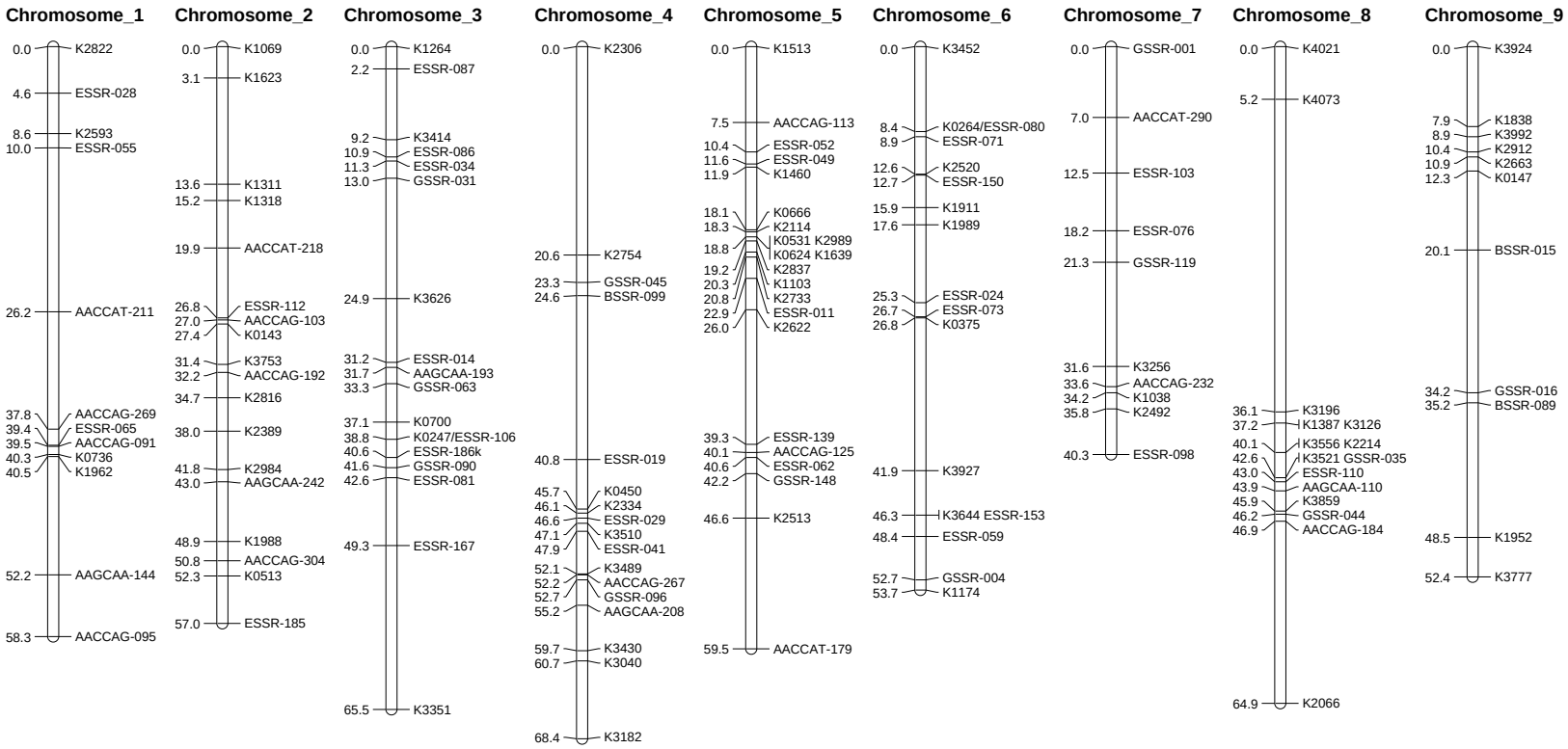

Figure 3.3 Linkage map for the F_2 mapping population SFFxHM2 (Chapter 2, this work).

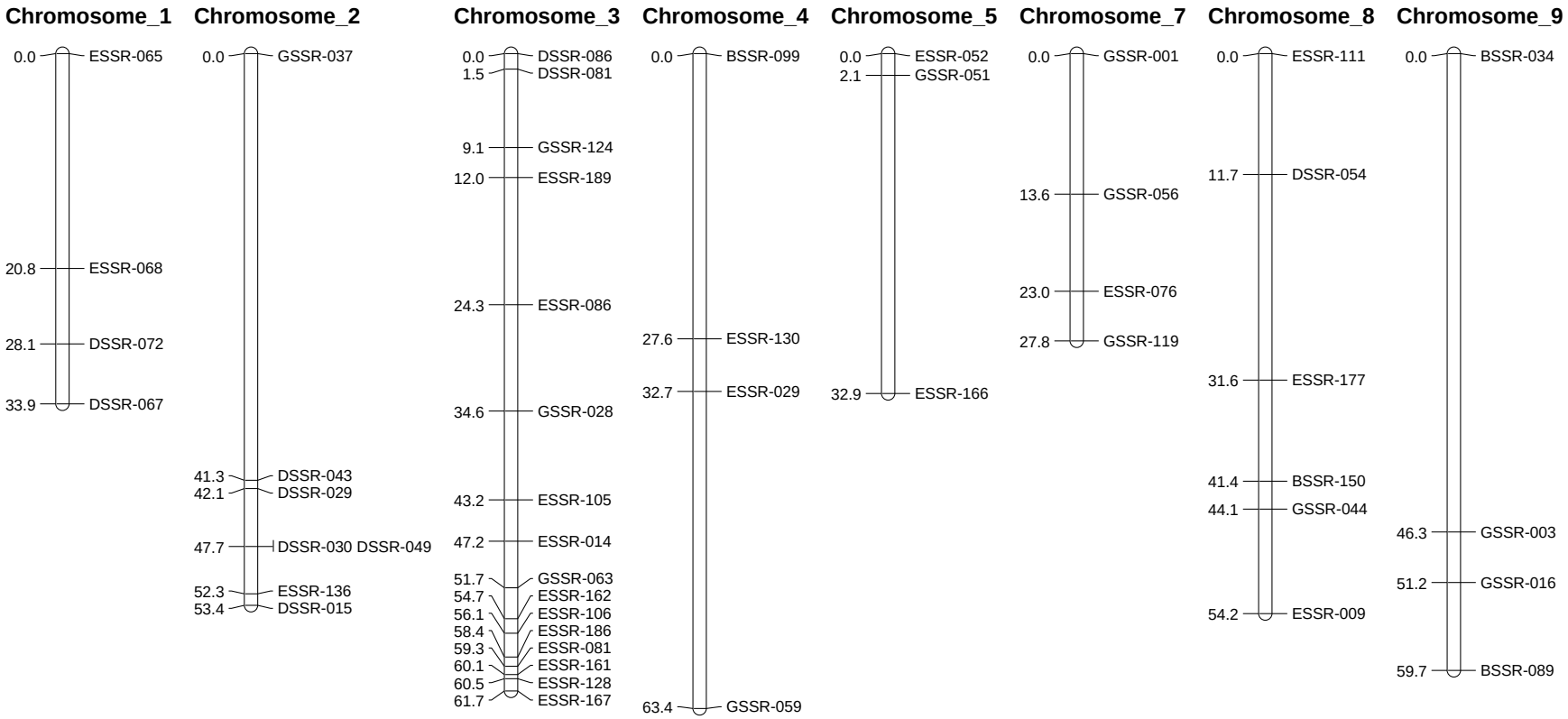

Figure 3.4 Linkage map for the F_3 mapping population Br1091xHM1.

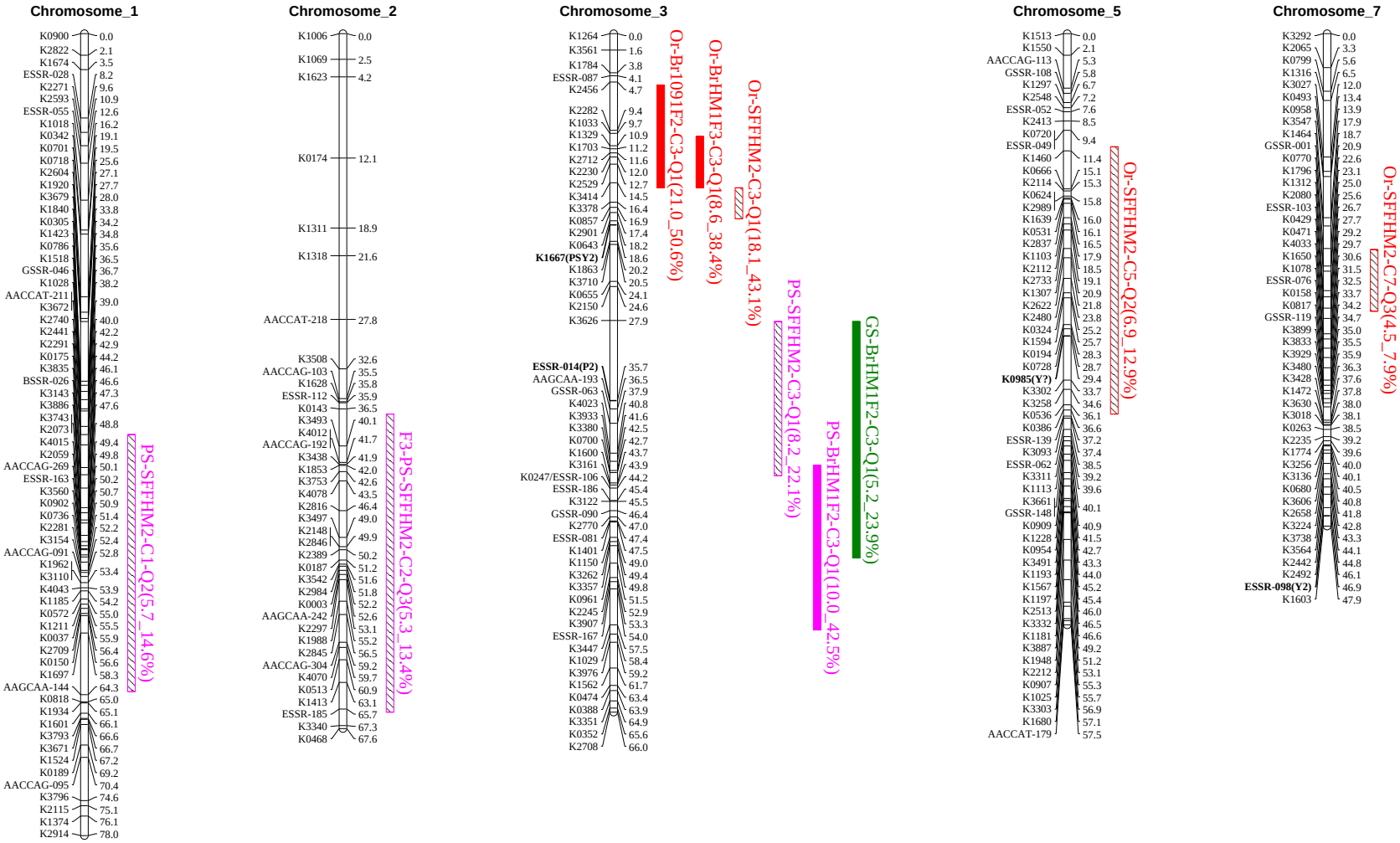

Figure 3.5 F_2 merged map with all QTL identified in this work from the F_2 populations Br1091xHM1 and SFFxHM2.

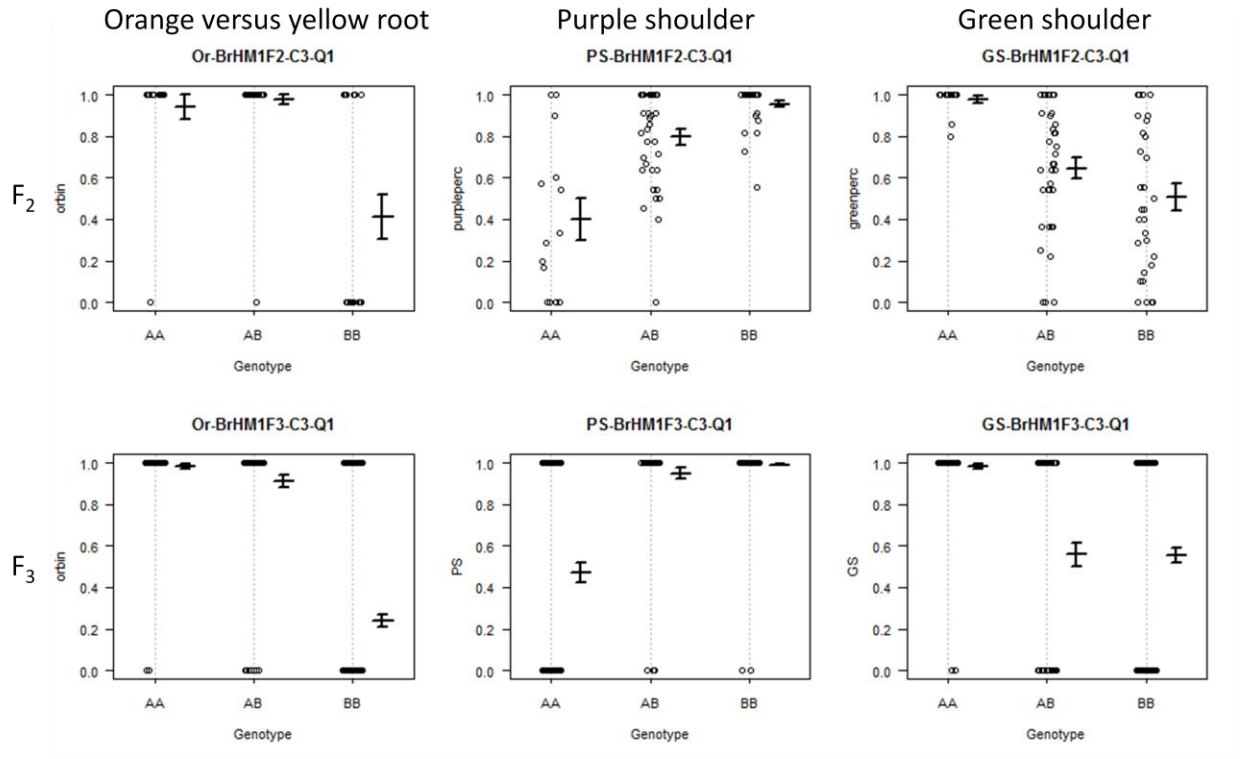

Figure 3.6 Phenotypic scores in the Br1091xHM1 population for orange versus yellow root color, purple shoulder, and green shoulder in the F_2 and F_3 populations of AA, AB, and BB individuals at the significant QTL. The bars are ± 1 standard error.

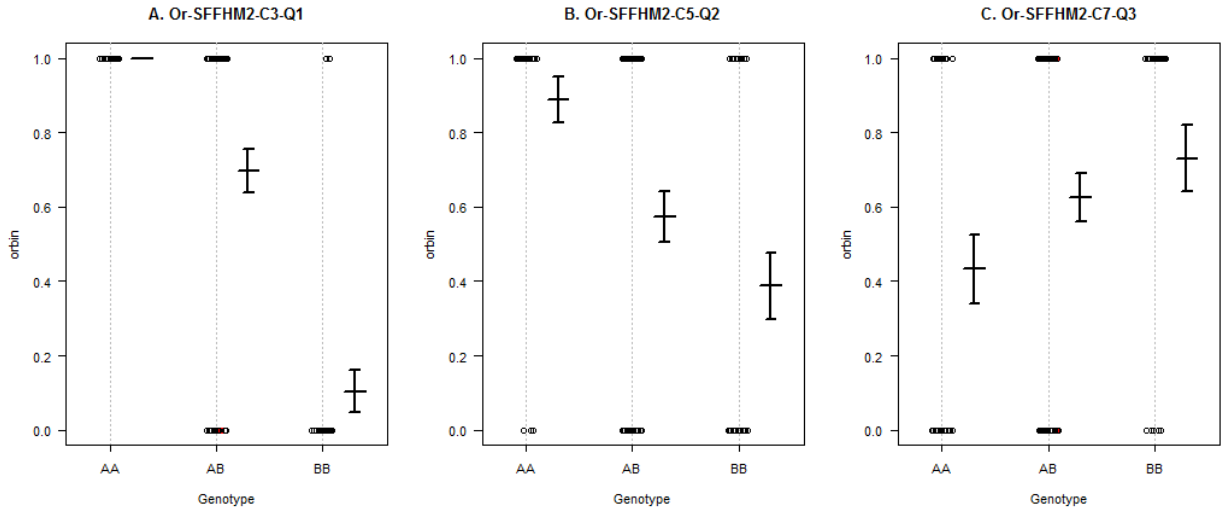

Figure 3.7 Phenotypic scores in the SFFxHM2 population for orange (1) versus yellow (0) exterior root color of AA, AB, and BB individuals on chromosomes (**A**) 3, (**B**) 5, and (**C**) 7. The bars are ± 1 standard error.

