ABSTRACT

FRAHER, SIMON PHILLIP. Advancing Molecular Tools for the Accelerated Release of Root-Knot Nematode Resistant Sweetpotato Varieties. (Under the direction of Dr. G. Craig Yencho).

Sweetpotato, *Ipomoea batatas* (L.) Lam. (2n=6x=90) is among the world's most important food crops, and it is especially important in North Carolina where 65% of US production occurs. Due to its highly heterozygous hexaploid and complicated breeding nature, sweetpotato lags far behind other crops in terms of genomic tools. However, recent advances in sweetpotato genetics have afforded opportunities to associate sequence data with phenotypic data though genomics analysis, especially the publication of a diploid reference genome for *I. trifida* (Kunth.) G. Don in 2018, and open-source software packages such as MAPpoly and QTLpoly, which respectively enable the construction of linkage maps and subsequent detection of quantitative trait loci (QTL) within the genome.

Root-knot nematodes, *Meloidogyne* spp., are a major pest of sweetpotato and they also affect many other crop and weed species. *Meloidogyne enterolobii* (Yang & Eisenback, 1983), the guava root-knot nematode, is an emergent pest in sweetpotato for which there is little resistance in elite breeding lines. Resistance does exist for *M. enterolobii* in exotic germplasm, as well as United States Department of Agriculture Agricultural Research Service (USDA ARS) breeding lines, other university breeding programs, and older US heirloom varieties. However, breeding with this germplasm sacrifices yield and other traits. Previous research revealed the presence of a single major QTL for resistance to the related southern root-knot nematode, *M. incognita*, on linkage group 7 explaining 58.3% of variation in gall counts in the 'Tanzania' x 'Beauregard' (TB) sweetpotato mapping population of 240 progeny. Here, we describe a bioassay for quantifying *M. enterolobii* resistance in sweetpotato in segregating progenies and report a single major QTL on linkage group 4 explaining 66.9% of the variation in production of

M. enterolobii eggs per gram of root tissue in the TB population. The TB population exhibited a 1:1 segregation pattern for resistance to *M. enterolobii*, suggesting simplex dominant inheritance.

Based on our QTL analyses, a bioinformatics pipeline was run using whole genome sequence data for 'Tanzania' and 'Beauregard'. Whole genome sequence data for 'Tanzania' and 'Beauregard' was aligned with the diploid *I. trifida* (NCNSP306) reference assembly to identify single dose SNP markers within the respective QTL regions. From a pool of 2,444 SNP markers detected by this pipeline, we narrowed the search to 24 SNPs likely to be PCR-compatible and closest to respective QTL peaks. DNA from sweetpotato genotypes of known *M. enterolobii* and/or *M. incognita* resistance, including 122 TB progeny and 82 other diverse germplasm, was assayed by LGC BioSearch Technologies using kompetitive allele-specific PCR (KASP) genotyping. Of the 24 putative markers submitted to LGC, 16 working assays were developed, and 11 were polymorphic. The genotype data was aligned with resistance information and chi-square analysis revealed four significant markers that predicted resistance with over 80% accuracy. We report two markers for *M. enterolobii* resistance (LG 4: 7038930 & 7039636) with greater than 96.8% accuracy and two markers for *M. incognita* resistance (LG7: 1699509 & 1700440) with 94.0% and 85.1% accuracy, respectively, in the evaluated germplasm. Further validation in broader germplasm and additional mapping populations could determine these markers to be suitable for implementation for marker-assisted breeding in sweetpotato.

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Advancing Molecular Tools for the Accelerated Release of Root-Knot Nematode Resistant Sweetpotato Varieties

by
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DEDICATION

I would like to dedicate this work to the many talented researchers who laid the foundations for my success. I stand on the shoulders of giants, and this research would not have been possible without your pioneering efforts. My gratitude is only surpassed by your patience.

BIOGRAPHY

Simon Fraher was born November 11th, 1993 in Washington State to parents Stephanie and Timothy Fraher. He was raised in Olympia, Washington, nestled amongst the trees near the mucky beaches of the Puget Sound. Surrounded by the whimsical wildlife of the Pacific Northwest, he had no choice but to pursue a career in the biological sciences.

Simon received his Bachelor of Science in Bioresource Research at Oregon State University in 2016, after completing an undergraduate thesis with Dr. Jennifer Parke in Plant Pathology. In his final year at OSU, Simon worked with barley breeder Dr. Pat Hayes. It was through Pat that he first learned of plant breeding as a career. After moving to Raleigh in 2019 and working with Craig Yencho and Ken Pecota in sweetpotato breeding, the decision was made. It is Simon's desire to work within in the nexus of field and lab work, team management, statistics, and networking which we call plant breeding.

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CHAPTER 1: Literature Review

Sweetpotato Overview

Sweetpotato, *Ipomoea batatas* (L.) Lam. (2n=6x=90), ranks among the world's top ten food crops (FAO, 2020; Loebenstein & Thottappilly, 2009; Woolfe, 1992). Sweetpotato is important in both developing and developed nations due to its drought tolerance, hardiness, adaptability, nutrition, and high yield (Ji et al., 2015; Laurie et al., 2015). Nutritionally, sweetpotato outranks many vegetables in carbohydrate, fiber, vitamin, mineral, antioxidant, and protein content (Ji et al., 2015). In developing nations sweetpotato is a staple crop, while in developed nations it is a secondary food source (Loebenstein & Thottappilly, 2009). Of the 111 nations that produce sweetpotato worldwide, 101 are considered developing nations (Woolfe, 1992).

In 2018, Asia and Africa were the foremost sweetpotato producing regions, accounting for 66% and 28.3% of world production. A vast majority of production occurred in China, amounting to over 50 million tons. Collectively, the Americas only produced 4.6% of the world's sweetpotatoes; however, the United States ranked highest in the world for export quantity in 2017 at approximately 292,000 tons (FAO, 2020). In much of the world sweetpotatoes are grown on small intercropped parcels in remote areas and consumed on site, so it is likely that the FAO underestimates the production of this crop in some areas (Woolfe, 1992). Sub-Saharan Africa (SSA) actually has a greater planted acreage than China, but yields are much lower, averaging 6 tons per hectare in SSA and 21 tons in China (Low et al., 2017). Because of its drought tolerance and high energy output, in Africa sweetpotato is treated as a backup crop for when maize fails (Low et al., 2017).

Regionally, preferences differ for sweetpotato. Sweetpotato storage root shapes can be round, ovoid, blocky, tapered; skin colors range from white, brown, purple, red, and rose; flesh color can be white, cream, yellow, orange, purple (Loebenstein & Thottappilly, 2009). The United States has a strong preference for blocky, orange, moist, sweet types with low dry matter (Cervantes-Flores et al., 2011). Orange-fleshed sweetpotatoes (OFSP) are so colored due to their high beta-carotene (provitamin A) content, and there are global efforts to biofortify sweetpotato through breeding, and increase consumption of OFSP in areas that suffer from vitamin A deficiency (Laurie et al., 2015; Loebenstein & Thottappilly, 2009). In SSA, most varieties grown are high dry matter white sweetpotatoes lacking beta-carotene, yet 100g of OFSP would be sufficient to meet daily requirements of vitamin A in children (Low et al., 2017).

Sweetpotato storage roots are an incredibly versatile ingredient. Primary uses for the storage roots are boiling, baking, and frying, while secondary products include starch, flour, and purees (Loebenstein & Thottappilly, 2009). Sweetpotatoes can be chipped, fried, canned (especially as baby food and 'yams'), frozen, pickled, made into jams and jellies, drinks, sauces, candies, noodles and more. The leaves and shoots are also edible and nutritious (Woolfe, 1992). Sweetpotatoes also have industrial applications and are used (in concert with microbes) to produce citric acid, MSG, and alcohol. In Asia, a major fraction of the crop is used for starch and ethanol production, as well as livestock feed (pig, cattle, and fish) (Loebenstein & Thottappilly, 2009). The sweetpotato is a festive food, an accompaniment to Thanksgiving in the United states; it is a ceremonial food, served to nobility in Tonga (Woolfe, 1992); in many SSA nations it is a famine food, a security crop or food for the poor.