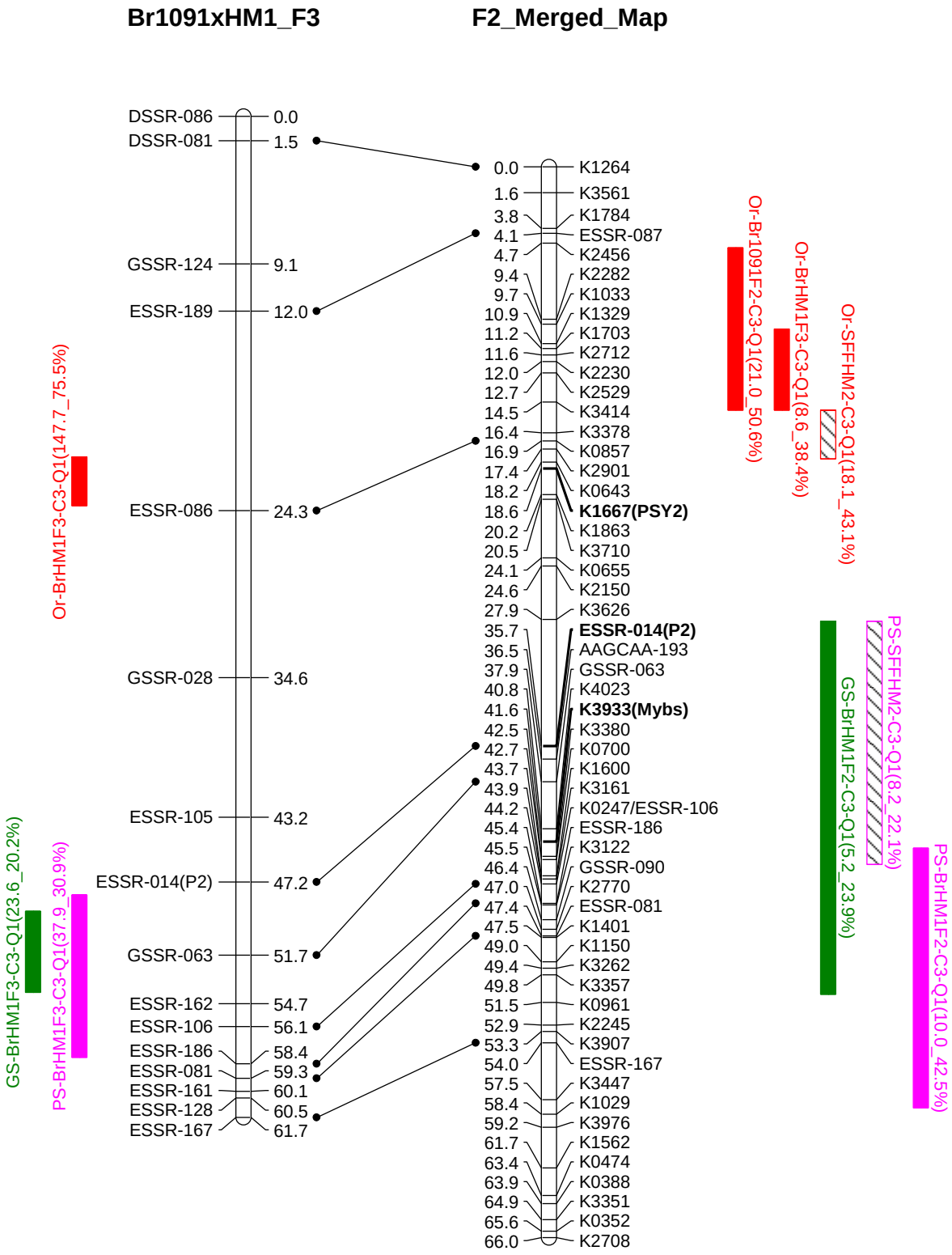

Figure 3.8 Br1091xHM1 F_3 map on the left and F_2 merged map on the right comparing the QTL regions for chromosome 3.

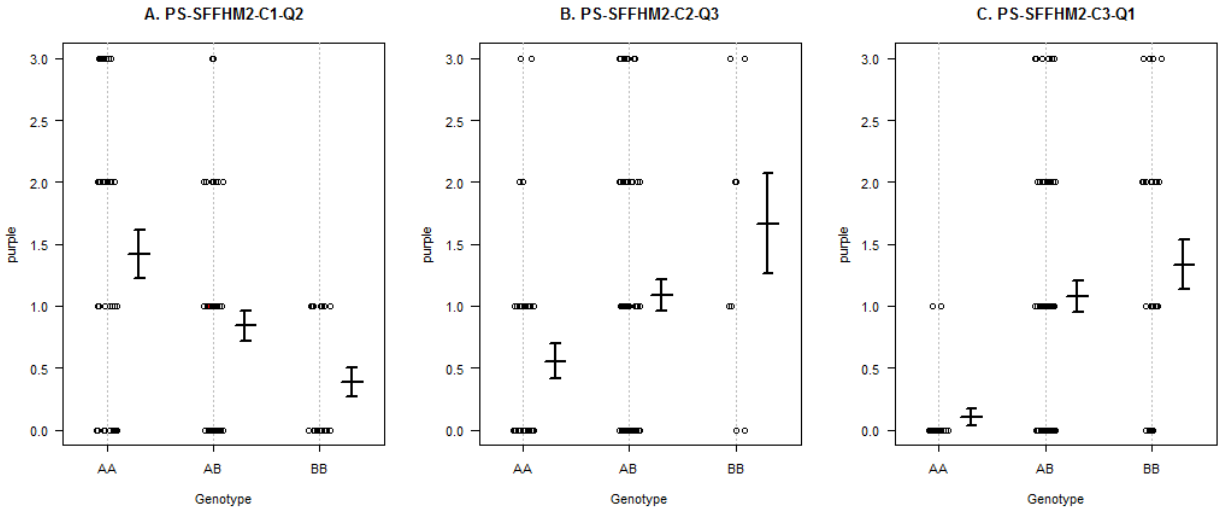

Figure 3.9 Phenotypic scores in the SFFxHM2 population for purple shoulder (0 to 3 scale with 0 being no purple shoulder and 3 being purple shoulder spreading to the rest of the root) of AA, AB, and BB individuals on chromosomes (**A**) 1, (**B**) 2, and (**C**) 3. The horizontal lines represent the population mean and the bars are ± 1 standard error.

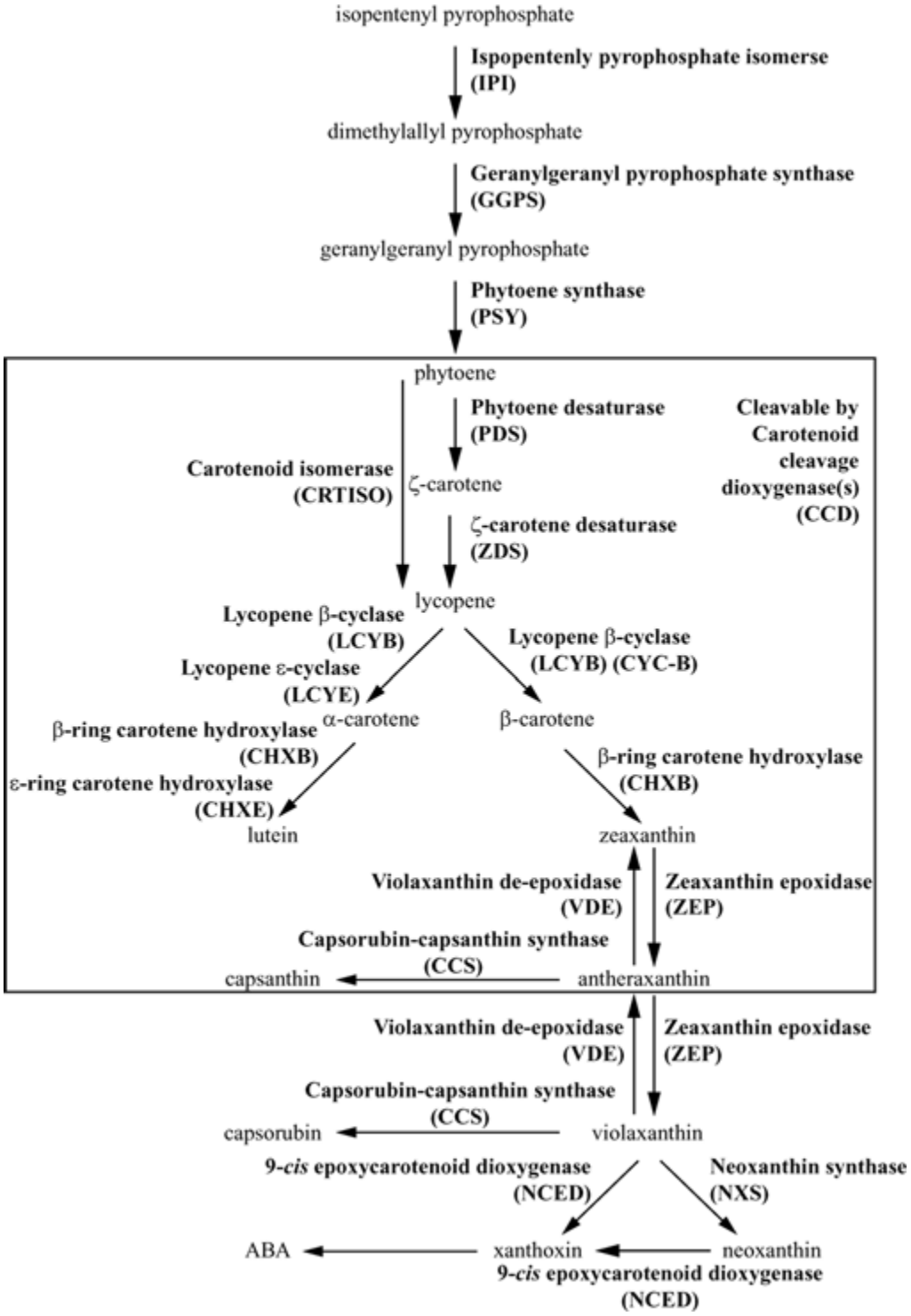

Figure 3.10 Carotenoid biosynthetic pathway. *PSY2* is upstream of the differentiation of carotenes.

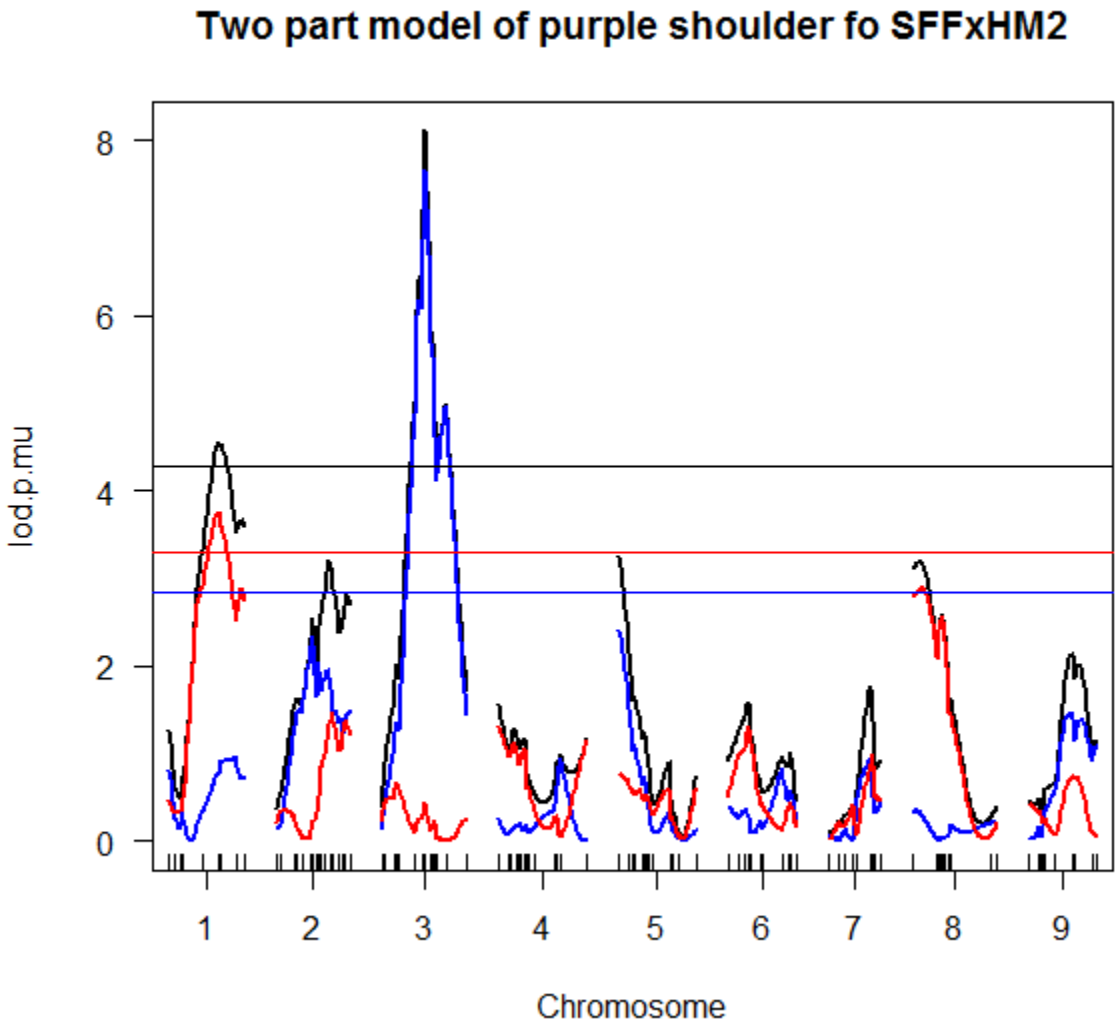

Figure 3.11 Graph of the two-part model LOD scores for purple shoulder in the SFFxHM2 population. The blue lines represent the LOD score for the presence or absence of purple shoulder (similar to a binary analysis), the red lines represent the LOD score for the amount of purple on the roots (on a 0-3 scale) that have purple shoulders, and the black line is the total LOD. The horizontal lines represent the 10% LOD thresholds after 1000 permutations of the respective colors.

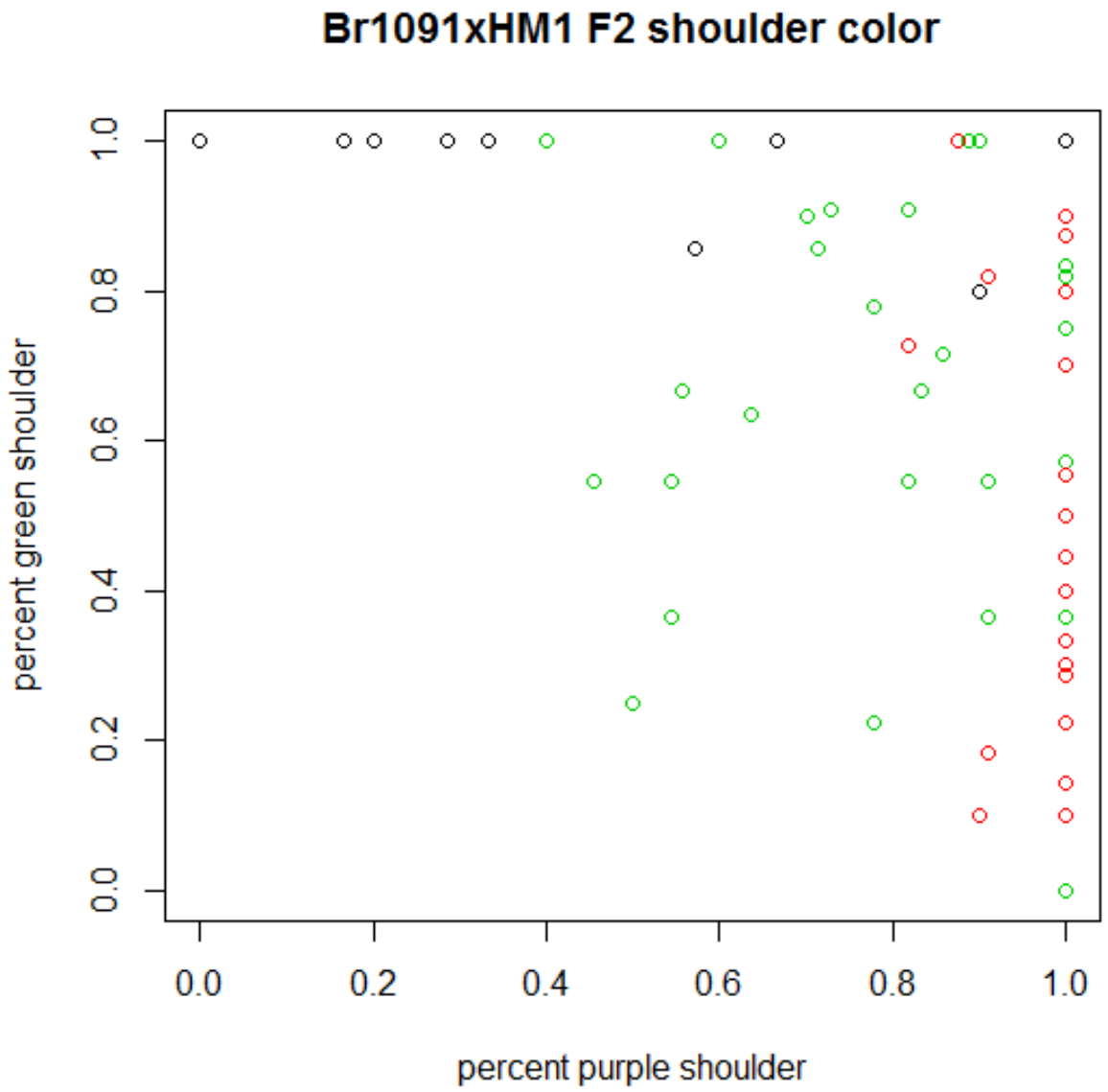

Figure 3.12 Plot of the Br1091xHM1 F_2 population with the percent of plants with green shoulder color in each F_3 family plotted against the percent of plants with purple shoulder color in each F_3 family. The black circles represent HM1 alleles, the red circles Br1091 alleles, and the green circles are heterozygous.

Table 3.1 Primers designed for use in the Br1091xHM1 F_3 population.

SSR	Motif	Forward Primer	Reverse Primer	Tm (°C)	Product Size, nt
DSSR-015	(TAT)12	AGGGAACTTTGGAGAAGAAG	ATATCAAACGGGAAACGTAA	60-55*	269
DSSR-029	(CA)9	CGTTTTTCTCAATCTTCACC	CAGACCCGATTTCACACTAT	60-55*	335
DSSR-030	(TGT)4	AGTTTCTTGCAAAAGCTGAA	GGATAAGTCAACTGCTTTGG	60-55*	298
DSSR-043	(GA)8	GGACGCACAAATATAAATCC	CTCTCAAGAATTTCCGTCAA	60-55*	248
DSSR-049	(ATC)4	TGTGTTAAATCTGGACGTGA	CTGATTTGTGCCATGAGTAA	60-55*	225
DSSR-054	(TG)7 (AT)19	AATTTGCTCCAAAATGGTTA	ATGAAGAAAATGCTCCAAGA	60-55*	418
DSSR-067	(AG)27	CGTGTTAATTAAATGCACGA	GTTGACACCAAACAGGAAAT	60-55*	262
DSSR-072	(AC)8	TATGGAGCATGGTGAACATA	TAATGGCTTCAGTTCCCTAA	60-55*	337
DSSR-081	(AT)6	TTCTTTGTCTTTCCCTTTGA	AATGGCAGCTTCAGAGTTAG	60-55*	296
DSSR-086	(CT)7 (TAC)4 (TAT)4	GCAGAGAAAGTCCAGTGAAG	TGGTTTGGTCTGGTTTAGTC	60-55*	346

*Touchdown PCR was used for all PCR reactions from 60°C to 55°C

Table 3.2 Summaries of the linkage maps for populations Br1091xHM1 F_2, SFFxHM2, and Br1091xHM1 F_3.

	Br1091xHM1 F_2	SFFxHM2	Br1091xHM1 F_3
Number of individuals	138	113	497
Number of polymorphic markers	389	138	48
AFLPs	0	20	0
SSRs	0	47	48
SNPs	389	71	0
Total distance (cM)	563.3	520.1	387.1
Average intermarker spacing (cM)	1.5	4.0	9.7
Maximum marker spacing (cM)	20.3	30.9	46.3

Table 3.3 Phenotypic scores for all traits treated as binary traits using a single gene model for the chi-square test. "F_2 from F_3" indicates the presence of the trait in at least one root from the F_3 family derived from the F_2.

Population	Trait	Total phenotyped	Present	Absent	Expected present	Expected absent	chi-square
SFFxHM2							
	Orange versus yellow root	112	67	45	84	28	2.0E-4
	Purple shoulder	112	64	48	84	28	1.3E-5
Br1091xHM1							
	Orange versus yellow root						
	F_2	137	108	29	102.75	34.25	0.30
	F_3	483	305	178	301.875	181.125	0.77
	Purple shoulder						
	F_2 from F_3	83	78	5	62.25	20.75	6.5E-5
	F_3	471	399	72	353.25	117.75	1.1E-6
	Green shoulder						
	F_2 from F_3	83	76	7	62.25	20.75	4.9E-4
	F_3	471	320	151	353.25	117.75	4.0E-4

Table 3.4 Chromosomal locations and effects of QTL for root color in three carrot populations.

(Population) Chromosome	QTL	Position (cM)	LOD	% VE[a]	Parental effect[b]	Closest marker	Marker interval	1.5 LOD[c]
(Br1091xHM1 F_2)								
Orange Root								
3	*Or-BrHM1F2-C3-Q1*	9.4	21	50.6	Br1091	K2282	K2456 - K3414	5 - 15
Purple shoulder								
3	*PS-BrHM1F2-C3-Q1*	51.5	10	42.5	HM1	K0961	K3933-K3447	42 - 58
Green shoulder								
3	*GS-BrHM1F2-C3-Q1*	42.7	5.2	23.9	Br1091	K0700	K3626-K0961	28 - 51
(SFFxHM2)								
Orange Root								
3	*Or-SFFHM2-C3-Q1*	16.4	18.1	43.1	SFF	K3378	K3414 - K0643	15 - 18
5	*Or-SFFHM2-C5-Q2*	21.8	6.9	12.9	SFF	K2622	K1460 - ESSR-139	11 - 37
7	*Or-SFFHM2-C7-Q3*	0.0	4.5	7.9	HM2	GSSR-001	GSSR-001 - ESSR-103	21-27
3*5			2.7	4.7				
	% Variation explained by multi-QTL model = 61.0%							
Purple shoulder								
1	*PS-SFFHM2-C1-Q2*	51.4	5.7	14.6	SFF	K0736	AACCAT-211 - AAGCAA-144	39 - 64
2	*PS-SFFHM2-C2-Q3*	46.4	5.3	13.4	HM2	K2816	K0143 - ESSR-185	37 - 66
3	*PS-SFFHM2-C3-Q1*	35.7	8.2	22.1	HM2	ESSR-014	K3626 - K0700	28 - 43
	% Variation explained by multi-QTL model = 45.2%							
(Br1091xHM1 F_3)								
Orange								
3	*Or-BrHM1F3-C3-Q1*	21.7	147.7	75.5	Br1091	ESSR-086	ESSR-086 - ESSR-189	21-24
Purple Shoulder								
3	*PS-BrHM1F3-C3-Q1*	53.7	37.9	30.9	HM1	ESSR-162	ESSR-186 - ESSR-014	48-58
Green Shoulder								
3	*GS-BrHM1F3-C3-Q1*	50.7	23.6	20.2	Br1091	GSSR-063	ESSR-162 - ESSR-014	49-54

[a]Percentage of the variation explained

[b]The parent allele responsible for the trait (eg. Br1091 is the parent that contributes to orange root color)

[c]1.5 LOD support interval (cM) (Broman and Sen 2009)

Chapter 4

QTL for early flowering and root cracking in carrot

Introduction

Carrot (*Daucus carota*) is a major root crop worldwide, yet little genetic characterization of root morphology or flowering traits has been performed to date. 34,000 hectares of carrots are produced in the US (USDA, National Agricultural Statistics Service, 2001) and many of these carrots are consumed as "baby" or "cut-and-peel" carrots providing a convenient source of fresh nutrients to consumers (Simon et al. 2008). Given the fresh nature of the product, carrot root quality as it relates to texture, color, and taste is extremely important. Carrot displays a wide variety of root shapes, colors, and quality traits (ex. Rubatzky et al. 1999) but only a few, major traits have been characterized with a focus on color. One trait related to root structure is root cracking, which was characterized in a 'Nantes' type cultivar as a dominant trait, best characterized after mechanical pricking of the root with a knife (Dickson 1966).

Floral initiation also affects root quality since the carrot root rapidly lignifies during flowering (Rubatzky et al. 1999). Carrot has traditionally been viewed as a biennial crop requiring exposure to a period of cold (vernalization) to induce flowering in temperate germplasm, but annual habit is prevalent in tropical accessions (Rubatzky et al. 1999). When plant breeders for temperate production utilize tropical germplasm for disease resistance, vigor, and other traits, annual flowering is typically observed in the progeny and biennial habit must be selected.

Genetic control of flowering has been examined in many plants, but most extensively in Arabidopsis (*Arabidopsis thaliana*) and maize (*Zea maize*). In crops requiring vernalization, exposure of the plant to cold temperatures is required to ensure seed set. The vernalization pathway is best characterized in Arabidopsis, and many crops have a similar pattern. In

Arabidopsis, *Flowering Locus C* (*FLC*) is a MADS-box transcription factor that inhibits flowering. *Frigida* (*FRI*) promotes up-regulation of *FLC* and is down-regulated during cold temperatures, with colder temperatures or longer exposure to mild temperatures fulfilling the vernalization requirement of the plant. This down-regulation of *FRI* leads to the down-regulation of *FLC*, which remains down-regulated after returning to warmer conditions, allowing flowering to proceed (Michaels and Amasino 1999). Genes important in the vernalization pathway have also been identified in several other species with a similar pattern of vernalization (summarized by Cockram et al. 2007).

After vernalization, and in crops that do not require vernalization to flower, days to flowering genes influence floral initiation timing. Many crops have a wide range of flowering times with diverse genetic backgrounds across diverse environmental conditions relying on several QTL for flower initiation. Days to flowering is the number of days after planting until flower initiation begins, normally phenotyped as the first visual sign of flowering. In Arabidopsis, flowering time genes appear to be due to relatively large effect alleles with many of the individual QTL effects exceeding one day (Salomé et al. 2011), while in maize, there are many, small effect alleles with very few individual QTL effects exceeding one day (Buckler et al. 2009). The comparison is even greater when looking at the effect of individual genes in the context of the development of the crop. With Arabidopsis having a much shorter life cycle than maize, a QTL effect of one day is a much larger effect on the life cycle of Arabidopsis than a QTL effect of one day in maize. This differences between Arabidopsis and maize is thought to typify differences in flowering times of self-pollinated and cross-pollinated crops more generally, and is proposed to have evolutionary advantages. With self-pollinated crops, synchronous flowering with neighboring

plants is not very important because they are not needed for fertilization. With cross-pollinated crops, synchronous flowering is very important for fertilization and numerous, small effect alleles allow a gradient of flowering times to ensure plants have partially synchronous flowering times with neighboring plants. This, in turn, contributes to cross-pollination and pollen flow (Buckler et al. 2009).

Vernalization requirement in carrot was characterized in one genetic background as a single, dominant gene (Alessandro and Galmarini 2007) and mapped to chromosome 2 (Alessandro et al. 2013). Evaluations for internode elongation, the first visible sign of flower initiation, were made weekly in the first year of growth in an F_2 population from a cross between an annual cultivar and a biennial cultivar resulting in segregation ratios that did not deviate from those expected for a single, dominant locus. While this is an important finding, the authors speculated that there are additional genes controlling flowering across diverse carrot genetic backgrounds and diverse populations derived from temperate x subtropical intercrosses of carrot exhibit quantitative segregation for both annual growth habit and days to flowering in both annual and biennial types (unpublished data).

In the course of evaluating nematode resistance in progeny of two diverse breeding stocks of carrots, patterns of inheritance were observed for root cracking and early flowering. This work was undertaken to begin characterizing days to flowering QTL through indirect phenotyping methods in a population previously characterized for *Meloidogyne incognita* root-knot nematode resistance (Chapter 2). This research lays the foundation for future research and validation of inheritance patterns observed for these traits.

Materials and methods

Plant materials

An F_2 population segregating and evaluated for *M. incognita* root-knot nematode resistance was also segregating for early flowering, root cracking, and annual growth habit. Br1091xHM1 is a population derived from the cross between the Brasilian cultivar 'Brasilia 1091' (Br1091) and the Syrian cultivar 'Homs' (HM1). The F_2 population (N=138) was developed from the self-pollination of a single F_1 plant, 103 F_3 families were derived from the self-pollination of individual F_2 plants, and 32 F_4 families were derived from the self-pollination of individual F_3 plants. All F_2 and F_3 plants used for genotyping were grown in pots in the greenhouse at the University of California, Riverside, California, and screened for nematode resistance as outlined in chapter 2 and also evaluated for root cracking and flowering characteristics as described below. Thirty eight F_3 and 32 F_4 families, derived from different F_2 plants, were also grown under field conditions on Paul Miller's Farm in Hancock, WI and evaluated for early flowering.

Phenotyping

Root cracking (RC) was phenotyped in F_3 individuals pot grown in the greenhouse as the presence or absence of longitudinal growth cracks on the primary root (these were the same roots used in the root-knot nematode evaluation from chapter 2). No effort was made to quantify the severity of the cracking. F_3 family averages were used to represent the phenotype for the F_2 genotypes. Annual growth habit (AH) was phenotyped in the same F_3 individuals as root cracking as the presence of limited storage root formation and was accompanied by early

flowering (Figure 4.1). F_3 family averages were used to represent the phenotype for the F_2 genotypes. Early flowering in the greenhouse (EFG) was phenotyped in the F_2 generation as a binary trait and represented plants that did not require vernalization and initiated flowering before being planted in the greenhouse in Arlington, WI for seed production. Early flowering in the field (EFF) was phenotyped as plants that did not require vernalization and initiated flowering during the growing season with at least visibly elongated internodes at the time the field plots were harvested. F_3 or F_4 family averages were used to represent the phenotype for the F_2 or F_3 genotype, respectively.

DNA extractions and marker evaluation

DNA extractions, marker evaluations, and genetic map construction for the F_2 population was performed as described in chapter 2. Phenotyped, vernalized roots were planted in pots in the University of Wisconsin, Department of Horticulture greenhouse at Arlington, Wisconsin. Leaf samples from plants that survived the vernalizing and transplanting process were harvested and lyophilized. DNA was extracted according to Murray and Thompson (1980), and quantified on 1% agarose gel electrophoresis.

Marker evaluations

Fluorescent SSR primers and PCR procedures were performed according to Cavagnaro et al. (2011), Iorizzo et al. (2011), and chapter 3. SSR markers were evaluated at the University of Wisconsin Biotechnology Center using an ABI 3730xl capillary sequencer. Polymorphic SSRs were identified by screening a subset of 10 individuals from the F_2 population. Select SSRs from

the QTL regions of several traits identified in the Br1091xHM1 F_2 population were selected for evaluation in the entire F_3 population. GeneMarker version 1.5 was used to score alleles (SoftGenetics, State College, Pennsylvania).

SNPs were evaluated using the KASPar system (http://www.KBioscience.co.uk). The entire Br1091xHM1 F_2 population (138 individuals) was evaluated on a panel of 4,000 SNPs previously developed by Iorizzo et al. (2013a).

Genetic map construction

Linkage maps were constructed with JoinMap 3.0 software (Van Ooijen 2001) as previously described in chapter 2. Briefly, linkage groups were obtained at a LOD threshold >3.0. The regression mapping algorithm was used with Haldane's mapping function to calculate distances between markers and individuals related to the parents of each population were used to phase the markers. A bin map was developed with the Br1091xHM1 F_2 population. For the Br1091xHM1 F_3 population, the F_2 parents were used to phase the map. SNPs and SSRs with known chromosome locations were used to anchor the linkage groups (Iorizzo et al. 2013b; Cavagnaro et al. 2011; Iovene et al. 2011;Yildiz et al. 2013). After being assigned to chromosomes, linkage groups were oriented and numbered following the chromosome orientation and classification of Iovene et al. (2011).