Sweetpotato disseminated from a center of diversity in the Americas, and may have been one of the earliest domesticated crops (Austin, 1988). There are several competing hypotheses on

the pre-Columbian dissemination of sweetpotato into Oceania. The tripartite hypothesis relies on archaeological, linguistic, and written evidence to posit that early Polynesian explorers collected sweetpotato during voyages to South America approximately 1000 years ago, while secondary and tertiary dispersal events had European explorers delivering sweetpotato into the Philippines (via Spanish galleons) and Indonesia (via Portuguese traders) (Roullier et al., 2013). Sweetpotato would later arrive in Europe during Columbian exchange events (Austin, 1988; Roullier et al., 2013). Despite tropical origins, the sweetpotato is now being produced across the globe, including in non-tropical growing regions. Only two food crops have emerged from the approximately 1600 species in Convolvulaceae: the sweetpotato *Ipomoea batatas*, and water spinach *Ipomoea aquatica* Forssk. (Wu et al., 2018). *Ipomoea batatas* is considered a cultigen, grown only in cultivation and with no known extant wild populations (Woolfe, 1992).

Sweetpotato in North Carolina

North Carolina has a rich history of sweetpotato production. The sweetpotato was grown in the Southeast region of what is now the United States at least as early as 1648, and Native Americans were growing it in the Caribbean and Central and South America when Europeans arrived in 1492. The sweetpotato was an important crop during the American Revolutionary and Civil wars, eaten predominantly by poorer classes and slaves (Loebenstein & Thottappilly, 2009). In 1995 the sweetpotato was declared the state vegetable of North Carolina.

‘Covington’ is currently the predominant sweetpotato variety (~90%) grown in North Carolina (Baselga et al., 2020), where roughly 65% of sweetpotatoes in the US were produced in 2019 (USDA, 2020). In 2019, North Carolina harvested 97,700 acres of sweetpotatoes, valued at approximately $324 million. Corn and soybean brought in more money than sweetpotatoes at

nearly $500 million each, though on significantly more land: 990 thousand acres of corn and 1.5 million acres of soybean were harvested in that same year (USDA/NASS, 2020). Sweetpotato production occurs for the most part in the coastal plain region of North Carolina. In 2019, Sampson, Johnson, and Lenoir counties, ranked in order of production, grew nearly half the sweetpotatoes in the state (Webb & Troxler, 2020).

A warm-season crop, sweetpotatoes thrive in the 90°F (32.2°C) summers of the coastal plains of North Carolina. Planting generally occurs from May-June, with harvest in late August through October (Jennings et al., 2019). After harvest, storage roots are cured and stored with specific temperature, moisture, and ventilation requirements (Edmunds et al., 2008). Sweetpotatoes in North Carolina are grown asexually from "seed" that are actually storage roots. Seed roots are bedded in the spring and adventitious sprouts emanating from the bedded storage roots (called "slips" or "plants"), are cut, transplanted, and grown as annuals in the field. Saving seed is discouraged, as it can lead to virus and other disease accumulation, and it is recommended to buy slips or storage roots from certified seed growers who use certified clean planting material from tissue culture to produce G0 stock (Jennings et al., 2019). Storage roots grown from G0 plants produce first generation (G1) sweetpotatoes.

In the United States, there is a preference for and familiarity with orange-fleshed moist sweetpotatoes. The major varieties (primarily 'Covington' and 'Beauregard') possess this trait, and have smooth textures with brown sugar or apricot-like flavors; other colors, flavors, and textures are less accepted in the United States (Leksrisompong et al., 2012). Increased awareness of nutrition and availability of new products is causing a "renaissance" in the popularity of sweetpotato in the United States (Clark et al., 2013). As demands change, breeding programs have shifted their focus as sweetpotato chips and fries require different varieties than table stock

or animal feed types (Martin & Jones, 1986). Breeders must consider a number of other traits, including pest and disease resistances, yield, uniformity, shape, appearance, earliness, composition, nutrition, and more. A variety which does well with these traits across a broad spectrum of environments is considered highly adapted to that growing region (Martin & Jones, 1986).

Of particular concern to the sweetpotato industry in North Carolina is the guava root-knot nematode, *Meloidogyne enterolobii* (Yang & Eisenback, 1983), a recently introduced pest that is threatening the entire regional industry. The Southern root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, is a more common but less damaging pest of sweetpotato. Breeding sweetpotatoes for resistance to these two nematodes has become a top priority for breeders in North Carolina and other sweetpotato-producing states and regions.

Root-Knot Nematodes in Sweetpotato

Meloidogyne, the root-knot nematode genus, is a diverse and highly adapted group of nearly 100 species of plant parasitic roundworms (Moens et al., 2009; OEPP/EPPO, 2011). Root-knot nematodes (RKN) infect roots and inhibit water and nutrient uptake, and while they rarely kill their host, growth and yield are severely impacted (Ye, 2018). RKN are among the most destructive nematodes in agriculture, affecting quality in addition to yield (Cervantes-Flores et al., 2008b). In sweetpotato, RKN affect storage root physiology, inducing blisters, root-knots, and black to brown corky tissue (Clark & Moyer, 1988; Overstreet et al., 2019). RKN can also create opportunities for secondary infections that can cause root rot and other further economic losses (Moens et al., 2009). RKN disrupt the uniformity of storage root development in sweetpotato and can influence storage root cracking, although other factors like rainfall can be

just as influential in cracked root occurrence (Lawrence et al., 1986), therefore this symptom should not be considered diagnostic of RKN damage. Growers often detect symptoms of RKN at the end of a growing season (Ye, 2018), after losses have occurred, when they lift plants from the ground to find the bumpy knotted roots that evoke the name "root-knot" nematode (Figure 1).

Meloidogyne spp. are sedentary endoparasites which can move intercellularly as well as intracellularly in plant tissues. Second stage juveniles (J2) infect sweetpotato roots, migrating to a feeding site where they become sedentary. Feeding induces hypertrophy in cells surrounding the nematode's head, causing multinucleate giant cells to form, resulting in visible galls on the roots (Clark & Moyer, 1988). In the case of *M. enterolobii*, a female within a gall can produce 400-600 eggs in a lifecycle of approximately four weeks, allowing multiple lifecycles in a growing season (Overstreet et al., 2019).

Meloidogyne enterolobii, the guava root-knot nematode, is one of the most damaging species in its genus, and total crop failure has been reported in sweetpotato due to this pest (Kirkpatrick et al., 2019; Ye, 2018). *Meloidogyne enterolobii* has a broad host range, extreme pathogenicity, and the ability to develop and reproduce on crops known to carry resistance genes for other *Meloidogyne* species (Castagnone-Sereno, 2012; Moens et al., 2009; OEPP/EPPO, 2011; Rutter et al., 2018). The host range of *M. enterolobii* consists of at least 121 plants, including many vegetable and herb crops, field crops, fruits and trees, ornamentals, and weeds (EPPO, 2021). Noteworthy is the short list of plants shown to be poor or non-hosts: garlic, peanut, maize, oat, wheat, sorghum, grapefruit, sour orange (Brito et al., 2004; Castagnone-Sereno, 2012; Gorny, 2020; Quesada-Ocampo, 2018).

Meloidogyne enterolobii (initially misidentified as *M. incognita*) was first identified in 1981 in the pacara earpod tree (*Enterolobium contortisiliquum* (Vell.) Morong), after which the

parasite was named (Yang & Eisenback, 1983). Later, a new species described as *M. mayaguensis* Rammah & Hirschmann, a parasite of eggplant (*Solanum melongena* L.), was identified in Puerto Rico in 1988 (Rammah & Hirschmann, 1988). *Meloidogyne mayaguensis* was later determined to be a synonym of *M. enterolobii* based on morphological, host, esterase, and DNA evidence (Xu et al., 2004) and the two were synonymized in 2009, with the species name *M. enterolobii* retained (Castagnone-Sereno, 2012; Hunt & Handoo, 2009). *Meloidogyne enterolobii* (as *M. mayaguensis*) has also been misidentified as *M. arenaria* Neal due to similarities in chromosome number and physiology (Brito et al., 2004). Difficulty in identifying *M. enterolobii* poses a major challenge to growers, who may not be aware of the infestation, or may attribute symptoms to another nematode. Thus, the prevalence of these pests is very likely under-reported.

Meloidogyne incognita, the southern root-knot nematode, like *M. enterolobii*, infects fibrous roots and grows rapidly, such that maximum nematode reproduction is concurrent with peak sweetpotato storage root growth (Lawrence et al., 1986). Field symptoms alone are not sufficient for species identification, as host response to *M. enterolobii* is similar to infection by *M. incognita* (Gorny, 2020). Species differentiation requires DNA analysis in a lab (Castagnone-Sereno, 2012; Gorny, 2020; OEPP/EPPO, 2011). Management strategies are different for *M. enterolobii* and *M. incognita*, so it is necessary to identify to the species level (Quesada-Ocampo, 2018).