To analyze early flowering in the field, a combination of data from F_2 and F_3 genotypes was used, representing the F_3 and F_4 families phenotyped, respectively. Markers used in the F_2 map construction differed from those evaluated in the F_3 population, so markers from the F_2 map that were closest to markers in the F_3 map according to the assembled carrot genome were used as the same marker as in the F_3 map, and the F_3 map order and distances were used in the combined map.

QTL mapping

QTL analysis was performed using R/qtl (Broman and Sen 2009) with interval mapping (using the multiple imputations method) or a binary model depending on the phenotype and population. QTL detection for each phenotype included preliminary QTL identification using scanone followed by QTL modeling. The largest LOD peak from the analysis was added to the QTL model and if the QTL model was significant, it was retained. This process was then repeated using addqtl, instead of scanone, followed by QTL modeling and testing for interactions until adding additional QTL to the model was no longer significant. The support intervals were calculated using a 1.5 LOD drop.

QTL were named *trait-population-C_-Q_* where "*trait*" is the phenotype being analyzed, "*population*" is the population the QTL was identified in, "*C_*" is the chromosome on which the QTL was identified and "*Q_*" is the QTL identifier from the QTL model in the order of percent variation explained. "F_2" or "F_3" was added after "BrHM1" to differentiate the F_2or F_3

population. For example, *Cr-BrHM1F$_2$-C5-Q1* is a QTL for root cracking, mapped in the Br1091xHM1 F_2 population, is on chromosome 5, and explains the most variation in the model.

Results

Genetic linkage maps

The Br1091xHM1 linkage map as reported in chapter 2 was generated from the evaluation of 138 individuals and included 389 SNP markers (Table 4.1, Figure 4.2). The total genetic distance covered 563.3 cM with an average marker spacing of 1.5 cM. Markers on chromosome 9 displayed significant segregation distortion from the 1:2:1 expected ratio (data not shown).

The Br1091xHM1 F_3 linkage map was generated from the evaluation of 497 individuals and included 48 SSR markers (Table 4.1, Figure 4.3). The total genetic distance covered 387.1 cM with an average marker spacing of 9.7 cM representing 8 chromosomes (chromosome 6 not represented). The combined F_2 and F_3 map generated to analyze early flowering in the field used the same F_3 map and included 70 individuals, 38 from the F_2 population and 32 from the F_3 population.

Phenotypic observations

Root cracking had the inheritance pattern of a recessive trait with cracking observed in 30 of 82 families (3:1 p-value = 0.09). Some families had one or two cracked roots while in some families the entire family was cracked, both of which were scored as cracked. Annual habit was

also recessive with 30 of 84 families displaying some annual habit (3:1 p-value = 0.34), but unlike root cracking, at most 2-3 plants displayed annual habit in any family. No families were completely annual in habit. Early flowering in the greenhouse segregated as a recessive trait with 53 out of 138 plants flowering early (3:1 p-value = 0.38). Early flowering in the field segregated quantitatively with some families completely flowering by the end of the season, some families with no flowering by the end of the season, and most families in between.

QTL mapping

One QTL for root cracking was detected in the F_2 population on chromosome 5 (Table 4.2, Figure 4.4). Initial evaluation of the percentage of cracked roots in each F_3 family using a two part model to scan for both presence/absence and amount of cracking, the binary presence/absence had the highest level of significance (data not shown). Therefore, QTL modeling was performed as a binary trait. *RC-BrHM1-C5-Q1* accounted for 20.8% of the variation with the root cracking attributed to the Br1091 parent with mostly additive effects (Figure 4.5).

One QTL for annual growth habit was detected in the F_2 population on chromosome 5 and accounted for 18.4% of the variation (Table 4.2, Figure 4.4). *AH-BrHM1-C5-Q1* attributed annual growth habit to the HM1 parent and displayed additive effects (Figure 4.6).

QTL for early flowering in the field using the combined F_2 and F_3 map were located on chromosomes 1, 2, and 4 with the multi-QTL model accounting for 44.9% of the variation (Table 4.2, Figure 4.4). QTL for early flowering attributed to the Br1091 parent included those on chromosomes 1 and 4 while the QTL for early flowering on chromosome 2 was attributed to the HM1 parent. *EFF-BrHM1-C1-Q1* displayed additive effects while *EFF-BrHM1-C2-Q3* displayed dominance for early flowering and *EFF-BrHM1-C4-Q2* displayed dominance for non-flowering (Figure 4.7).

QTL for early flowering in the greenhouse in the F_2 population were located on chromosomes 5 and 7 and they accounted for 28.5% of the variation (Table 4.2, Figure 4.4). *EFG-BrHM1-C5-Q2* attributed early flowering to the HM1 parent and displayed dominance with slight over-dominance for flowering while *EFG-BrHM1-C7-Q1* attributed early flowering to the Br1091 parent and displayed recessive early flowering (Figure 4.8).

Discussion

One QTL for root cracking was identified on chromosome 5 that explained 20.8% of the variation using the presence of root cracking in F_3 families and the F_2 genotypes. While this is not a very large effect, it is significant given the phenotyping method used and relies on expression of root growth cracks in the local environment. Root cracking is dependent upon genetics, but environmental and management factors such as fertility and irrigation can enhance or limit root cracking expression in a crop (Hartz et al. 2005). Root cracking has been attributed to rapid growth due to over-fertilization or varying water levels resulting in uneven growth

(Hartz et al. 2005). While no effort was made to manipulate the environmental conditions to favor or limit cracking, the conditions were similar for all plants.

Previous researchers found roots displayed their full genetic predisposition to cracking only when mechanically stimulated (Dickson 1966; Hartz et al. 2005). Because no effort was made to physically cause cracking as in other studies on carrot root cracking, little can be said in comparison to those previous studies. While only roots that cracked naturally in the field were used in the analysis, it represents a starting point for further research not only for the presence or absence of root cracking, but also the severity of the cracking. Some F_4 families derived from this population have completely split open roots with the phloem separated from the xylem core (data not shown, Figure 4.9 is a representative picture).

Three QTL for early flowering in the field, phenotyped as the percentage of plants flowering in the field at the end of the season, two QTL for early flowering in the greenhouse, phenotyped as the plants that initiated flowering before vernalization and did not flower after vernalization, and one QTL for annual growth habit, phenotyped as the presence of a reduced storage root, were detected. None of these traits were directly phenotyped to account for days to flowering or vernalization requirement, but for the purpose of this analysis, we consider all three traits contribute to days to flowering or vernalization requirement, with early flowering in the field being the most direct comparison to days to flowering QTL previously characterized in other crops. Because flowering was only noted at harvest, the QTL identified here would represent the sum effect of all days to flowering QTL instead of a direct measure of the individual days to flower QTL. ‘Brasilia’ cultivars are known to be early flowering, and two of the three QTL for

early flowering in the field are attributed to the B1091 parent (Table 4.2). Three significant QTL were identified, but because of the very small population size (N=70), verification of the QTL in future work will be needed.

One QTL for annual growth habit was detected on chromosome 5 attributing annual habit to the HM1 parent. While phenotyping this trait, it was observed that many of the plants were already bolting with the elongated stem severed when the plants were defoliated (data not shown), and with a reduced storage root, the plant is not able to supply energy and nutrients after vernalization. When removing early flowering plants from the field in the general breeding program, many of the earliest flowering plants have smaller storage roots as well (author's observation).

Early flowering in the greenhouse could represent vernalization requirement QTL because of the way the plants were handled and DNA samples taken. The F_2 plants were pot grown in a greenhouse in California and vernalized after the leaves were removed. The vernalized roots were replanted in a greenhouse in Wisconsin and only when the leaves started growing after vernalization were leaf samples taken for DNA extraction. Plants that have initiated flowering and are then defoliated and vernalized will often not flower again, even though they will produce new leaves (unpublished data). The QTL on chromosome 7 attributes early flowering in the California greenhouse and then not flowering again in the Wisconsin greenhouse after vernalization to the Br1091 parent. While *EFG-BrHM1-C7-Q1* only explains 19.1% of the variation (Table 4.2), only two F_2 plants that produced seed were homozygous for the Br1091 allele at the QTL (data not shown). *EFG-BrHM1-C7-Q1* along with this large segregation

distortion could point to a larger effect QTL for vernalization requirement that needs further analysis. In the F_3 individuals, a similar situation occurred with Br1091 alleles on chromosome 7 underrepresented in plants that produced F_4 seed (data not shown). This is completely circumstantial evidence for vernalization QTL as anything that would cause the carrot plants to not flower or die in the greenhouse before flowering could also explain the QTL due to the phenotyping method, but it is a testable hypothesis.

Each QTL individually represents a specific trait, but considering the suite of QTLs detected for early flowering in the field, early flowering in the greenhouse, and annual growth habit, it represents a starting point for further research into vernalization requirement and days to flowering QTL. If these QTL do represent days to flowering, this work identified six QTLs with small effect for flowering. Given the varied phenotyping methods, if there was a large effect QTL controlling a lot of the variation, it should have been detected in more than one analysis. The six individual QTL here represent six small effect QTL and represent a small percent of the total variation for days to flowering. This finding of different, small effect QTL in different environments is characteristic of a trait controlled by many, small effect QTL (Broman and Sen 2009). This is expected for flowering in out-crossing crops. In self-pollinating crops like *Arabidopsis*, relatively few QTL explain the majority of the variation (Salomé et al. 2011), while in out crossing crops like maize, many QTL have very small effect (Buckler et al. 2009).

Interestingly, *AH-BrHM1-C5-Q1* and *RC-BrHM1-C5-Q1* were located in the same chromosomal region with opposite parental effects (Figure 4.4, Table 4.2). HM1 alleles promote annual growth habit and Br1091 alleles promote root cracking (Figure 4.10). These QTL may be two

distinct, linked loci, but the relationship between the traits is worth noting. While plants displaying annual growth habit that do not form a storage root would be highly resistant to cracking due to the small root size and limited radial growth, plants that grow rapidly and form a large storage root would be prone to root cracking. The chromosomal region here could affect root growth and cracking is only a visual phenotype of large and rapid root growth. Further research is needed to discern if these are two, linked loci or a pleiotropic effect of the same locus regulating root growth.

Comparison to Meloidogyne incognita resistance QTL

The F_2 and F_3 populations used in this work were previously characterized for *M. incognita* root-knot nematode resistance. While this work identifies many QTL, only 2 QTL for early flowering in the field are on the same chromosomes as QTL for *M. incognita* root knot nematode resistance. *EFF-BrHM1-C1-Q1* is on the same chromosome as *Mi-BrHM1-C1-Q3*, and *EFF-BrHM1-C2-Q3* is on the same chromosome as *Mi-BrHM1-C2-Q1*, but because of the large support intervals in the QTLs identified here due to the small population size, it is difficult to make predictions about how linked the traits are. The nematode resistance at each QTL is attributed to the parent that also contributes to early flowering in the field, and this observation correlates to some observations that as you remove plants that flower annually in the breeding program, the nematode resistance is indirectly selected against as well (unpublished data).

Future directions

This work represents a start to further study days to flowering and root cracking QTL with much more work needed to draw firm conclusions. Using this Br1091xHM1 population, a set of 100

recombinant inbred lines (RILs) is being developed that will be very useful for characterizing days to flowering and vernalization QTL. Growing the complete set of RILs when they are at the F_6 generation in the summer of 2016 and following the phenotyping convention of Alessandro and Galmarini (2007), days to flowering can be recorded and analyzed. Following vernalization for those roots that did not flower annually, days to flowering after vernalization can also be phenotyped using the same criteria. Phenotyping the RILs as a family should allow a more precise phenotype and allow for accurate QTL characterization.

To verify the specific claim that the *EFG-BrHM1-C7-Q1* is an important vernalization QTL, F_3 or F_4 families that are heterozygous for the region on chromosome 7 should be grown and evaluated. Evaluating several families heterozygous on chromosome 7 will allow a more complete picture of the effect of this QTL, along with families homozygous for each parental allele. Genotyping individuals before vernalization will allow the analysis of DNA samples that might not make it through vernalization or that might not flower after vernalization, one of the difficulties encountered in this current work.

The QTL for root cracking and annual habit on chromosome 5 warrant further analysis with the benefit that both QTL are in the same chromosomal region, allowing evaluation of both traits at the same time. Families that are fixed homozygous for each parent allele and several segregating F_3 or F_4 families should be grown. Phenotyping each plant as phenotyped in this work and genotyping for this region on chromosome 5 will allow characterization of both QTL. Evaluating both traits in the set of RILs when they are fully developed will also be useful in determining if there are modifier QTL along with the QTL identified here influencing the trait.

Conclusions

Several QTL representing days to flowering and vernalization requirement were identified in the Br1091xHM1 cross with two QTL linked to *M. incognita* resistance QTL. While not initially designed to study days to flowering and vernalization QTL, this work represents a starting point to further characterize days to flowering and vernalization requirement QTL. With the development of 100 RILs and remnant seed from many F_2, F_3, and F_4 generations of known parental genotype, this germplasm represents an ideal population to study the QTL identified here. Setting up further experiments specifically designed to look at days to flowering using the RILs will aid the detection of additional QTL, as well as evaluating days to flowering in other genetic backgrounds. Using mapping populations designed for other traits and evaluating the vernalized roots for days to flowering, several additional populations could be evaluated with minimal additional work for genotyping and mapping. Adding the QTL identified here to new QTL identified in future studies will build a framework to understand days to flowering and vernalization requirement in carrot.

Figure 4.1 A Br1091xHM1 F_3 family segregating for annual growth habit. From left to right, roots 3, 5, and 7 of this picture are representative of the annual growth habit phenotype.

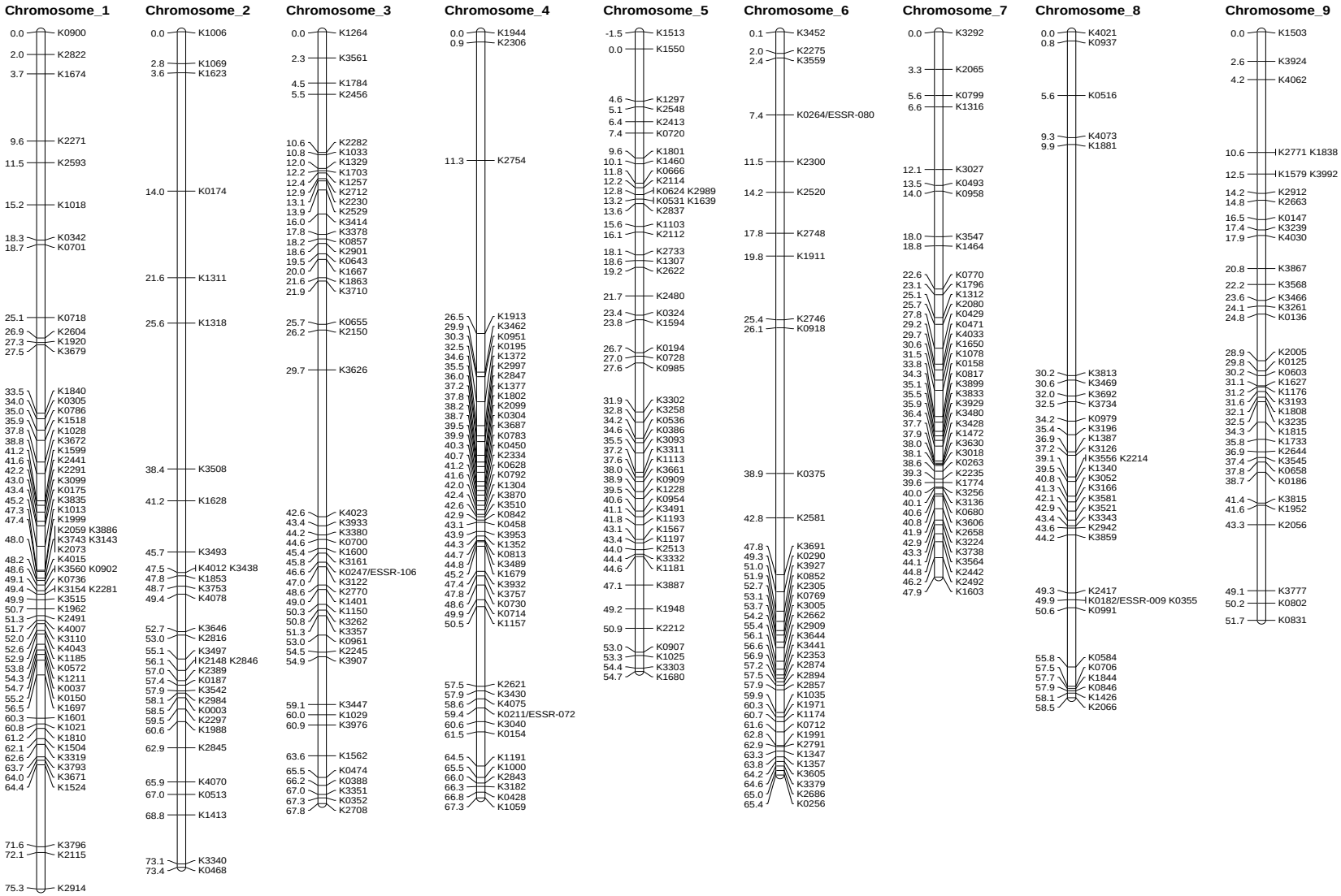

Figure 4.2 Linkage map for the F_2 mapping population Br1091xHM1 (Chapter 2, this work).

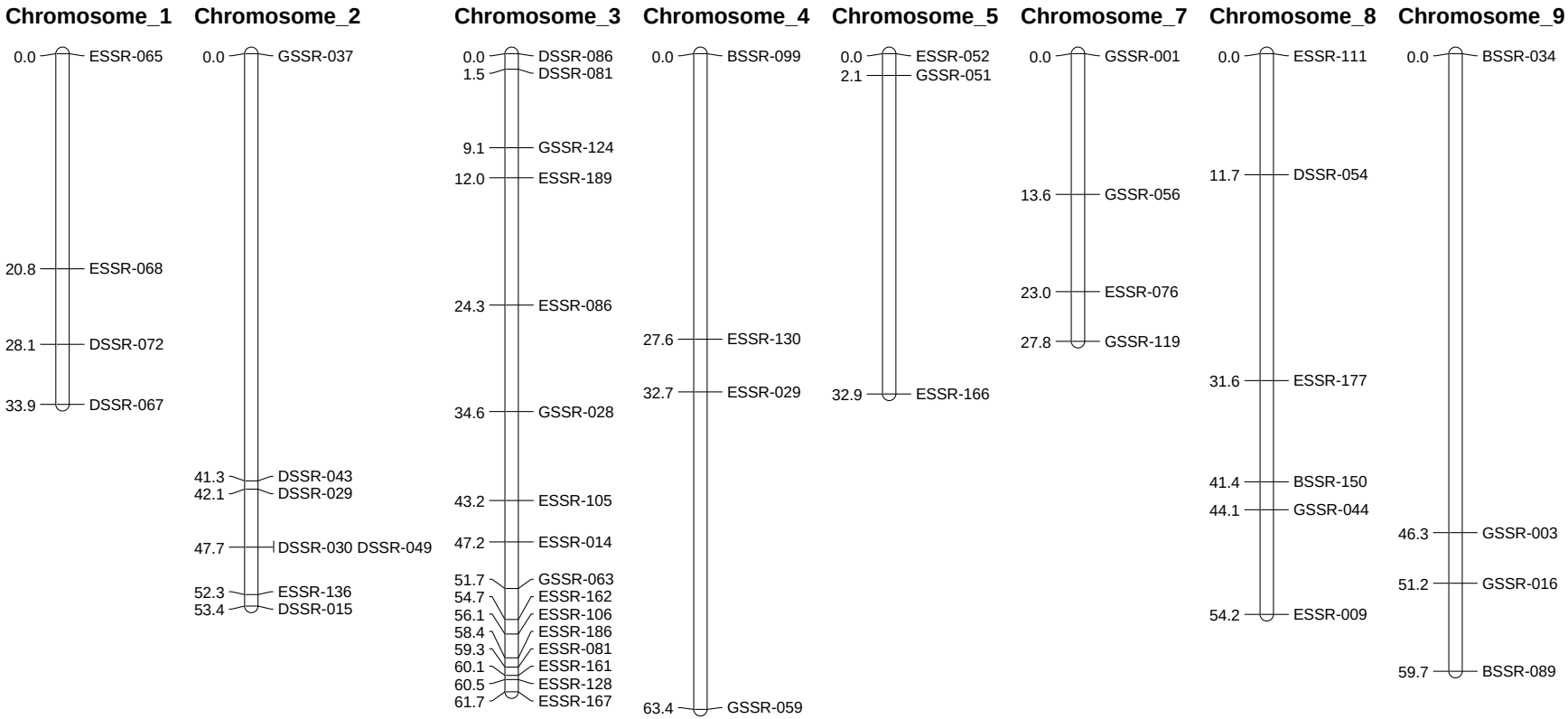

Figure 4.3 Linkage map for the F_3 mapping population Br1091xHM1 and also the map for the combined F_2:F_3 analysis of early flowering in the field (chapter 3, this work).

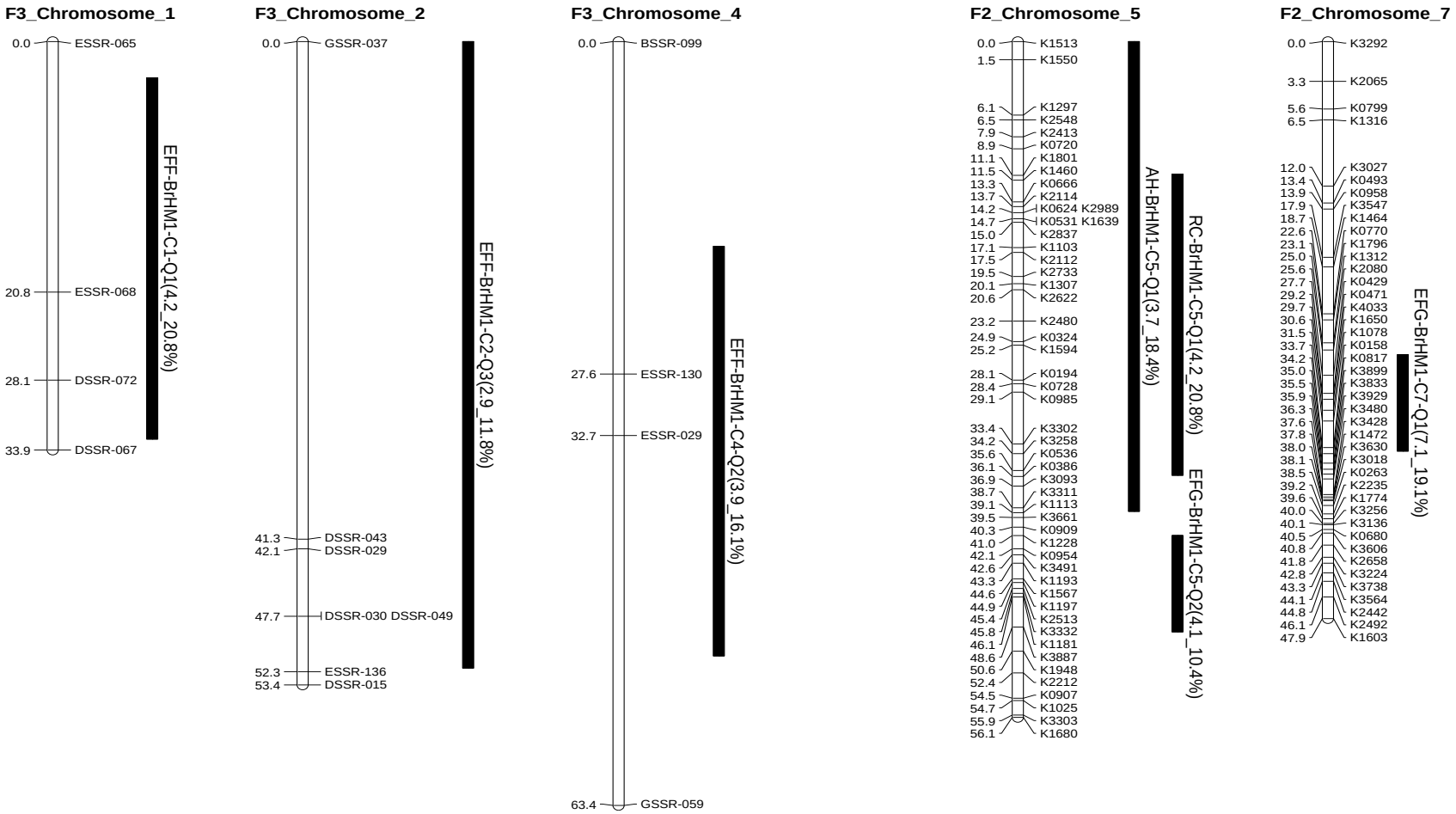

Figure 4.4 Linkage map of carrot chromosomes that incorporates significant QTL for early flowering and root cracking traits from a Br1091xHM1 cross. The first three chromosomes are from the combined F_2:F_3 map and the last two are from the F_2 map. The bars represent 1.5 LOD support intervals and the numbers in parenthesis indicate the largest LOD score followed by the percent phenotypic variation explained by that QTL.

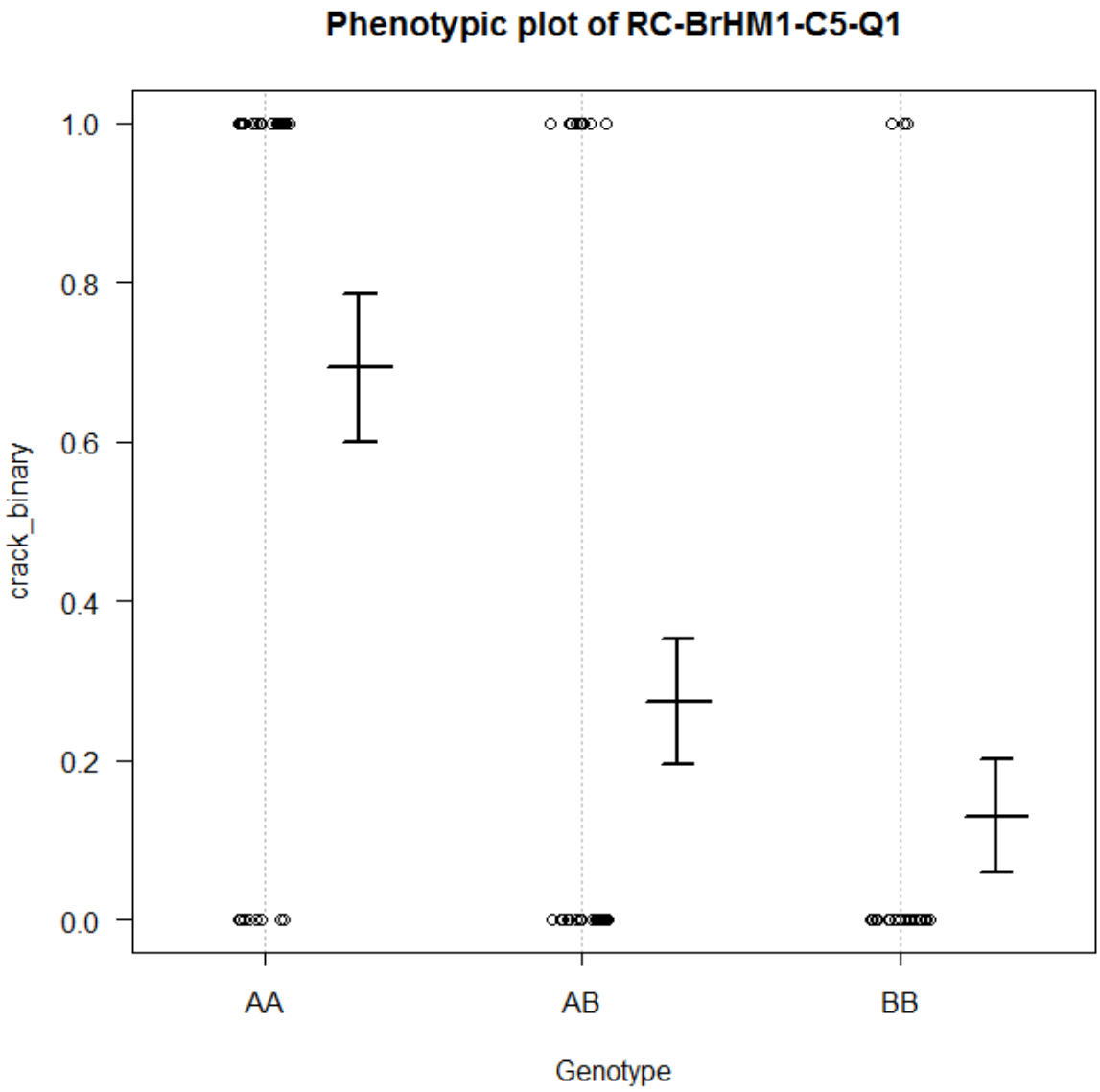

Figure 4.5 Phenotypic scores for root cracking as a binary trait (0 or 1 with 0 being no roots with cracks in the F_3 family and 1 being at least one root with a crack in the F_3 family) of AA, AB, and BB individuals at *RC-BrHM1-C5-Q1*. The bars represent the means of each genotypic class.

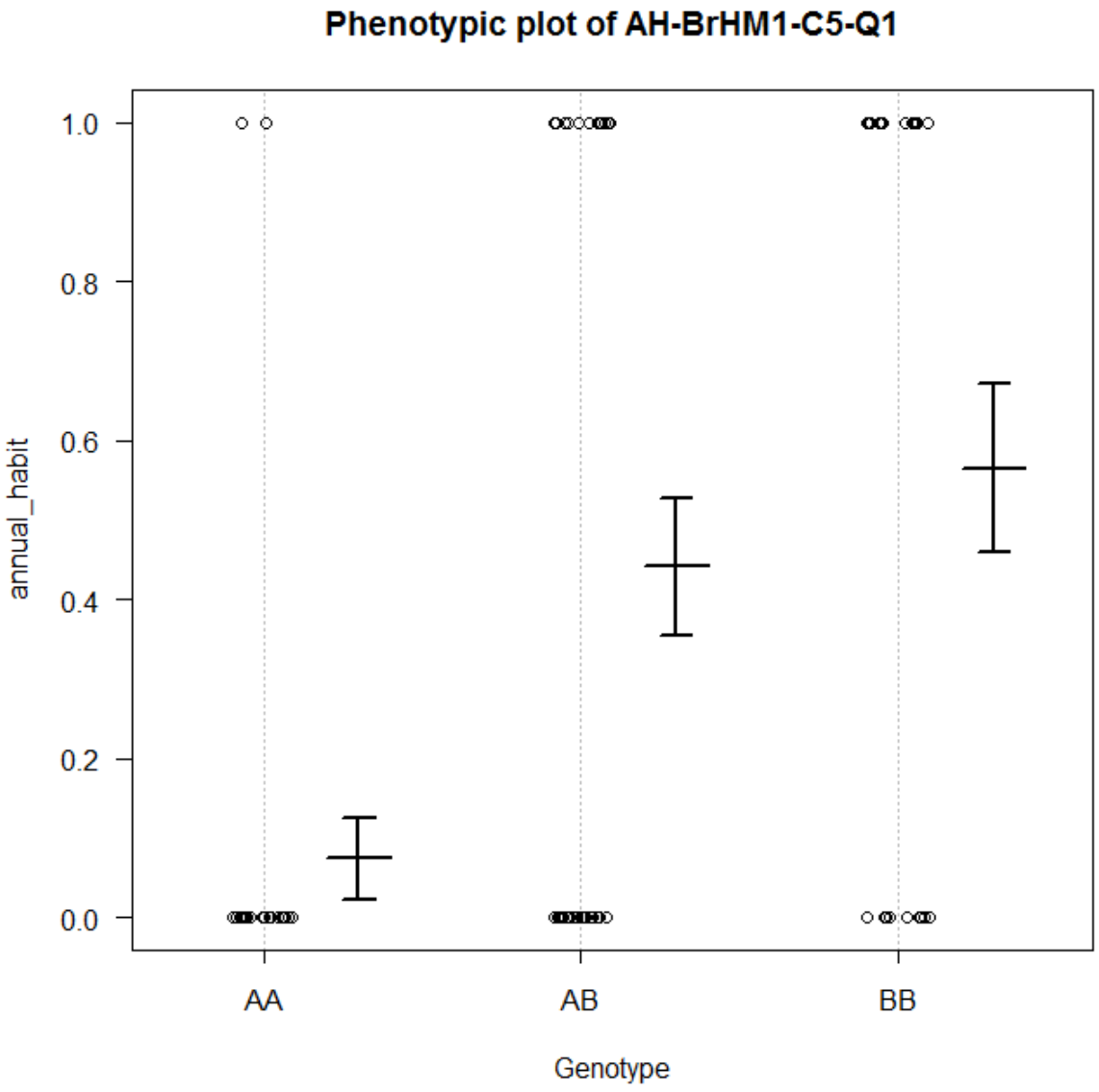

Figure 4.6 Phenotypic scores for annual growth habit as a binary trait (0 or 1 with 0 being no roots with annual habit in the F_3 family and 1 being at least one root with annual habit in the F_3 family) of AA, AB, and BB individuals at *AH-BrHM1-C5-Q1*. The bars represent the means of each genotypic class.