Meloidogyne enterolobii has been detected in Africa, Europe, and North, Central, and South America (Moens et al., 2009). In 2010, the European and Mediterranean Plant Protection Organization added *M. enterolobii* to their EPPO A2 list (pest present in region), recommending quarantine (EPPO, 2020). A map of known affected nations and states is included (Figure 2).

The EPPO global database also lists *M. enterolobii* as A1-listed (not yet present) in Egypt, Morocco, Chile, Georgia, Russia, Ukraine, as well as the Eurasian Economic Union (EAEU) nations (EPPO, 2021). In the continental United States, *M. enterolobii* was first detected in Florida in 2001 on root samples from unidentified ornamental plants, as well as on the shrub *Tibouchina elegans* (Brito et al., 2004).

The major vector for plant pathogenic nematodes is humans, as root-knot nematodes are generally unable to move more than one meter unassisted in their lifetime. Soil on shoes, tires, farm equipment, and plant parts are major pathways for nematode introduction into previously uninfested fields. Environmental factors like flooding and irrigation can also vector nematodes (Lambert & Bekal, 2002). Preventative action by farmers is among the best controls for preventing the spread of RKN. Preventative actions include dipping plant materials in hot water, which can be effective but impractical due to damage to vegetative material. Crop rotation, use of clean stock, and nematicides can also be effective at preventing and eliminating RKN (Clark & Moyer, 1988). Due to the abundance of potential hosts, especially weed species, crop rotation may not always be possible or effective. Fumigant and non-fumigant nematicides (e.g., fluopyram, fluensulfone, fluazaindolizine, and oxamyl) have been effective controls for *M. enterolobii* under laboratory conditions (Watson, 2021), but are costly and can be neurotoxic to humans and animals; host resistance offers a more sustainable and economical solution (Cervantes-Flores et al., 2008b). In developing nations in tropical and subtropical regions, host resistance may be the only economically viable solution for RKN control (Roberts, 1992). However, breeding for nematode resistance may sacrifice yield, quality, maturation time, or other traits. A resistant variety may also be limited in nematode race specificity and durability.

The genetic basis of RKN resistance in sweetpotato is not clear (Cervantes-Flores et al., 2002). It has been hypothesized that RKN resistance is quantitative, involving multiple genes or alleles (Cervantes-Flores et al., 2002). *Meloidogyne incognita* has 4 races which are distinguished by host range, with races 1 and 3 being most prevalent in sweetpotato (Clark & Moyer, 1988). Plant resistance to RKN is typically race-specific, so it is imperative to identify the nematode race used in resistance screening and match it to the predominant race in the region (Ukoskit et al., 1997). For example, the release papers for the sweetpotato variety 'Covington,' specify that this variety is resistant to *Meloidogyne incognita* (Kofoid & White) Chitwood race 3 (Yencho et al., 2008). *Meloidogyne incognita* race 3 is the predominant race in North Carolina (Cervantes-Flores et al., 2008b), the growing region for which and in which 'Covington' was bred.

Currently, it is not clear if distinct races exist for *M. enterolobii.* Tigano et al. (2010) described *M. enterolobii* populations on a diversity of crops from sixteen nations in North and South America and Africa with very little genetic diversity. A separate phylogenetic analysis and nucleotide sequencing study of five African *M. enterolobii* populations found no differences between groups (Onkendi & Moleleki, 2013). Four populations of *M. enterolobii* isolated in North Carolina were found to be homogeneous in virulence (Schwarz et al., 2020). It may be that diversification of this pest has yet to occur, as introductions are believed to be recent or from similar sources in much of the world. Further, *M. enterolobii* reproduce via obligate mitotic parthenogenetic reproduction, and extremely low polymorphism would be expected in this asexually reproducing species (Tigano et al., 2010; Yang & Eisenback, 1983). However, *M. incognita* reproduces in the same fashion and has several identified races (Yang & Eisenback, 1983). The success of these asexually reproducing parasites has been considered an

"evolutionary paradox" (Castagnone-Sereno & Danchin, 2014), though, there is some evidence to suggest transposable elements and unique genome structure allow for high genetic plasticity without sexual reproduction (Koutsovoulos et al., 2019). Sex can occur, and the ratio of males in *Meloidogyne* spp. appears to increase under adverse conditions for the nematode (e.g. intraspecific competition, scarcity of fibrous roots to infect) (Lawrence et al., 1986). Due to the low level of polymorphism in *M. enterolobii* populations worldwide, it has been hypothesized that host resistance against one *M. enterolobii* population could be effective against many (Tigano et al., 2010).

Root-Knot Nematodes in North Carolina

Root-knot nematodes, *Meloidogyne* spp., are the most common plant parasitic nematodes in North Carolina (Ye, 2018). *Meloidogyne enterolobii* has been under internal quarantine in this state since 2018 (Wilson, 2018). The first detection of *M. enterolobii* in North Carolina was in 2011 in a Wayne County cotton field. Abnormally large galls were reported, as compared to symptoms of *Meloidogyne incognita*. In 2012, soybean in Wayne and Johnson counties was also found to be infested. Each of these cases was confirmed by PCR and DNA sequencing (Ye et al., 2013). The soybean and cotton varieties reported were RKN resistant (Rutter et al., 2018), and *M. enterolobii* had overcome or was not affected by this resistance.

'Covington', the major variety grown in North Carolina, was released in 2005 and is of the orange-fleshed moist sweet type of sweetpotato that are predominant in the United States (Yencho et al., 2008). This variety currently constitutes over 90% of commercial acreage in the state (Baselga et al., 2020), and about 40% of production in the nation (Barkley et al., 2017; Jiang et al., 2015). One major accomplishment with this release was resistance to *M. incognita*

race 3 (Chitwood 1949) (Yencho et al., 2008). However, ‘Covington’ is susceptible to *M. enterolobii* (Rutter et al., 2018; Schwarz et al., 2021). In fact, all major sweetpotato varieties grown in North Carolina have tested susceptible to *M. enterolobii*, including ‘Covington’, ‘Beauregard’, ‘Bonita’, ‘Bayou Belle’, and ‘Bellevue’ (Rutter et al., 2021; Schwarz et al., 2021). The establishment of *M. enterolobii* in North Carolina has already occurred, and growers need a resistant and adapted variety of the orange-fleshed sweet type to remain profitable. Some older varieties carry *M. enterolobii* resistance, including ‘Carver’, ‘Caromex’, ‘Centennial’, ‘Pelican Processor’, ‘Jewel’, as do some USDA ARS releases including ‘Regal’, ‘Resisto’, ‘Red Resisto’, and ‘Southern Delite’ (Rutter et al., 2021; Schwarz et al., 2021). Unfortunately, none of these lines are market-competitive with ‘Covington’ or ‘Beauregard’, and all have major flaws which, if used as breeding parents, would stymie the creation of a new superior variety with *M. enterolobii* and *M. incognita* resistance.

Currently, there is no *M. enterolobii* resistant variety that fills the agronomic niche that ‘Covington’ occupies. Of the commercial varieties grown in North Carolina, only the specialty variety ‘Murasaki-29’ (starchy, cream-flesh) is resistant (Schwarz et al., 2021). ‘Tanzania’, a starchy high dry matter Ugandan landrace not adapted to the growing regions of North Carolina, also tested resistant in independent trials (Rutter et al., 2021; Schwarz et al., 2021). For breeding purposes, cultivars like these present a challenge. A major issue in breeding with exotic lines, and even with older, less refined domestic lines (like ‘Centennial’ or ‘Jewel’), is linkage drag. Linkage drag describes the effects of deleterious off-target genes which are linked to (or “dragged” with) a target gene during introgression by breeding (Stam & Zeven, 1981; Tanksley et al., 1992). Typically, linkage drag refers to inheritance of undesirable traits (e.g., diminished yield or other quantitative traits) along with the acquisition of tolerance or resistance genes. For

example, crosses with cultivated tomato *Solanum lycopersicum* L. and wild-type tomato *Solanum pennellii* Correll can increase insect resistance at the cost of reduced horticultural quality (Mutschler et al., 2005). The same theory applies to intraspecific crosses. For example, the sweetpotato variety ‘Tanzania’ crossed with ‘Beauregard’ is unlikely to produce progeny as horticulturally adapted to the Southeast as ‘Beauregard’ due to the exotic background of ‘Tanzania’. However, many of these progenies would presumably exhibit some of the resistance traits carried by ‘Tanzania’, perhaps including RKN resistance. Nevertheless, material from other public breeding programs like USDA ARS may represent the best-adapted material for breeding purposes. Integrating host resistance into regionally adapted material is imperative for North Carolina. A sweetpotato variety resistant to *M. enterolobii* is several breeding cycles away from release, and each cycle can take three to five years. High throughput phenotyping and early-generation resistance detection would dramatically accelerate the timeline for the release of a *M. enterolobii* resistant variety.

Sweetpotato Improvement

Ipomoea batatas is generally self-incompatible and cross-incompatibilities are also frequent, especially in lines with common ancestry. Where crosses are successful, seed capsules yield less than two seed on average (Cervantes-Flores et al., 2011). Outdoor polycross nurseries, utilizing insects for pollination, can be used to increase seed production, yet pollen donors can be difficult or impossible to track. Greenhouse paired-cross pollinations done by hand enables tracking of both parental lines, with greatly increased costs of labor and resources. The preferred strategy in sweetpotato breeding has been to select amongst progeny based on desirable traits, and to eliminate lines with any unfavorable traits (Jones et al., 1986). As a highly heterozygous

complex polyploid, segregation can be observed within F1 progeny of sweetpotato (Wu et al., 2010). However, due to the practice of asexual propagation, one selected seed can become a major variety. There is no need to inbreed this crop for genetically stable seed, in fact, inbreeding is difficult to impossible due to frequent self-incompatibility (Yada et al., 2017).

Mass selection and polycross nurseries have been effective breeding strategies to tap resistance to *Meloidogyne* (Jones et al., 1986). Mass selection chooses superior parental lines and bulks seed from these individuals. Because most traits in sweetpotato are considered independent (i.e., unlinked), it is possible through mass selection to increase the prevalence of a trait across generations, as well as increasing the level of that trait's expression (Jones et al., 1986). Paired crossing, however, may become more efficient with the advent of molecular breeding approaches, eliminating the uncertain parentage that polycross techniques introduce, resulting in more targeted breeding efforts. Molecular tools would allow indirect selection, further increasing breeding efficiency. There is a need for marker assisted breeding (MAB) tools in sweetpotato breeding (Cervantes-Flores et al., 2011). Currently, no such markers have been used in sweetpotato breeding.

Origin, Genetics, and Marker Assisted Breeding

Ipomoea batatas (2n=6X=90) has a large genome size of approximately 3.3Gb (Arumuganathan & Earle, 1991). It is the only known hexaploid in the *Convolvulaceae* (Jones et al., 1986). The hexaploid nature, high degree of heterozygosity, and large genome size of *I. batatas* have made genome assembly for this species difficult (Wu et al., 2018). Also, assembly algorithms are rarely optimized for polyploid genomes due to the challenges created by multiple homoeologous pairs of chromosomes (Isobe et al., 2019). In 2018, Wu et al. described the de-

novo assembly of genome scaffolds for *Ipomoea trifida* (Kunth.) G. Don and *Ipomoea triloba* L., two diploid relatives of *I. batatas*. These reference genomes enabled tools like MAPpoly, a novel algorithm optimized for multilocus linkage map assembly in full-sib autohexaploid populations (https://mmollina.github.io/MAPpoly/) (Mollinari et al., 2020). MAPpoly was used to assemble a linkage map for the 'Tanzania' x 'Beauregard' mapping population that was aligned with *I. trifida* (Mollinari et al., 2020).

Although the precise genetic origin of *I. batatas* is not certain, recent publications support the emergence of *I. batatas* from allo- and or autopolyploidization events from diploid relatives (Cervantes-Flores et al., 2008a; Isobe et al., 2019; Shiotani & Kawase, 1989; Wu et al., 2018). Another hypothesis is that *I. batatas* contains three closely related diploid subgenomes that resemble *Ipomoea trifida* (Gao et al., 2020). Some might argue in favor of a strict autopolyploid origin for *I. batatas* based on segregation patterns, with some preferential pairing of chromosomes (Cervantes-Flores et al., 2008a). This distinction is important, as in autopolyploids, analogous chromosomes can pair randomly during meiosis while in allopolyploids, pairing occurs between homologs. Alleles of paired chromosomes of an allopolyploid would be expected to segregate independently, as in diploids (Ramsey & Schemske, 2002). Shiotani and Kawase (1989) proposed introgression of desirable traits from wild diploid relatives into *I. batatas* through novel synthesis of and subsequent crosses with hexaploid *I. batatas*-like plants. Their model proposes creation of a tetraploid hybrid from selected diploid relatives, a subsequent cross of this tetraploid by a desirable diploid resulting in triploid progeny, and then generation of a hexaploid plant which might be compatible with *I. batatas*. Their proposed genome structure for *I. batatas* is $B_1B_1B_2B_2B_2B_2$, though additional homology may exist between the B_1 and B_2 genomes due to the frequency of hexavalent

associations in their study (Shiotani & Kawase, 1989). Whole genome sequencing of *I. batatas* will further illuminate crop history and genetic origins, and will provide a vital understanding of genetics for breeding and physiological studies (Isobe et al., 2019).

In an autohexaploid (e.g. Aaaaaa), segregation ratios are 1:1 (simplex), 4:1 (duplex), or 19:1 (triplex), or 1:0 (quadruplex) for the respective genotypes Aaaaaa, AAaaaa, AAAaaa, and AAAAaa. In an allohexaploid (e.g. Aa aa aa), these ratios are different for all genotypes save the simplex and the quadruplex, and in the case of a tetradiploid (e.g. Aaaa aa) these ratios again are different in all scenarios save for the simplex (Aaaa aa, aaaa Aa) and quadruplex (AAAA aa) (Kriegner et al., 2003). The sweetpotato genome demonstrates both auto- and allopolyploid characteristics, but due to ongoing and historic hybridization events, it fits fully into neither category, existing with its relatives on a "network with reticulations" of relatedness (Wu et al., 2018). Nevertheless, a single dominant gene can demonstrate 1:1 phenotypic segregation in any population of *I. batatas* – allohexaploid, autohexaploid, or tetradiploid. The relative ease of breeding with a simplex dominant trait makes quantitative trait locus (QTL) analysis a strong foundation for the development of MAB in sweetpotato. QTL analysis helps to determine if phenotypic variation is due to many loci with relatively small effects, or few loci (or a single locus) with large effects. One limitation to such pursuits in the past, beyond the lack of reference genomes, was limited marker data (Yada, et al., 2017). Another limitation is that single nucleotide polymorphism (SNP) data has only recently become more available for sweetpotato (Gemenet et al., 2020). The recent publication of reference genomes for diploid wild relatives *I. trifida* and *I. triloba* (Wu et al., 2018) was a critical first step that has enabled improved linkage map construction and subsequent QTL analysis in sweetpotato, which may further lead to the

implementation of genomic tools in sweetpotato breeding through the identification of trait-associated DNA markers.

Genome assemblies of *I. trifida* and *I. triloba* serve as robust references for sweetpotato, in lieu of a full genome assembly for hexaploid *I. batatas* (Wu et al., 2018). *Ipomoea trifida* was hypothesized to be dually responsible for autopolyploid sweetpotato, with evidence that *I. trifida* contributed both nuclear and chloroplast genomes to sweetpotato (Muñoz-Rodríguez et al., 2018). Another hypothesis involves a hybridization event and allopolyploidization, whereby *I. trifida* crossed with another diploid relative like *I. triloba* (Austin, 1988). Whole genome sequence (10X Genomics Chromium) reads of the sweetpotato variety 'Tanzania' align better with *I. trifida* (NCNSP0306) than with *I. triloba* (NCNSP0323) (Wu et al., 2018). A SNP-based genetic map generated from the cross 'Beauregard' x 'Tanzania' (BT) aligned more closely with *I. trifida* (96.5% coverage) than with *I. triloba* (83.1% coverage) (Mollinari et al., 2020). *Ipomoea trifida* is regarded as the primary reference genome due to better alignment and a higher quality genome assembly (Mollinari et al., 2020; Wu et al., 2018), and the autopolyploid origin of *I. batatas* from *I. trifida* is well supported. Having these genomic resources available for *I. batatas* will greatly enhance our ability to identify trait-associated markers for use in MAB. For example, in tetraploid blueberry (*Vaccinium corymbosum*) a chromosome-scale haplotype-phased genome assembly showed demonstrably improved precision in identifying QTL as compared to a previous highly fragmented genome draft (Benevenuto et al., 2019).