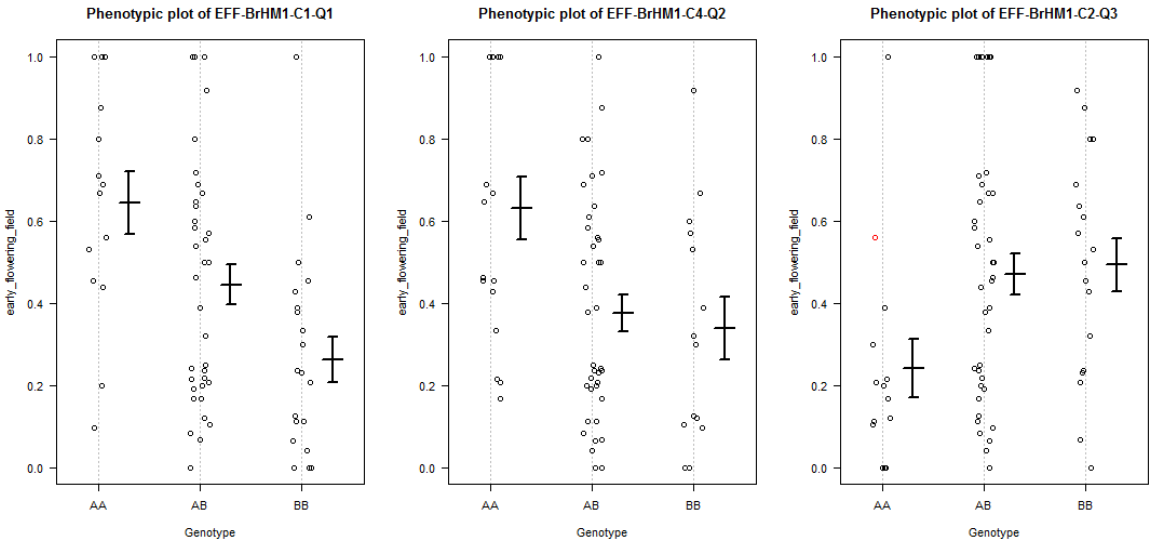

Figure 4.7 Phenotypic scores for early flowering in the field as a percentage of roots in the F_3 family that flowered in the field without requiring vernalization of AA, AB, and BB individuals at the three significant QTL on chromosomes 1, 4, and 2 (in order of % variation explained). The bars represent the means of each genotypic class.

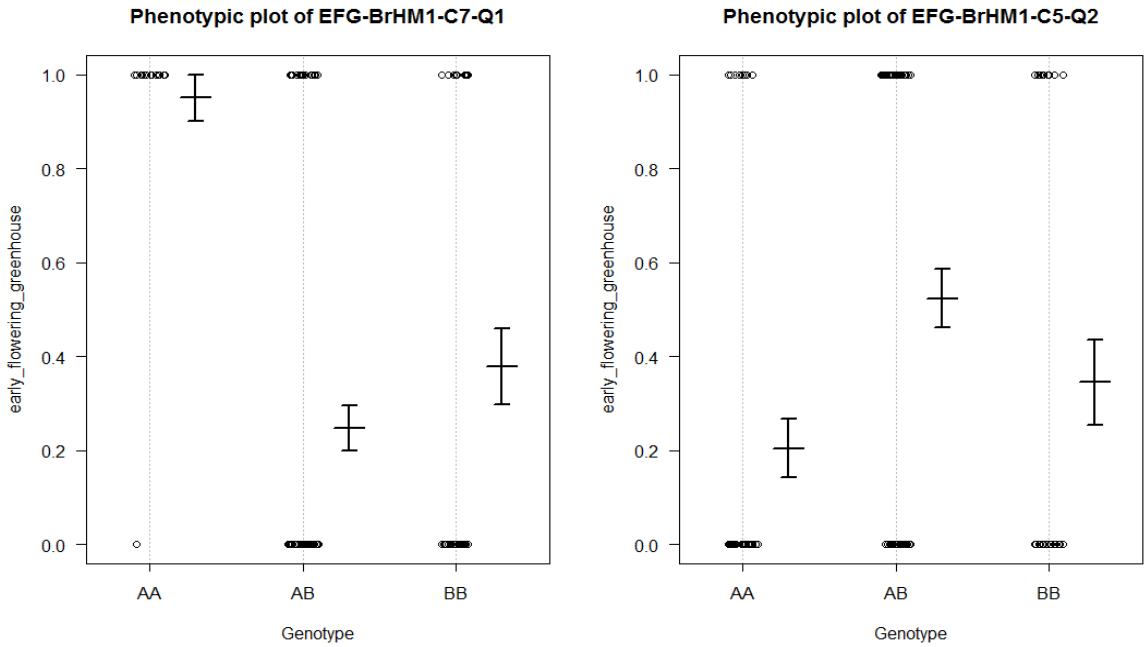

Figure 4.8 Phenotypic scores for early flowering in the greenhouse as a percentage of roots in the F_3 family that flowered in the greenhouse without requiring vernalization of AA, AB, and BB individuals at the two significant QTL on chromosomes 7 and 5 (in order of % variation explained). The bars represent the means of each genotypic class.

Figure 4.9 Germplasm related to Br1091 segregating for root cracking. From left to right, the first root is not cracked while the second and third roots have extreme root cracking. In some Br1091xHM1 F_4 families, all plants are completely split open as in the third carrot above.

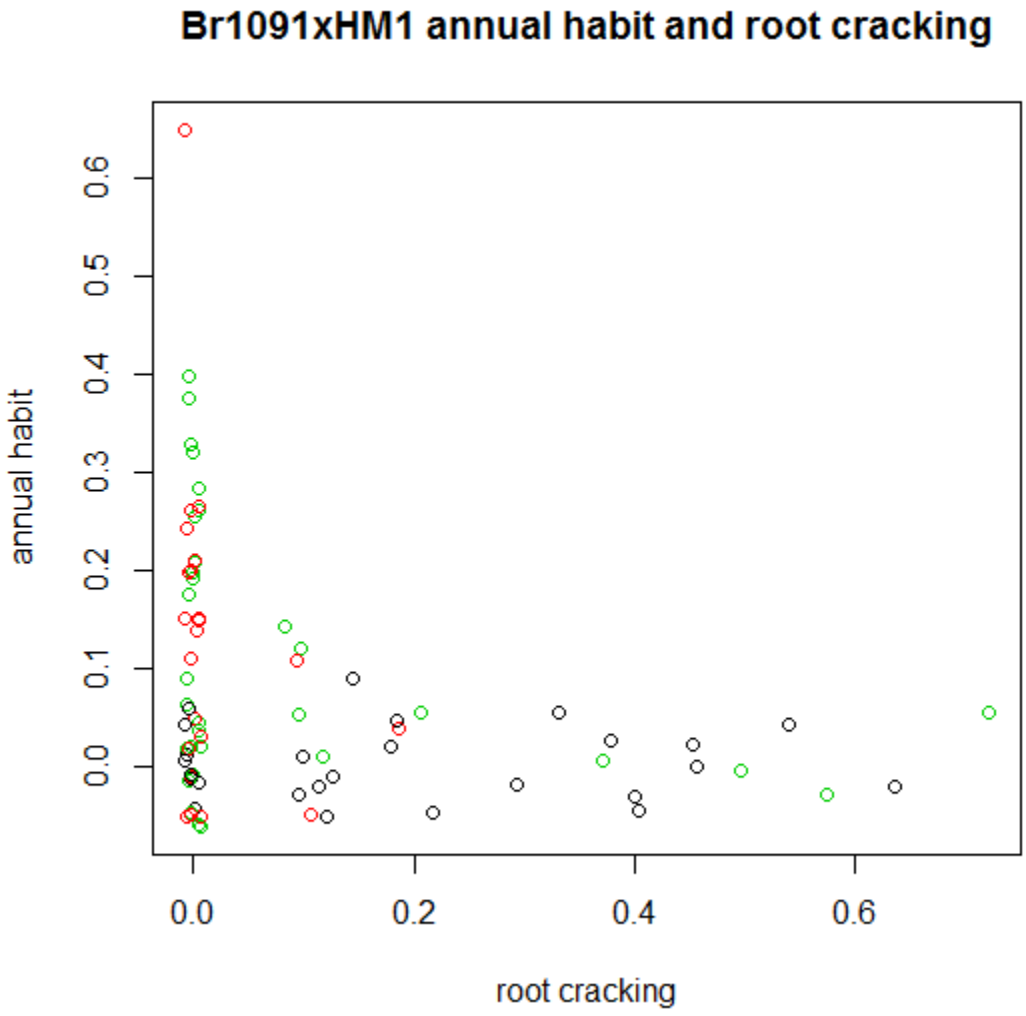

Figure 4.10 Plot of the Br1091xHM1 F_2 population with the percent of plants with annual growth habit in each F_3 family plotted against the percent of plants displaying root cracking in each F_3 family. The black circles represent Br1091 alleles, the red circles HM1 alleles, and the green circles are heterozygous.

Table 4.1 Summaries of the linkage maps for populations Br1091xHM1 F_2, Br1091xHM1 F_3, and Br1091xHM1 F_2:F_3 combined.

	Br1091xHM1 F_2	Br1091xHM1 F_3	Br1091xHM1 combined
Number of individuals	138	497	70
Number of polymorphic markers	389	48	48
AFLPs	0	0	0
SSRs	0	48	48
SNPs	389	0	0
Total distance (cM)	563.3	387.1	387.1
Average intermarker spacing (cM)	1.5	9.7	9.7
Maximum marker spacing (cM)	20.3	46.3	46.3

Table 4.2 Chromosomal locations and effects of QTL for early flowering and root cracking in Br1091xHM1 carrot populations.

(Trait) Chromosome	QTL	(Map) Position (cM)	LOD	% VE[a]	Parental effect[b]	Closest marker	Marker interval	1.5 LOD[c]
(Root cracking)		(F_2)						
5	*RC-BrHM1-C5-Q1*	20.1	4.2	20.8	Br1091	K1307	K1801 - K0386	11-37
(Annual Habit)		(F_2)						
5	*AH-BrHM1-C5-Q1*	23.2	3.7	18.4	HM1	K2480	K1513 - K3661	0 - 40
(Early Flowering Field)		(F_2-F_3)						
1	*EFF-BrHM1-C1-Q1*	20.8	4.2	17.7	Br1091	ESSR-068	ESSR-065 - DSSR-067	3-33
2	*EFF-BrHM1-C2-Q3*	2.0	2.9	11.8	HM1	GSSR-037	GSSR-037 - ESSR-136	0-52
4	*EFF-BrHM1-C4-Q2*	29.0	3.9	16.1	Br1091	ESSR-130	ESSR-130 - GSSR-059	17-51
		% Variation explained by multi-QTL model = 44.9%						
(Early Flowering Greenhouse)		(F_2)						
5	*EFG-BrHM1-C5-Q2*	45.4	4.1	10.4	HM1	K2513	K1228 - K3887	41 - 49
7	*EFG-BrHM1-C7-Q1*	29.7	7.1	19.1	Br1091	K4033	K2080 - K0158	26 - 34
		% Variation explained by multi-QTL model = 28.5%						

[a]Percentage of the variation explained

[b]The parent allele responsible for the trait (eg. HM1 is the parent that contributes to annual habit)

[c]1.5 LOD support interval (cM) (Broman and Sen 2009)

Chapter 5

Conclusions and recommendations for future work

This work identified at least five QTL regions for resistance to *M. incognita* RKN in carrot as well as QTL for other traits of interest in the same populations. Future work for the other traits was previously discussed in the respective chapters on root and shoulder color (chapter 3) and early flowering and root cracking (chapter 4), but several projects to continue the *M. incognita* RKN resistance work initiated here are outlined below. A general summary is followed by discussing the development of a Br1091xHM1 recombinant inbred line (RIL) population, marker assisted selection (MAS) to integrate resistance into desirable cultivars, and strategies to identify candidate genes in these populations.

One QTL for *M. incognita* resistance from each population was mapped to the same region as *Mj-1*, a previously characterized locus conferring resistance to *M. javanica*. The QTL co-localize to *Mj-1*, but the genes responsible for resistance remain unknown. Along with the resistance identified on chromosome 8 in the region of *Mj-1*, four other QTL regions were identified in three segregating populations on chromosomes 1, 2, 4, and 9. Four QTL were detected in Br1091xHM1, three in SFFxHM2 and three in HM3 with each parent contributing at least one QTL to *M. incognita* resistance. While work in all three populations would be ideal, the work outlined below focuses on Br1091xHM1 due to better performance of the population in the breeding program.

To further study the QTL identified in the Br1091xHM1 population, a set of 95 RILs is being developed that will allow accurate phenotyping on a family basis for RKN resistance as well as root and shoulder color, early flowering, and several other traits. RILs are a set of unselected, related inbred lines, normally derived from a single segregating F_2 population by single seed

descent to at least the F_6 generation. After the F_6 generation, most loci in each family will be homozygous, resulting in a set of related inbred lines. The benefit of RILs is being able to grow replicated trials of a segregating population, with multiple individuals representing each genotype. This replication is especially important for traits that have high variability in the plant response, like RKN resistance. The evaluation of multiple plants with the same genotype allows using the family mean as the phenotype and improves the phenotypic data used in a QTL analysis.

To characterize the QTL for RKN resistance, the full set of RILs should be evaluated in the greenhouse for both *M. incognita* and *M. javanica* RKN, phenotyping both gall rating and egg production. This test, along with dense genotyping, will allow characterization of the QTL identified here as influencing root galling or egg production, as well as possible detection of other QTL. Comparing the resistance to *M. incognita* and *M. javanica* will also help discern if *Mi-BrHM1-C8-Q2* is the same as *Mj-1*. *Mj-1* confers dominant resistance to *M. javanica* with unknown effect against *M. incognita*. If RILs with the Br1091 allele on chromosome 8 are susceptible to *M. javanica*, then resistance to *M. incognita* is either a different allele of *Mj-1* or a different locus. If RILs with the Br1091 allele at *Mi-BrHM1-C8-Q2* are resistant to *M. javanica*, then further work is needed to compare the QTL to *Mj-1*.

After genotyping the RILs, lines with all combinations of resistance and susceptible alleles at the four QTL identified in Br1091xHM1 should be selected for further evaluation to characterize the QTL. These 16 combinations should be evaluated under field conditions, evaluated with several other RKN species or races, and studied histologically to see what happens within the root when

RKN infection occurs. Crosses to susceptible and resistant sterile hybrids to test the QTL as they would be used in industry three-way hybrids will further characterize the usefulness of the QTL. These 16 would also be useful for test-crossing to resistance QTL identified in the future and if all 16 lines are not present in the final set of RILs, crosses to recover all 16 combinations should be made to have the complete set available for genetic study. The line homozygous resistant at each QTL can also be used as the foundation for introgressing other RKN resistant genes or QTL into one line, pyramiding as many RKN resistance genes as possible in one population.

To discern if *Mj-1* and the QTL on chromosome 8 are the same locus, including *Mj-1*/*Mj-1* germplasm in the histological studies would be beneficial. If the histological studies reveal differences in the plant response to nematode attack between *Mj-1* and *Mi-BrHM1-C8-Q2*, then the QTL on chromosome 8 and *Mj-1* are either different alleles of the same locus or distinct loci. If this histological study is not conclusive, crosses between RILs with the resistant allele at *Mi-BrHM1-C8-Q2* and germplasm with *Mj-1* should be made. Due to the close linkage of the traits, a large segregating population would be necessary to look for recombination between the loci. *M. javanica* should be used for the test because it produces a more obvious plant response compared to the quantitative resistance observed with *M. incognita*. If susceptible recombinants are identified, then *Mj-1* and *Mi-BrHM1-C8-Q2* are distinct loci.

Several other traits have been observed segregating in the population as the RILs are being developed including top size height and width, leaf angle, extended hypocotyls, flower stalk height, flower stalk habit (erect or prostrate), emergence and early season vigor, root shape, lateral rooting, root color, petiole color, and shoulder color and size. While not directly related

to this work, phenotyping and mapping as many other traits as possible in the RILs will be an economical way to add traits and QTL to the carrot linkage map.