The genetic basis for plant phenotypic variation can be quantitative (or continuous), whereby many genes with small effects influence a trait's expression, or qualitative (or discrete), where one gene (or few tightly linked genes) controls expression (Tanksley, 1993). In plant breeding, quantitative traits are more difficult to work with, as segregation is occurring at many

loci. However, the advent of molecular breeding has provided breeders powerful tools to work with quantitative traits. Genetic markers are distinct DNA sequences that are found in specific places on the genome, and while they do not often affect the plant's phenotype in a directly observable way, markers can be detected in DNA. The implications of this are tremendous for studying quantitative traits: we can study segregation patterns of individual markers which are linked to genes controlling for quantitative traits, and thus estimate the magnitude of that gene's effect and assign it a position relative to these markers (Tanksley, 1993). By measuring the recombination frequencies between linked markers, they can be organized by relative position to one another into linkage groups (like chromosomes) which are further assembled into linkage maps. In particular, linkage disequilibrium can be measured by crossing two phenotypically different plants, and studying the linkage of their progeny (Tanksley, 1993). A genome region with a significant effect on the expression of a quantitative trait is called a quantitative trait locus (QTL). Identifying these regions has been made possible through analytical methods like QTL analysis, providing a base from which to develop MAB tools (Ribaut & Hoisington, 1998). For example, hundreds of African sweetpotato landraces represent a useful pool to tap for resistance to sweetpotato virus disease (SPVD), and importantly, molecular markers have been used to identify known resistant genotypes (Miano et al., 2008).

Single nucleotide polymorphism (SNP) markers are the most abundant polymorphism in a given genome, and they can be used to construct high-density linkage maps, which can be used to identify quantitative trait loci (QTL) (Covarrubias-Pazaran et al., 2016). Dense linkage maps and QTL analyses have helped sweetpotato breeders associate genome regions with phenotypic expression (Amankwaah, 2019; Cervantes-Flores et al., 2011). Linkage maps are often derived from segregation analysis on inbred lines, which are obtained over multiple generations of

inbreeding or through doubled haploid techniques (Grattapaglia & Sederoff, 1994; Wu et al., 2010); however, producing inbred lines may not be feasible in plants with long generation times, compatibility barriers, or higher ploidy. Development of inbred lines in sweetpotato is difficult due to the prevalence of self-incompatibility and polyploidy (Yada et al., 2017). One benefit of working with plants of higher ploidy is that crossing highly heterozygous lines can show segregation at the F1 level (Wu et al., 2010), as is the case with sweetpotato (Figure 3). Hackett et al. (1998) simulated a biparental cross of autotetraploid potato (*Solanum tuberosum* L. subsp. *tuberosum*) to test novel linkage mapping methods for simplex and duplex marker data in polyploid crops. Notably, they found a population size of at least 250 progeny of the F1 cross would have a high chance of identifying homologous chromosomes (Hackett et al., 1998). That same year, Meyer et al. (1998) constructed a partial linkage map for tetraploid potato using amplified fragment length polymorphism (AFLP) markers, which are limited in their ability to detect dosage. In particular, SNP data is useful for QTL analysis in polyploids as it can be used to estimate dosage, or the number of copies of that allele in the genome (Hackett et al., 2013). Thus, SNP data derived from a population of roughly 200 or more full-sib progeny of an F1 cross in a polyploid crop can be appropriate for QTL analysis (Hackett et al., 2013). A biparental cross of sweetpotato can make a segregating population fit for QTL analysis.

Using SNP data, Oloka et al., (2021) discovered a major QTL for *M. incognita* race 3 within the 'Tanzania' x 'Beauregard' (TB) sweetpotato mapping population, explaining 58.3% of the variation in *M. incognita* gall counts. They hypothesized that resistance was mediated by the presence of single gene with a duplex allele (AAaaaa) present in 'Tanzania' and a nulliplex allele (aaaaaa) in 'Beauregard', which would segregate 1 aaaaaa : 3 Aaaaaa : 1 AAaaaa. In the TB progenies they observed 4:1 segregation (approximately 75% resistant progenies) for

resistance to *M. incognita*, which fit the hypothesized inheritance pattern (Oloka et al., 2021). Such a QTL makes an appealing target for the implementation of marker-assisted selection in a sweetpotato breeding program.

In this thesis, I studied the TB population as it segregates for resistance to the emergent plant parasitic nematode, *Meloidogyne enterolobii*. In Chapter 2, I describe our approach to phenotyping resistance to this pest using improved protocols by Schwarz et al. (2021), and discuss different strategies for measuring resistance to this new pest. I also describe how we associated this phenotypic data with genomic data using QTL analysis to identify a single major QTL on chromosome 4 that explains 66.9% of the variation for resistance to *M. enterolobii*. This single major QTL, and that found by Oloka et al. (2021) on chromosome 7 for resistance to *M. incognita*, motivated an attempt to identify markers within these QTL regions that could be used predict resistance in the TB population and in broader germplasm. In Chapter 3, we aligned whole genome sequence data for 'Tanzania' and 'Beauregard' with the *I. trifida* reference genome published by Wu et al. (2018) and identified a pool of 2,444 biallelic simplex or pentaplex SNP markers within these QTL regions. As a pilot test, a small pool of markers (24) nearest the QTL peaks was submitted to LGC BioSearch Technologies (Hoddesdon, UK) to genotype known resistant and susceptible sweetpotato clones, including many TB lines and a diverse mix of germplasm. A chi-square goodness of fit test revealed two markers for *M. enterolobii* resistance (LG 4: 7038930 & 7039636) with greater than 96% predictive accuracy, and two markers for *M. incognita* resistance (LG7: 1699509 & 1700440) with 95.2% and 86.4% accuracy, respectively ($P < 0.001$). Further validation of these markers in broader germplasm could determine if they are suitable for implementation as the first marker assisted breeding efforts in sweetpotato.

REFERENCES

Amankwaah, V. A. (2019). *Phenotyping and Genetic Studies of Storage Root Chemistry Traits in Sweetpotato* [Ph.D., North Carolina State University]. http://www.proquest.com/docview/2385636619/abstract/B1E1FF11D3044EC7PQ/1

Arumuganathan, K., & Earle, E. D. (1991). Nuclear DNA Content of some Important Plant Species. *Plant Molecular Biology Reporter*, *9*(3), 11.

Austin, D. (1988). The Taxonomy Evolution and Genetic Diversity of Sweet Potatoes and Related Wild Species. *P. Gregory (Ed.) Exploration, Maintenance, and Utilization of Sweet Potato Genetic Resources, International Potato Center, Lima, Peru*, 27-59.

Barkley, S. L., Schultheis, J. R., Chaudhari, S., Johanningsmeier, S. D., Jennings, K. M., Truong, V.-D., & Monks, D. W. (2017). Yield and Consumer Acceptability of 'Evangeline' Sweetpotato for Production in North Carolina. *HortTechnology*, *27*(2), 281–290. https://doi.org/10.21273/HORTTECH03533-16

Baselga, F. M. de E., Schultheis, J. R., Boyette, M. D., Quesada-Ocampo, L. M., Starke, K. D., & Monks, D. W. (2020). Vine Removal Prior to Harvest, and Curing Duration and Temperature Affect the Incidence and Severity of Internal Necrosis in 'Covington' Sweetpotato. *HortTechnology*, *30*(5), 544–551. https://doi.org/10.21273/HORTTECH04408-19

Benevenuto, J., Ferrão, L. F. V., Amadeu, R. R., & Munoz, P. (2019). How can a High-quality Genome Assembly Help Plant Breeders? *GigaScience*, *8*(6). https://doi.org/10.1093/gigascience/giz068

Brito, J., Powers, T. O., Mullin, P. G., Inserra, R. N., & Dickson, D. W. (2004). Morphological and Molecular Characterization of *Meloidogyne mayaguensis* Isolates from Florida. *Journal of Nematology*, *36*(3), 232–240.

Castagnone-Sereno, P. (2012). *Meloidogyne enterolobii* (= *M. mayaguensis*): Profile of an Emerging, Highly Pathogenic, Root-knot Nematode Species. *Nematology*, *14*(2), 133–138. https://doi.org/10.1163/156854111X601650

Castagnone-Sereno, P., & Danchin, E. G. J. (2014). Parasitic Success without Sex – the Nematode Experience. *Journal of Evolutionary Biology*, *27*(7), 1323–1333. https://doi.org/10.1111/jeb.12337

Cervantes-Flores, J. C., Sosinski, B., Pecota, K. V., Mwanga, R. O. M., Catignani, G. L., Truong, V. D., Watkins, R. H., Ulmer, M. R., & Yencho, G. C. (2011). Identification of Quantitative Trait Loci for Dry-matter, Starch, and β-carotene Content in Sweetpotato. *Molecular Breeding*, *28*(2), 201–216. https://doi.org/10.1007/s11032-010-9474-5

Cervantes-Flores, J. C., Yencho, G. C., & Davis, E. L. (2002). Host Reactions of Sweetpotato Genotypes to Root-knot Nematodes and Variation in Virulence of *Meloidogyne incognita* Populations. *HortScience*, *37*(7), 1112–1116. https://doi.org/10.21273/HORTSCI.37.7.1112

Cervantes-Flores, J. C., Yencho, G. C., Kriegner, A., Pecota, K. V., Faulk, M. A., Mwanga, R. O. M., & Sosinski, B. R. (2008a). Development of a Genetic Linkage Map and Identification of Homologous Linkage Groups in Sweetpotato Using Multiple-dose AFLP Markers. *Molecular Breeding*, *21*(4), 511–532. https://doi.org/10.1007/s11032-007-9150-6

Cervantes-Flores, J. C., Yencho, G. C., Pecota, K. V., Sosinski, B., & Mwanga, R. O. M. (2008b). Detection of Quantitative Trait Loci and Inheritance of Root-knot Nematode Resistance in Sweetpotato. *Journal of the American Society for Horticultural Science*, *133*(6), 844–851. https://doi.org/10.21273/JASHS.133.6.844

Clark, C. A., Ferrin, D. M., Smith, T. P., & Holmes, G. J. (Eds.). (2013). *Compendium of Sweetpotato Diseases, Pests, and Disorders, Second Edition*. The American Phytopathological Society. https://doi.org/10.1094/9780890544952

Clark, C. A., & Moyer, J. W. (1988). *Compendium of Sweet Potato Diseases*. APS Press.

Covarrubias-Pazaran, G., Diaz-Garcia, L., Schlautman, B., Deutsch, J., Salazar, W., Hernandez-Ochoa, M., Grygleski, E., Steffan, S., Iorizzo, M., Polashock, J., Vorsa, N., & Zalapa, J. (2016). Exploiting Genotyping by Sequencing to Characterize the Genomic Structure of the American Cranberry Through High-density Linkage Mapping. *BMC Genomics*, *17*(1), 451. https://doi.org/10.1186/s12864-016-2802-3

Edmunds, B., Boyette, M., Clark, C., Ferrin, D., Smith, T., & Holmes, G. (2008, August 1). *Postharvest Handling of Sweetpotatoes | NC State Extension Publications*. https://content.ces.ncsu.edu/postharvest-handling-of-sweetpotatoes

EPPO. (2020, May). *EPPO Alert List*. https%3A%2F%2Fwww.eppo.int%2FACTIVITIES%2Fplant_quarantine%2Falert_list

EPPO. (2021). *Meloidogyne enterolobii*. Retrieved December 3, 2021, from https://gd.eppo.int/taxon/MELGMY/categorization

FAO. (2020). *FAOSTAT Crops*. http://www.fao.org/faostat/en/#data/QC/visualize

Gao, M., Soriano, S. F., Cao, Q., Yang, X., & Lu, G. (2020). Hexaploid Sweetpotato (*Ipomoea batatas* (L.) Lam.) May not Be a True Type to Either Auto- or Allopolyploid. *PLOS ONE*, *15*(3), e0229624. https://doi.org/10.1371/journal.pone.0229624

Gemenet, D. C., Kitavi, M. N., David, M., Ndege, D., Ssali, R. T., Swanckaert, J., Makunde, G., Yencho, G. C., Gruneberg, W., Carey, E., Mwanga, R. O., Andrade, M. I., Heck, S., & Campos, H. (2020). Development of Diagnostic SNP Markers for Quality Assurance and Control in Sweetpotato [*Ipomoea batatas* (L.) Lam.] Breeding Programs. *PLOS ONE*, *15*(4), e0232173. https://doi.org/10.1371/journal.pone.0232173

Gorny, A. (2020, February 24). *Root-knot Nematodes in Sweetpotatoes: Information and Updates*. Sweetpotato Regional Production Meeting, Goldsboro, NC.

Grattapaglia, D., & Sederoff, R. (1994). Genetic Linkage Maps of *Eucalyptus grandis* and *Eucalyptus urophylla* Using a Pseudo-Testcross: Mapping Strategy and RAPD Markers. *Genetics*, *137*(4), 1121–1137.

Hackett, C. A., Bradshaw, J. E., Meyer, R. C., McNICOL, J. W., Milbourne, D., & Waugh, R. (1998). Linkage Analysis in Tetraploid Species: A Simulation Study. *Genetical Research*, *71*(2), 143–153. https://doi.org/10.1017/S0016672398003188

Hackett, C. A., McLean, K., & Bryan, G. J. (2013). Linkage Analysis and QTL Mapping Using SNP Dosage Data in a Tetraploid Potato Mapping Population. *PLoS ONE*, *8*(5), e63939. https://doi.org/10.1371/journal.pone.0063939

Hunt, D., & Handoo, Z. (2009). Taxonomy, Identification and Principal Species. *Root-Knot Nematodes*, 55–97. https://doi.org/10.1079/9781845934927.0055

Isobe, S., Shirasawa, K., & Hirakawa, H. (2019). Current Status in Whole Genome Sequencing and Analysis of *Ipomoea* spp. *Plant Cell Reports*, *38*(11), 1365–1371. https://doi.org/10.1007/s00299-019-02464-4

Jennings, K., Quesada-Ocampo, L., Schultheis, J. R., Woodley, A., Yencho, G. C., Pecota, K. V., Huseth, A., Smith, S. C., & Boyette, M. (2019, June 24). *Chapter 8: Crop Production Management - Sweetpotatoes | NC State Extension Publications*. https://content.ces.ncsu.edu/north-carolina-organic-commodities-production-guide/chapter-8-crop-production-management-sweetpotatoes

Ji, H., Zhang, H., Li, H., & Li, Y. (2015). Analysis on the Nutrition Composition and Antioxidant Activity of Different Types of Sweet Potato Cultivars. *Food and Nutrition Sciences*, *06*(01), 161. https://doi.org/10.4236/fns.2015.61017

Jiang, C., Perkins-Veazie, P., Blankenship, S. M., Boyette, M. D., Pesic-VanEsbroeck, Z., Jennings, K. M., & Schultheis, J. R. (2015). Occurrence, Severity and Initiation of Internal Necrosis in 'Covington' Sweetpotato. *HortTechnology*, *25*(3), 340–348. https://doi.org/10.21273/HORTTECH.25.3.340

Jones, A., Dukes, P. D., & Schalk, J. M. (1986). Sweet Potato Breeding. In *Breeding Vegetable Crops*. AVI Publishing Co.

Kirkpatrick, T., Lee, J., & Faske, T. (2019). The Guava Root-Knot Nematode (*Meloidogyne enterolobii*), a Potential Threat to Arkansas Sweet Potatoes and Other Crops. *University of Arkansas Cooperative Extension Service Printing Services*, 2.

Koutsovoulos, G. D., Poullet, M., Ashry, A. E., Kozlowski, D. K., Sallet, E., Rocha, M. D., Martin-Jimenez, C., Perfus-Barbeoch, L., Frey, J.-E., Ahrens, C., Kiewnick, S., & Danchin, E. G. J. (2019). *The Polyploid Genome of the Mitotic Parthenogenetic Root-*

knot Nematode Meloidogyne enterolobii [Preprint]. Genomics. https://doi.org/10.1101/586818

Kriegner, A., Cervantes, J. C., Burg, K., Mwanga, R. O. M., & Zhang, D. (2003). A Genetic Linkage Map of Sweetpotato [*Ipomoea batatas* (L.) Lam.] Based on AFLP Markers. *Molecular Breeding*, *11*, 18.

Lambert, K., & Bekal, S. (2002). *Introduction to Plant-Parasitic Nematodes*. Introduction to Plant-Parasitic Nematodes. https://www.apsnet.org/edcenter/disandpath/nematode/intro/Pages/IntroNematodes.aspx

Laurie, S., Faber, M., Adebola, P., & Belete, A. (2015). Biofortification of Sweet Potato for Food and Nutrition Security in South Africa. *Food Research International*, *76*, 962–970. https://doi.org/10.1016/j.foodres.2015.06.001

Lawrence, G. W., Clark, C. A., & Wright, V. L. (1986). Influence of *Meloidogyne incognita* on Resistant and Susceptible Sweet Potato Cultivars. *Journal of Nematology*, *18*(1), 59–65.