The set of RILs is currently setting seed in the greenhouses in Arlington, WI with half the RILs at the F_4 and half at the F_5 seed generation (winter 2013-2014). All lines should be at the F_6 generation by the summer of 2016 for grow-out in the field in Wisconsin and California for horticultural traits and evaluation in the greenhouse in California for the nematode response. Genotyping by sequencing (GBS) or low coverage whole genome re-sequencing of each line will allow economical, dense genotyping of the RILs. Our lab has successfully used GBS with 80-95 individuals with more than 1,500 high-quality SNPs identified with one run of one lane lane of Illumina sequencing. With more inbreeding in the set of RILs than the previous populations used for GBS, more SNPs should be identified and the limit to map resolution will be recombination events and not marker coverage. One difficulty at the F_6 generation might be limited seed production because of inbreeding depression. If there is not enough seed to grow replicated trials, sib-mating four or more carrots from each line in one cage will allow greater seed production with the same representation of alleles in each line, but will add a year to the production of the finished set of RILs. If the whole set of RILs is not increased in this way, the set of 16 lines representing all combinations of QTL should be increased to provide seed for future studies.

The set of RILs will be a useful tool for characterizing RKN resistance and genetic mapping of other traits, but to develop useful germplasm for plant breeders, marker assisted selection (MAS) is currently underway. Using Br1091xHM1 progeny and SSR markers flanking the four

significant QTL, five F_4 plants were identified with resistant alleles at all four QTL. Four plants were sib-mated and crossed with the sterile inbred 2566A, and the remaining plant was inter-mated with the fertile inbred 2566B to begin developing Nb2566A and Nb2566B ("Nb" stands for nematode resistance from a Brasilia background). Using SSR markers flanking the QTL regions will allow backcrossing to susceptible 2566B while maintaining the QTL in the heterozygous condition. After several backcrosses, self-pollinating Nb2566B and crossing Nb2566B to Nb2566A will allow recovery of individuals homozygous for the resistant allele at each QTL region. In the final self-pollination of Nb2566B, all 16 combinations of resistant and susceptible alleles can be recovered from the same progeny allowing characterization of the individual QTL effects similar to the testing suggested for the RILs. One of the difficulties in applying QTL studies to other populations is the QTL effects often change or are not significant in different backgrounds. Comparing the QTL effects in the RILs and the Nb2566B progeny will allow characterizing the QTL effects in different genetic backgrounds. The first cross was made this winter in Arlington, WI and will be ready for grow-out this summer and the first backcross the winter of 2014-2015.

Identifying candidate genes within the QTL regions will be difficult due to the quantitative nature of the trait, but is possible as seen in soybeans (chapter 1). With quantitative resistance, the genes responsible for resistance do not always follow known resistance motifs so they are more difficult to identify when analyzing a genomic region. For each population, the most significant QTLs represent the most useful QTL for breeding and are the best subjects for candidate gene analysis. These QTL include *Mi-BrHM1-C2-Q1*, *Mi-BrHM1-C8-Q2*, *Mi-SFFHM2-C4-Q1*, *Mi-HM3-C9-Q1*, and *Mi-HM3-C8-Q2*. Of these, *Mi-BrHM1-C2-Q1* has the

largest effect and represents the best opportunity to identify the causative gene. To fine map the QTL, large populations represent one way to identify candidate genes, ideally with only one QTL segregating in the population to limit the background noise in QTL detection and refinement. The larger populations allow identification of individuals with recombination closer to the causative genomic region, limiting the number of candidate genes identified in the QTL region. In the Br1091xHM1 and SFFxHM2 populations, both parents were identified as resistant, and there might be other QTL affecting the resistance to RKN, so crossing plants with each QTL to a susceptible background will allow more precise characterization of the effect of each individual QTL. To that end, the development of Nb2566B will be useful in identifying candidate genes under *Mi-BrHM1-C2-Q1* and *Mi-BrHM1-C8-Q2*. As the backcrossing progresses, individuals heterozygous for only one QTL region and 2566B alleles at the rest of the QTLs can be self-pollinated to develop large segregating populations for one QTL at a time. Evaluating seedlings from the population with markers for the specific heterozygous QTL will allow identifying individuals with recombination events within the QTL support interval. Screening those individuals for their reaction to *M. incognita* RKN (along with several plants homozygous resistant and susceptible over the whole QTL region) will allow refining the QTL using less resources than evaluating the entire population with RKN and then genotyping, but will provide the same information for fine mapping. Comparing the fine-mapping results to the assembled carrot genome will allow the identification of candidate genes (as long as the causative genes are present in the sequenced line). To confirm the candidate genes, RNAi can be used to knock-down the expression of the resistance genes. Projects to develop optimized methods for RNAi in carrot are currently underway (unpublished data). Other techniques exist to identify candidate genes including RNA-seq and larger segregating population sizes in the

initial QTL detection, but the fine-mapping outlined here using the germplasm currently being developed represent a good chance to identify candidate genes.

This work identified several QTL for *M. incognita* RKN resistance in diverse backgrounds, but much work remains to integrate the resistance into desirable germplasm useful to commercial breeders. The next step to bring *M. incognita* RKN resistance into commercial cultivars is introgressing the QTL identified here into desirable cultivars, and discerning how many QTL are needed for field level resistance. Using the set of RILs and the MAS outlined here, the foundation is being laid for future research. To ensure the durability of resistance to *M. incognita* (and other RKN) now and in the future, other sources of resistance are currently being characterized. Efforts to compare those populations to the populations discussed in this work, and developing germplasm with several QTL from different populations are ongoing with the end goal of developing germplasm useful for carrot breeders to integrate into commercial cultivars.

References

Alessandro MS, Galmarini CR (2007) Inheritance of vernalization requirement in carrot. J Am Soc Hortic Sci 132:525–529

Alessandro MS, Galmarini CR, Iorizzo M, Simon PW (2013) Molecular mapping of vernalization requirement and fertility restoration genes in carrot. Theor Appl Genet 126:415–423. doi: 10.1007/s00122-012-1989-1

Ali A, Matthews WC, Cavagnaro PF, Iorizzo M, Roberts PA, Simon PW (2014) Inheritance and mapping of *Mj-2*, a new source of root-knot nematode (*Meloidogyne javanica*) resistance in carrot. J Hered 105:288–291. doi: 10.1093/jhered/est090

Ammiraju JSS, Veremis JC, Huang X, Roberts PA, Kaloshian I (2003) The heat-stable root-knot nematode resistance gene *Mi-9* from *Lycopersicon peruvianum* is localized on the short arm of chromosome 6. Theor Appl Genet 106:478–484. doi: 10.1007/s00122-002-1106-y

Angell F, Gabelman W (1970) Inheritance of purple petiole in carrot, *Daucus carota* var. *sativa*. Hort Science 5:175–176

Bailey D (1941) The seedling test method for root-knot nematode resistance. Proc Amer Soc Hort Sci 573–575

Barcala M, Garcia A, Cabrera J, Casson S, Lindsey K, Favery B, Garcia-Casado G, Solano R, Fenoll C, Escobar C (2010) Early transcriptomic events in microdissected Arabidopsis nematode-induced giant cells. Plant J 61:698–712. doi: 10.1111/j.1365-313X.2009.04098.x

Belair G, Parent LE (1996) Using crop rotation to control *Meloidogyne hapla* chitwood and improve marketable carrot yield. Hortscience 31:106–108

Bernoux M, Ellis JG, Dodds PN (2011) New insights in plant immunity signaling activation. Current Opinion in Plant Biology 14:512–518. doi: 10.1016/j.pbi.2011.05.005

Berthou F, Kouassi A, Bossis M, Dantec JP, Eddaoudi M, Ferji Z, Pelle R, Taghzouti M, Ellisseche D, Mugniery D (2003) Enhancing the resistance of the potato to southern root-knot nematodes by using *Solanum sparsipilum* germplasm. Euphytica 132:57–65. doi: 10.1023/A:1024624402333

Bleve-Zacheo T, Bongiovanni M, Melillo MT, Castagnone-Sereno P (1998) The pepper resistance genes *Me1* and *Me3* induce differential penetration rates and temporal sequences of root cell ultrastructural changes upon nematode infection. Plant Sci 133:79–90. doi: 10.1016/S0168-9452(98)00021-1

Boiteux LS, Belter JG, Roberts PA, Simon PW (2000) RAPD linkage map of the genomic region encompassing the root-knot nematode (*Meloidogyne javanica*) resistance locus in carrot. Theor Appl Genet 100:439–446. doi: 10.1007/s001220050057

Boiteux LS, Hyman JR, Bach IC, Fonseca MEN, Matthews WC, Roberts PA, Simon PW (2004) Employment of flanking codominant STS markers to estimate allelic substitution effects of a

nematode resistance locus in carrot. Euphytica 136:37–44. doi: 10.1023/B:EUPH.0000019508.78153.dd

Bridge J, Page SLJ (1980) Estimation of root-knot infestation levels on roots using a rating chart. Trop Pest Management 26:296–298

Broman KW, Sen S (2009) A guide to QTL mapping with R/qtl. Springer, New York. doi: 10.1007/978-0-387-92125-9

Brown C, Mojtahedi H, Santo G (1989) Comparison of reproductive efficiency of *Meloidogyne chitwoodi* on *Solanum bulbocastanum* in soil and in vitro tests. Plant Disease 73:957–959

Brown C, Mojtahedi H, Santo G (1991) Resistance to Columbia root-knot nematode in *Solanum* ssp and in hybrids. Am Potato J 68:445–452. doi: 10.1007/BF02853782

Buckler ES, Holland JB, Bradbury PJ, Acharya CB, Brown PJ, Browne C, Ersoz E, Flint-Garcia S, Garcia A, Glaubitz JC, Goodman MM, Harjes C, Guill K, Kroon DE, Larsson S, Lepak NK, Li H, Mitchell SE, Pressoir G, Peiffer JA, Rosas MO, Rocheford TR, Cinta Romay M, Romero S, Salvo S, Sanchez Villeda H, da Silva HS, Sun Q, Tian F, Upadyayula N, Ware D, Yates H, Yu J, Zhang Z, Kresovich S, McMullen MD (2009) The genetic architecture of maize flowering time. Science 325:714–718. doi: 10.1126/science.1174276

Buishand J, Gabelman W (1979) Investigations on the inheritance of color and carotenoid content in phloem and xylem of carrot roots (*Daucus carota* L). Euphytica 28:611–632. doi: 10.1007/BF00038928

Cavagnaro PF, Chung S-M, Manin S, Yildiz M, Ali A, Alessandro MS, Iorizzo M, Senalik DA, Simon PW (2011) Microsatellite isolation and marker development in carrot genomic distribution, linkage mapping, genetic diversity analysis and marker transferability across *Apiaceae*. BMC Genomics 12:386. doi: 10.1186/1471-2164-12-386

Cockram J, Jones H, Leigh FJ, O'Sullivan D, Powell W, Laurie DA, Greenland AJ (2007) Control of flowering time in temperate cereals: genes, domestication, and sustainable productivity. J Exp Bot 58:1231–1244. doi: 10.1093/jxb/erm042

Cook DE, Lee TG, Guo X, Melito S, Wang K, Bayless AM, Wang J, Hughes TJ, Willis DK, Clemente TE, Diers BW, Jiang J, Hudson ME, Bent AF (2012) Copy number variation of multiple genes at *Rhg1* mediates nematode resistance in soybean. Science 338:1206–1209. doi: 10.1126/science.1228746

Cosgrove DJ (1997) Assembly and enlargement of the primary cell wall in plants. Annu Rev Cell Dev Biol 13:171–201. doi: 10.1146/annurev.cellbio.13.1.171

Cousins P, Lauver M, Boyden L (2003) Genetic analysis of root-knot nematode resistance derived from *Vitis mustangensis*. Acta Horticulturae 603:149–155

Dangl JL, Horvath DM, Staskawicz BJ (2013) Pivoting the plant immune system from dissection to deployment. Science 341:746–751. doi: 10.1126/science.1236011

Darvasi A, Weinreb A, Minke V, Weller J, Soller M (1993) Detecting marker QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134:943–951

Dickson M (1966) Inheritance of longitudinal cracking in carrots. Euphytica 15:99–101

Djian-Caporalino C, Fazari A, Arguel MJ, Vernie T, VandeCasteele C, Faure I, Brunoud G, Pijarowski L, Palloix A, Lefebvre V, Abad P (2007) Root-knot nematode (*Meloidogyne* spp.) *Me* resistance genes in pepper (*Capsicum annuum* L.) are clustered on the P9 chromosome. Theor Appl Genet 114:473–486. doi: 10.1007/s00122-006-0447-3

Djian-Caporalino C, Pijarowski L, Fazari A, Samson M, Gaveau L, O'Byrne C, Lefebvre V, Caranta C, Palloix A, Abad P (2001) High-resolution genetic mapping of the pepper (*Capsicum annuum* L.) resistance loci *Me-3* and *Me-4* conferring heat-stable resistance to root-knot nematodes (*Meloidogyne* spp.). Theor Appl Genet 103:592–600. doi: 10.1007/PL00002914

Dropkin VH, Helgeson JP, Upper CD (1969) The hypersensitivity reaction of tomatoes resistant to *Meloidogyne incognita*: reversal by cytokinins. Journal of Nematology 1:55–61

Ehlers JD, Matthews WC, Hall AE, Roberts PA (2000) Inheritance of a broad based form of root-knot nematode resistance in cowpea. Crop Sci 40:611–618

Elling AA (2013) Major emerging problems with minor *Meloidogyne* species. Phytopathology 103:1092–1102. doi: 10.1094/PHYTO-01-13-0019-RVW

Fazari A, Palloix A, Wang L, Hua MY, Sage-Palloix A-M, Zhang BX, Djian-Caporalino C (2012) The root-knot nematode resistance *N* gene co-localizes in the *Me* genes cluster on the pepper (*Capsicum annuum* L.) P9 chromosome. Plant Breed 131:665–673. doi: 10.1111/j.1439-0523.2012.01994.x

Frazier W, Dennet R (1949) Isolation of *Lycopersicon esculentum* type tomato lines essentially homozygous resistant to root-knot. Proceedings of the American Society of Horticultural Science 54:225–236

Gomez R, Angel F, Bonierbale MW, Rodriguez F, Tohme J, Roca WM (1996) Inheritance of random amplified polymorphic DNA markers in cassava (*Manihot esculenta* Crantz). Genome 39:1039–1043. doi: 10.1139/g96-130

Gutierrrez OA, Jenkins JN, McCarty JC, Wubben MJ, Hayes RW, Callahan FE (2010) SSR markers closely associated with genes for resistance to root-knot nematode on chromosomes 11 and 14 of upland cotton. Theor Appl Genet 121:1323–1337. doi: 10.1007/s00122-010-1391-9

Hall Q, Cannon MC (2002) The cell wall hydroxyproline-rich glycoprotein RSH is essential for normal embryo development in Arabidopsis. Plant Cell 14:1161–1172. doi: 10.1105/tpc.010477

Hallmann J, Davies KG, Sikora R (2010) Biological control using microbial pathogens, endophytes and antagonists. In: Perry RN (ed), Moens M (ed) Root-knot Nematodes. CABI, Wallingford pp 380-411

Harborne J (1976) A unique pattern of anthocyanins in *Daucus carota* and other *Umbelliferae*. Biochem Sys Ecol 4:31–35

Hare W (1956) Resistance in pepper to *Meloidogyne incognita*. Phytopathology 46:98–104

Hartz TK, Johnstone PR, Nunez JJ (2005) Production environment and nitrogen fertility affect carrot cracking. Hortscience 40:611–615

Hendy H, Dalmasso A, Cardin M (1985) Differences in resistant *Capsicum annuum* attacked by different *Meloidogyne* species. Nematologica 31:72–78

Huang C, Charchar J (1982) Pre-planting inoculum densities of root-knot nematode related to carrot yield in greenhouse. Plant Dis 66:1064–1066

Huang S (1986) Penetration, development, reproduction, and sex ratio of *Meloidogyne Javanica* in 3 carrot cultivars. J Nematol 18:408–412

Huang S, Dellavecchia P, Ferreira P (1986) Varietal response and estimates of heritability of resistance to *Meloidogyne javanica* in carrots. J Nematol 18:496–501

Hunt DJ, Handoo ZA (2010) Taxonomy, Identification and Principal Species. In: Perry RN (ed), Moens M (ed) Root-knot Nematodes. CABI, Wallingford pp 55-97

Iorizzo M, Senalik DA, Ellison SL, Grzebelus D, Cavagnaro PF, Allender C, Brunet J, Spooner DM, Van Deynze A, Simon PW (2013a) Genetic structure and domestication of carrot (*Daucus carota* subsp *sativus*) (*Apiaceae*). Am J Bot 100:930–938. doi: 10.3732/ajb.1300055