Leksrisompong, P. P., Whitson, M. E., Truong, V. D., & Drake, M. A. (2012). Sensory Attributes and Consumer Acceptance of Sweet Potato Cultivars with Varying Flesh Colors. *Journal of Sensory Studies*, *27*(1), 59–69. https://doi.org/10.1111/j.1745-459X.2011.00367.x

Loebenstein, G., & Thottappilly, G. (Eds.). (2009). *The Sweetpotato*. Springer Netherlands. https://doi.org/10.1007/978-1-4020-9475-0

Low, J. W., Mwanga, R. O. M., Andrade, M., Carey, E., & Ball, A.-M. (2017). Tackling Vitamin A Deficiency with Biofortified Sweetpotato in Sub-Saharan Africa. *Global Food Security*, *14*, 23–30. https://doi.org/10.1016/j.gfs.2017.01.004

Martin, F. W., & Jones, A. (1986). Breeding Sweet Potatoes. In *Plant Breeding Reviews* (pp. 313–345). John Wiley & Sons, Ltd.

Moens, M., Perry, R. N., & Starr, J. L. (2009). *Meloidogyne* Species—A Diverse Group of Novel and Important Plant Parasites. In R. N. Perry, M. Moens, & J. L. Starr (Eds.), *Root-knot nematodes* (pp. 1–17). CABI. https://doi.org/10.1079/9781845934927.0001

Mollinari, M., Olukolu, B. A., Pereira, G. da S., Khan, A., Gemenet, D., Yencho, G. C., & Zeng, Z.-B. (2020). Unraveling the Hexaploid Sweetpotato Inheritance Using Ultra-Dense Multilocus Mapping. *G3 Genes|Genomes|Genetics*, *10*(1), 281–292. https://doi.org/10.1534/g3.119.400620

Muñoz-Rodríguez, P., Carruthers, T., Wood, J. R. I., Williams, B. R. M., Weitemier, K., Kronmiller, B., Ellis, D., Anglin, N. L., Longway, L., Harris, S. A., Rausher, M. D., Kelly, S., Liston, A., & Scotland, R. W. (2018). Reconciling Conflicting Phylogenies in the Origin of Sweet Potato and Dispersal to Polynesia. *Current Biology*, *28*(8), 1246-1256.e12. https://doi.org/10.1016/j.cub.2018.03.020

Mutschler, M. A., Xiong, J. H., Wally, A., & Lobato-Ortiz, R. (2005). Dissection of Linkage Drag in Breeding for Acylsugar-mediated Multiple Pests in Tomato. *HortScience*, *40*(4), 1098C – 1098. https://doi.org/10.21273/HORTSCI.40.4.1098C

Mwanga, R. O. M., Odongo, B., p'Obwoya, C. O., Gibson, R. W., Smit, N. E. J. M., & Carey, E. E. (2001). Release of Five Sweetpotato Cultivars in Uganda. *HortScience*, *36*(2), 385–386. https://doi.org/10.21273/HORTSCI.36.2.385

OEPP/EPPO. (2011). *Meloidogyne enterolobii*. *EPPO Bulletin*, *41*(3), 329–339. https://doi.org/10.1111/j.1365-2338.2011.02497.x

Oloka, B. M., da Silva Pereira, G., Amankwaah, V. A., Mollinari, M., Pecota, K. V., Yada, B., Olukolu, B. A., Zeng, Z.-B., & Craig Yencho, G. (2021). Discovery of a Major QTL for Root-knot Nematode (*Meloidogyne incognita*) Resistance in Cultivated Sweetpotato (*Ipomoea batatas*). *Theoretical and Applied Genetics*, *134*(7), 1945–1955. https://doi.org/10.1007/s00122-021-03797-z

Onkendi, E. M., & Moleleki, L. N. (2013). Detection of *Meloidogyne enterolobii* in Potatoes in South Africa and Phylogenetic Analysis Based on Intergenic Region and the Mitochondrial DNA Sequences. *European Journal of Plant Pathology*, *136*(1), 1–5. https://doi.org/10.1007/s10658-012-0142-y

Overstreet, C., McGawley, E. C., Clark, C., Rezende, J., Smith, T., & Sistrunk, M. (2019). *Guava Root-knot Nematode a Potentially Serious New Pest in Louisiana*. 3.

Quesada-Ocampo, L. (2018, May 24). *Sweetpotato Root Knot Nematode | NC State Extension Publications*. https://content.ces.ncsu.edu/sweetpotato-root-knot-nematode

Rammah, A., & Hirschmann, H. (1988). *Meloidogyne mayaguensis* n. Sp. (Meloidogynidae), a Root-knot Nematode from Puerto Rico. *Journal of Nematology*, *20*(1), 58–69.

Ramsey, J., & Schemske, D. W. (2002). Neopolyploidy in Flowering Plants. *Annual Review of Ecology and Systematics*, *33*(1), 589–639. https://doi.org/10.1146/annurev.ecolsys.33.010802.150437

Ribaut, J.-M., & Hoisington, D. (1998). Marker-assisted Selection: New Tools and Strategies. *Trends in Plant Science*, *3*(6), 236–239. https://doi.org/10.1016/S1360-1385(98)01240-0

Roberts, P. A. (1992). Current Status of the Availability, Development, and Use of Host Plant Resistance to Nematodes. *Journal of Nematology*, *24*(2), 213–227.

Roullier, C., Benoit, L., Mckey, D., & Lebot, V. (2013). Historical collections reveal patterns of diffusion of sweet potato in Oceania obscured by modern plant movements and recombination. *Proceedings of the National Academy of Sciences of the United States of America*, *110*. https://doi.org/10.1073/pnas.1211049110

Rutter, W. B., Skantar, A. M., Handoo, Z. A., Mueller, J. D., Aultman, S. P., & Agudelo, P. (2018). *Meloidogyne enterolobii* Found Infecting Root-knot Nematode Resistant Sweetpotato in South Carolina, United States. *Plant Disease*, *103*(4), 775–775. https://doi.org/10.1094/PDIS-08-18-1388-PDN

Rutter, W., Wadl, P., Mueller, J. D., & Agudelo, P. (2021). Identification of Sweetpotato Germplasm Resistant to Pathotypically Distinct Isolates of *Meloidogyne enterolobii* from the Carolinas. *Plant Disease*, PDIS-02-20-0379-RE. https://doi.org/10.1094/PDIS-02-20-0379-RE

Schwarz, T., Li, C., Ye, W., & Davis, E. (2020). Distribution of *Meloidogyne enterolobii* in Eastern North Carolina and Comparison of Four Isolates. *Plant Health Progress*, *21*(2), 91–96. https://doi.org/10.1094/PHP-12-19-0093-RS

Schwarz, T. R., Li, C., Yencho, G. C., Pecota, K. V., Heim, C. R., & Davis, E. L. (2021). Screening Sweetpotato Genotypes for Resistance to a North Carolina Isolate of *Meloidogyne enterolobii*. *Plant Disease*, *105*(4), 1101–1107. https://doi.org/10.1094/PDIS-02-20-0389-RE

Shiotani, I., & Kawase, T. (1989). *Genomic Structure of the Sweet Potato and Hexaploids in Ipomoea trifida*. https://doi.org/10.1270/jsbbs1951.39.57

Stam, P., & Zeven, A. C. (1981). The Theoretical Proportion of the Donor Genome in Near-isogenic Lines of Self-fertilizers Bred by Backcrossing. *Euphytica*, *30*(2), 227–238. https://doi.org/10.1007/BF00033982

Tanksley, S. D. (1993). Mapping Polygenes. *Annual Review of Genetics*, *27*(1), 205–233. https://doi.org/10.1146/annurev.ge.27.120193.001225

Tanksley, S. D., Ganal, M. W., Prince, J. P., de Vicente, M. C., Bonierbale, M. W., Broun, P., Fulton, T. M., Giovannoni, J. J., Grandillo, S., & Martin, G. B. (1992). High Density Molecular Linkage Maps of the Tomato and Potato Genomes. *Genetics*, *132*(4), 1141–1160. https://doi.org/10.1093/genetics/132.4.1141

Tigano, M., Siqueira, K. D., Castagnone-Sereno, P., Mulet, K., Queiroz, P., Santos, M. D., Teixeira, C., Almeida, M., Silva, J., & Carneiro, R. (2010). Genetic Diversity of the Root-knot Nematode *Meloidogyne enterolobii* and Development of a SCAR Marker for This Guava-damaging Species. *Plant Pathology*, *59*(6), 1054–1061. https://doi.org/10.1111/j.1365-3059.2010.02350.x

Ukoskit, K., Thompson, P. G., Watson, C. E., & Lawrence, G. W. (1997). *Identifying a Randomly Amplified Polymorphic DNA (RAPD) Marker Linked to a Gene for Root-knot Nematode Resistance in Sweetpotato*. https://doi.org/10.21273/jashs.122.6.818

USDA/NASS. (2020, June 9). *2019 State Agriculture Overview for North Carolina*. https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH%20CAROLINA

Watson, T. T. (2021). Sensitivity of *Meloidogyne enterolobii* and *M. incognita* to Fluorinated Nematicides. *Pest Management Science*. https://doi.org/10.1002/ps.6756

Webb, D., & Troxler, S. (2020). *Sweet Potatoes County Estimates*. 2.