Iorizzo M, Ellison S, Grzebelus D, Cavagnaro PF, Spooner D, Van Deynze A, Simon P (2013b) Development of a high-throughput SNP resource to advance genomic, genetic and breeding research in carrot (*Daucus carota* L.). XXI Plant and Animal Genome Meeting, San Diego, USA:P0762

Iorizzo M, Senalik DA, Grzebelus D, Bowman M, Cavagnaro PF, Matvienko M, Ashrafi H, Van Deynze A, Simon PW (2011) *De novo* assembly and characterization of the carrot transcriptome reveals novel genes, new markers, and genetic diversity. BMC Genomics 12:389. doi: 10.1186/1471-2164-12-389

Iovene M, Cavagnaro PF, Senalik D, Buell CR, Jiang J, Simon PW (2011) Comparative FISH mapping of *Daucus* species (*Apiaceae* family). Chromosome Res 19:493–506. doi: 10.1007/s10577-011-9202-y

Jablonska B, Ammiraju JSS, Bhattarai KK, Mantelin S, Martinez de Ilarduya O, Roberts PA, Kaloshian I (2007) The *Mi-9* gene from *Solanum arcanum* conferring heat-stable resistance to root-knot nematodes is a homolog of *Mi-1*. Plant Physiol 143:1044–1054. doi: 10.1104/pp.106.089615

Janssen GJW, vanNorel A, VerkerkBakker B, Janssen R (1996) Resistance to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* in wild tuber bearing *Solanum* spp. Euphytica 92:287–294

Janssen GJW, vanNorel A, Janssen R, Hoogendoorn J (1997a) Dominant and additive resistance to the root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax* in Central American *Solanum* species. Theor Appl Genet 94:692–700. doi: 10.1007/s001220050467

Janssen GJW, vanNorel A, VerkerkBakker B, Janssen R, Hoogendoorn J (1997b) Introgression of resistance to root-knot nematodes from wild Central American *Solanum* species into *S. tuberosum* ssp. *tuberosum*. Theor Appl Genet 95:490–496. doi: 10.1007/s001220050588

Jenkins JN, McCarty JC, Wubben MJ, Hayes R, Gutierrez OA, Callahan F, Deng D (2012) SSR markers for marker assisted selection of root-knot nematode (*Meloidogyne incognita*) resistant plants in cotton (*Gossypium hirsutum* L). Euphytica 183:49–54. doi: 10.1007/s10681-011-0470-6

Jones J, Wright S, Newsom L (1958) Sources of tolerance to and inheritance of resistance to root-knot nematodes in cotton. National Cotton Council of America, Memphis, Houston, TX, USA, pp 34–39

Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329. doi: 10.1038/nature05286

Just BJ, Santos C a. F, Fonseca MEN, Boiteux LS, Oloizia BB, Simon PW (2007) Carotenoid biosynthesis structural genes in carrot (*Daucus carota*): isolation, sequence characterization, single nucleotide polymorphism (SNP) markers and genome mapping. Theor Appl Genet 114:693–704. doi: 10.1007/s00122-006-0469-x

Just BJ, Santos CAF, Yandell BS, Simon PW (2009) Major QTL for carrot color are positionally associated with carotenoid biosynthetic genes and interact epistatically in a domesticated x wild carrot cross. Theor Appl Genet 119:1155–1169. doi: 10.1007/s00122-009-1117-z

Kaloshian I, Lange W, Williamson V (1995) An aphid resistance locus is tightly linked to the nematode resistance gene, *Mi*, in tomato. Proc Natl Acad Sci U S A 92:622–625. doi: 10.1073/pnas.92.2.622

Kouassi AB, Kerlan MC, Caromel B, Dantec JP, Fouville D, Manzanares-Dauleux M, Ellisseche D, Mugniery D (2006) A major gene mapped on chromosome XII is the main factor of a quantitatively inherited resistance to *Meloidogyne fallax* in *Solanum sparsipilum* (vol 112, pg 699, 2006). Theor Appl Genet 112:1400–1400. doi: 10.1007/s00122-006-0230-5

Lamport DTA, Kieliszewski MJ, Chen Y, Cannon MC (2011) Role of the extensin superfamily in primary cell wall architecture. Plant Physiol 156:11–19. doi: 10.1104/pp.110.169011

Li Z, Jakkula L, Hussey RS, Tamulonis JP, Boerma HR (2001) SSR mapping and confirmation of the QTL from PI96354 conditioning soybean resistance to southern root-knot nematode. Theor Appl Genet 103:1167–1173. doi: 10.1007/s001220100672

Luzzi B, Boerma H, Hussey R (1994a) Inheritance of resistance to the southern root-knot nematode in soybean. Crop Sci 34:1240–1243

Luzzi B, Boerma H, Hussey R (1994b) A gene for resistance to the southern root-knot nematode in soybean. J Hered 85:484–486

Matthews W, Simon P, Roberts P (1999) Influence of temperature on expression of resistance in carrot to *Meloidogyne javanica*. J Nematol 31:553

McDonald BA, Linde C (2002) Pathogen population genetics, evolutionary potential, and durable resistance. Annu Rev Phytopathol 40:349–379. doi: 10.1146/annurev.phyto.40.120501.101443

Medina-Filho H, Stevens M (1980) Tomato breeding for nematode resistance: survey of resistant varieties for horticultural characteristics and genotype of acid phosphatase. Acta Hortic 100:383–391

Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11:949–956. doi: 10.1105/tpc.11.5.949

Michelmore RW, Meyers BC (1998) Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res 8:1113–1130

Milligan SB, Bodeau J, Yaghoobi J, Kaloshian I, Zabel P, Williamson VM (1998) The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant genes. Plant Cell 10:1307–1319

Moens M, Perry RN, Starr JL (2010) *Meloidogyne* species - a diverse group of novel and important plant parasites. In: Perry RN (ed), Moens M (ed) Root-knot Nematodes. CABI, Wallingford pp 1-17

Murray M, Thompson W (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325. doi: 10.1093/nar/8.19.4321

Nombela G, Williamson VM, Muniz M (2003) The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. Mol Plant-Microbe Interact 16:645–649. doi: 10.1094/MPMI.2003.16.7.645

Nyczepir AP, Thomas SH (2010) Current and future management strategies in intensive crop production systems. In: Perry RN (ed), Moens M (ed) Root-knot Nematodes. CABI, Wallingford pp 412-443

Omwega C, Roberts P (1992) Inheritance of resistance to *Meloidogyne* spp in common bean and the genetic basis of its sensitivity to temperature. Theor Appl Genet 83:720–726

Parlevliet J (1989) Identification and evaluation of quantitative resistance. In: Leonard K (ed), Fry W (ed) Plant Disease Epidemiology. McGraw-Hill, New York, pp 215–248

Paulson RE, Webster JM (1972) Ultrastructure of the hypersensitive reaction in roots of tomato, *Lycopersicon esculentum* L., to infection by the root-knot nematode, *Meloidogyne incognita*. Physiological Plant Pathology 2:227–234

Pegard A, Brizzard G, Fazari A, Soucaze O, Abad P, Djian-Caporalino C (2005) Histological characterization of resistance to different root-knot nematode species related to phenolics accumulation in *Capsicum annuum*. Phytopathology 95:158–165. doi: 10.1094/PHYTO-95-0158

Pham A-T, McNally K, Abdel-Haleem H, Boerma HR, Li Z (2013) Fine mapping and identification of candidate genes controlling the resistance to southern root-knot nematode in PI 96354. Theor Appl Genet 126:1825–1838. doi: 10.1007/s00122-013-2095-8

Potenza C, Thomas SH, Sengupta-Gopalan C (2001) Genes induced during early response to *Meloidogyne incognita* in roots of resistant and susceptible alfalfa cultivars. Plant Sci 161:289–299. doi: 10.1016/S0168-9452(01)00415-0

Rick C, Fobes J (1974) Association of an allozyme with nematode resistance. Tomato genet coop rep p 25

Riggs R, Winstead N (1959) Studies on resistance in tomato to root-knot nematodes and on occurrence of pathogenic biotypes. Phytopathology 716–724

Roberts P (1987) The influence of planting date of carrot on *Meloidogyne incognita* reproduction and injury to roots. Nematologica 33:335–342

Roberts PA, Matthews WC, Ehlers JD (1996) New resistance to virulent root-knot nematodes linked to the *Rk* locus of cowpea. Crop Sci 36:889–894

Roberts PA, Matthews WC, Ehlers JD, Helms D (2008) Genetic determinants of differential resistance to root-knot nematode reproduction and galling in lima bean. Crop Sci 48:553–561. doi: 10.2135/cropsci2007.07.0384

Rossi M, Goggin FL, Milligan SB, Kaloshian I, Ullman DE, Williamson VM (1998) The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. Proc Natl Acad Sci USA 95:9750–9754. doi: 10.1073/pnas.95.17.9750

Rubatzky VE (1999) Carrots and related vegetable *Umbelliferae*. New York : CABI Pub, Wallingford, Oxon, UK

Salome PA, Bomblies K, Laitinen RAE, Yant L, Mott R, Weigel D (2011) Genetic architecture of flowering time variation in *Arabidopsis thaliana*. Genetics 188:421–U313. doi: 10.1534/genetics.111.126607

Santos CAF, Simon PW (2002) QTL analyses reveal clustered loci for accumulation of major provitamin A carotenes and lycopene in carrot roots. Mol Genet Genomics 268:122–129. doi: 10.1007/s00438-002-0735-9

Schaff JE, Nielsen DM, Smith CP, Scholl EH, Bird DM (2007) Comprehensive transcriptome profiling in tomato reveals a role for glycosyltransferase in *Mi* mediated nematode resistance. Plant Physiol 144:1079–1092. doi: 10.1104/pp.106.090241

Severin AJ, Woody JL, Bolon Y-T, Joseph B, Diers BW, Farmer AD, Muehlbauer GJ, Nelson RT, Grant D, Specht JE, Graham MA, Cannon SB, May GD, Vance CP, Shoemaker RC (2010) RNA-Seq Atlas of *Glycine max*: A guide to the soybean transcriptome. BMC Plant Biol 10:160. doi: 10.1186/1471-2229-10-160

Simon PW (1996) Inheritance and expression of purple and yellow storage root color in carrot. J Hered 87:63–66

Simon PW, Freeman RE, Vieira JV, Boiteux LS, Briard M, Nothnagel T, Michalik B, Kwon Y-S (2008) Carrot. In: Prohens J (ed), Nuez F (ed) Vegetables II. Springer New York, pp 327–357

Simon PW, Matthews WC, Roberts PA (2000) Evidence for simply inherited dominant resistance to *Meloidogyne javanica* in carrot. Theor Appl Genet 100:735–742. doi: 10.1007/s001220051346

Smith P (1944) Embryo culture of a tomato species hybrid. Proc Am Soc Hortic Sci 413–416

Stange M, Utz HF, Schrag TA, Melchinger AE, Wuerschum T (2013) High-density genotyping: an overkill for QTL mapping? Lessons learned from a case study in maize and simulations. Theor Appl Genet 126:2563–2574. doi: 10.1007/s00122-013-2155-0

Tamulonis JP, Luzzi BM, Hussey RS, Parrott WA, Boerma HR (1997) RFLP mapping of resistance to southern root-knot nematode in soybean. Crop Sci 37:1903–1909

Taylor LR, Sasser JN, Nelson LA (1982) Relationships of climate and soil characteristics to geographical distribution of *Meloidogyne* species in agricultural soils. Cooperative Publication, Department of Plant Pathology, North Carolina State University and US Agency for International Development, Raleigh, North Carolina

Truco MJ, Ashrafi H, Kozik A, van Leeuwen H, Bowers J, Wo SRC, Stoffel K, Xu H, Hill T, Van Deynze A, Michelmore RW (2013) An ultra high density, transcript based, genetic map of lettuce. G3-Genes Genomes Genet 3:617–631. doi: 10.1534/g3.112.004929

Van Ooijen JW, Voorrips R (2001) JoinMap ® 3.0, Software for the calculation of genetic linkage maps. Plant Research International, Wageningen, The Netherlands

Vivek BS, Simon PW (1999) Linkage relationships among molecular markers and storage root traits of carrot (*Daucus carota* L. ssp *sativus*). Theor Appl Genet 99:58–64. doi: 10.1007/s001220051208

Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78. doi: 10.1093/jhered/93.1.77

Vos P, Simons G, Jesse T, Wijbrandi J, Heinen L, Hogers R, Frijters A, Groenendijk J, Diergaarde P, Reijans M, Fierens-Onstenk J, de Both M, Peleman J, Liharska T, Hontelez J, Zabeau M (1998) The tomato *Mi-1* gene confers resistance to both root-knot nematodes and potato aphids. Nat Biotechnol 16:1365–1369. doi: 10.1038/4350

Vrain T (1982) Relationship between *Meloidogyne hapla* density and damage to carrots in organic soils. J Nematol 14:50–57

Wang C, Matthews WC, Roberts PA (2006b) Phenotypic expression of *rkn1* mediated *Meloidogyne incognita* resistance in *Gossypium hirsutum* populations. J Nematol 38:250–257

Wang C, Ulloa M, Mullens TR, Yu JZ, Roberts PA (2012) QTL analysis for transgressive resistance to root-knot nematode in interspecific cotton (*Gossypium* spp.) progeny derived from susceptible parents. PLoS One 7:e34874. doi: 10.1371/journal.pone.0034874

Wang C, Ulloa M, Roberts PA (2006a) Identification and mapping of microsatellite markers linked to a root-knot nematode resistance gene (*rkn1*) in Acala NemX cotton (*Gossypium hirsutum* L.). Theor Appl Genet 112:770–777. doi: 10.1007/s00122-005-0183-0

Wang C, Ulloa M, Roberts PA (2008) A transgressive segregation factor (*RKN2*) in *Gossypium barbadense* for nematode resistance clusters with gene *rkn1* in *G. hirsutum*. Mol Genet Genomics 279:41–52. doi: 10.1007/s00438-007-0292-3

Wang M, Gabelman W (1993) Screening for resistance to root-knot nematode (*Meloidogyne hapla*) in carrot (Daucus carota L.). HortScience 28:449

Wang M, Goldman IL (1996) Resistance to root knot nematode (*Meloidogyne hapla* Chitwood) in carrot is controlled by two recessive genes. J Hered 87:119–123

Watts V (1947) The use of *Lycopersicon peruvianum* as a source of nematode resistance in tomatoes. Proceedings of the American Society of Horticultural Science 49:233–234

Widmer TL, Mitkowski NA, Abawi GS (2002) Soil organic matter and management of plant-parasitic nematodes. J Nematol 34:289–295

Williamson VM (1998) Root-knot nematode resistance genes in tomato and their potential for future use. Annu Rev Phytopathol 36:277–293. doi: 10.1146/annurev.phyto.36.1.277

Williamson VM, Hussey RS (1996) Nematode pathogenesis and resistance in plants. Plant Cell 8:1735–1745

Xua X, Zeng L, Tao Y, Vuong T, Wan J, Boerma R, Noe J, Li Z, Finnerty S, Pathan SM, Shannon JG, Nguyen HT (2013) Pinpointing genes underlying the quantitative trait loci for root-knot nematode resistance in palaeopolyploid soybean by whole genome resequencing. Proc Natl Acad Sci U S A 110:13469–13474. doi: 10.1073/pnas.1222368110

Yaghoobi J, Kaloshian I, Wen Y, Williamson V (1995) Mapping a new nematode resistance locus in *Lycopersicon Peruvianum*. Theor Appl Genet 91:457–464

Yaghoobi J, Yates JL, Williamson VM (2005) Fine mapping of the nematode resistance gene *Mi-3* in *Solanum peruvianum* and construction of a *S. lycopersicum* DNA contig spanning the locus. Mol Genet Genomics 274:60–69. doi: 10.1007/s00438-005-1149-2

Yildiz M, Willis DK, Cavagnaro PF, Iorizzo M, Abak K, Simon PW (2013) Expression and mapping of anthocyanin biosynthesis genes in carrot. Theor Appl Genet 126:1689–1702. doi: 10.1007/s00122-013-2084-y

Ynturi P, Jenkins JN, McCarty JC, Gutierrez OA, Saha S (2006) Association of root-knot nematode resistance genes with simple sequence repeat markers on two chromosomes in cotton. Crop Sci 46:2670–2674. doi: 10.2135/cropsci2006.05.0319

Yu MH, Heijbroek W, Pakish LM (1999) The sea beet source of resistance to multiple species of root-knot nematode. Euphytica 108:151–155. doi: 10.1023/A:1003616612201