Wilson, P. (2018, October 5). *NCDA&CS Declares an Internal Quarantine for all North Carolina Counties for the Guava Root Knot Nematode*. NCDA&CS. http://www.ncagr.gov/paffairs/release/2018/10-18guavarootknotnematode.htm

Woolfe, J. A. (1992). *Sweet Potato: An Untapped Food Resource*. Cambridge University Press.

Wu, S., Lau, K. H., Cao, Q., Hamilton, J. P., Sun, H., Zhou, C., Eserman, L., Gemenet, D. C., Olukolu, B. A., Wang, H., Crisovan, E., Godden, G. T., Jiao, C., Wang, X., Kitavi, M., Manrique-Carpintero, N., Vaillancourt, B., Wiegert-Rininger, K., Yang, X., Bao, K., Schaff, J., Kreuze, J., Gruneberg, W., Khan, A., Ghislain, M., Ma, D., Jiang, J., Wmanga, R., Leebens-Mack, J., Coin, L., Yencho, G. C., Buell, C. R., Fei, Z. (2018). Genome Sequences of Two Diploid Wild Relatives of Cultivated Sweetpotato Reveal Targets for Genetic Improvement. *Nature Communications*, *9*(1), 4580. https://doi.org/10.1038/s41467-018-06983-8

Wu, S., Yang, J., Huang, Y., Li, Y., Yin, T., Wullschleger, S. D., Tuskan, G. A., & Wu, R. (2010). An Improved Approach for Mapping Quantitative Trait Loci in a Pseudo-Testcross: Revisiting a Poplar Mapping Study. *Bioinformatics and Biology Insights*, *4*, BBI.S4153. https://doi.org/10.4137/BBI.S4153

Xu, J., Liu, P., Meng, Q., & Long, H. (2004). Characterisation of Meloidogyne species from China using Isozyme Phenotypes and Amplified Mitochondrial DNA Restriction Fragment Length Polymorphism. *European Journal of Plant Pathology*, *110*(3), 309–315. https://doi.org/10.1023/B:EJPP.0000019800.47389.31

Yada, B., Alajo, A., Ssemakula, G. N., Mwanga, R. O. M., Brown-Guedira, G., & Yencho, G. C. (2017). Selection of Simple Sequence Repeat Markers Associated with Inheritance of

Sweetpotato Virus Disease Resistance in Sweetpotato. *Crop Science*, *57*(3), 1421–1430. https://doi.org/10.2135/cropsci2016.08.0695

Yang, B., & Eisenback, J. D. (1983). *Meloidogyne enterolobii* n. Sp. (Meloidogynidae), a Root-knot Nematode Parasitizing Pacara Earpod Tree in China. *Journal of Nematology*, *15*(3), 381–391.

Ye, W. (2018). Nematodes of Agricultural Importance in North and South Carolina. In S. A. Subbotin & J. J. Chitambar (Eds.), *Plant Parasitic Nematodes in Sustainable Agriculture of North America* (pp. 247–276). Springer International Publishing. https://doi.org/10.1007/978-3-319-99588-5_10

Ye, W. M., Koenning, S. R., Zhuo, K., & Liao, J. L. (2013). First Report of *Meloidogyne enterolobii* on Cotton and Soybean in North Carolina, United States. *Plant Disease*, *97*(9), 1262. https://doi.org/10.1094/PDIS-03-13-0228-PDN

Yencho, G. C., Pecota, K. V., Schultheis, J. R., VanEsbroeck, Z.-P., Holmes, G. J., Little, B. E., Thornton, A. C., & Truong, V.-D. (2008). 'Covington' Sweetpotato. *HortScience*, *43*(6), 1911–1914. https://doi.org/10.21273/HORTSCI.43.6.1911

CHAPTER 1: FIGURES

Figure 1. *Meloidogyne enterolobii*, the guava root-knot nematode, on 'Covington' sweetpotato fibrous roots (left) and galls on a 'Covington' storage root (right).

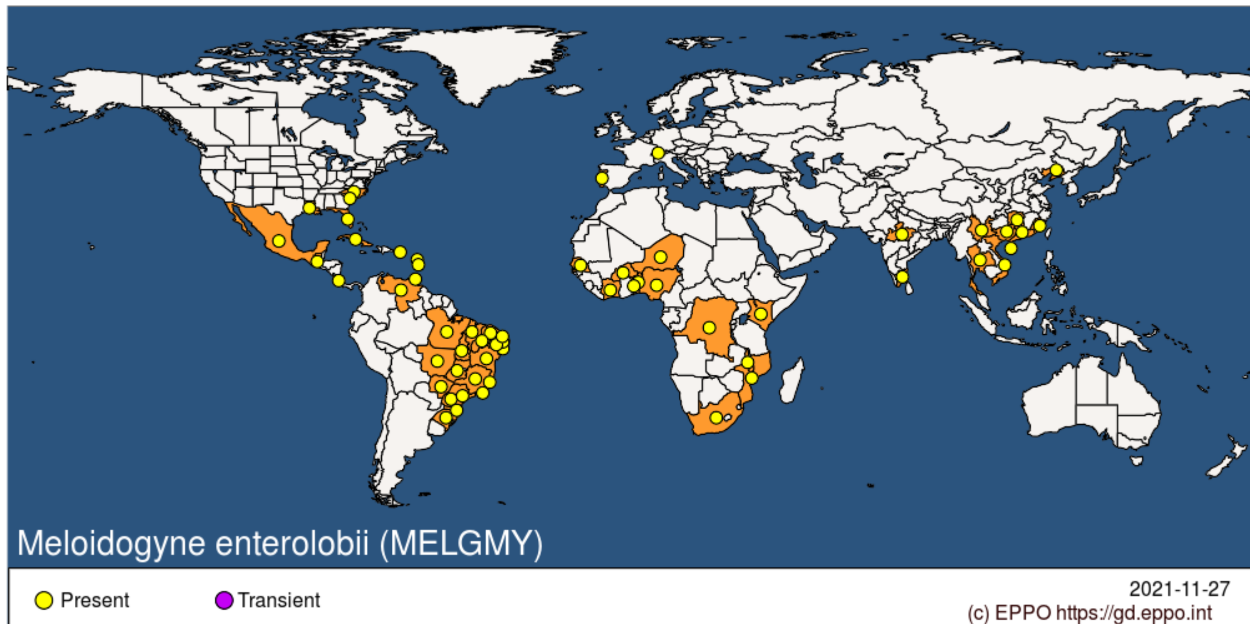

Figure 2. Nations or states which have confirmed occurrences of *M. enterolobii* in any plant. From: EPPO, 2021. Available at: https://gd.eppo.int/taxon/MELGMY/datasheet

Figure 3. Segregation at the F1 level in sweetpotato. These storage roots represent two biparental mapping populations segregating for resistance to *M. enterolobii* and many other traits (e.g., flesh and skin color, yield, storage root shape. (Left) Progeny of a cross between 'Monaco', an orange-fleshed, rose-skinned line, and L14-31 a red-skinned, orange fleshed *M. enterolobii* resistant line. (Right) Progeny of a cross between L14-41p, a purple *M. enterolobii* resistant line and 'Beauregard', an orange-fleshed, rose-skinned line.

CHAPTER 2: QTL analysis of the 'Tanzania' x 'Beauregard' sweetpotato mapping population for resistance to *Meloidogyne enterolobii*

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Abstract

Sweetpotato, *Ipomoea batatas* (L.) Lam. (2n=6x=90) is among the world's most important food crops. Due to its complex genetics, sweetpotato lags far behind other crops in terms of genomic tools. However, recent advances in sweetpotato genomics provided new opportunities to associate genotypic and phenotypic data though quantitative trait loci (QTL) analysis and candidate gene identification.

We screened the biparental mapping population, 'Tanzania' x 'Beauregard' (TB), for resistance to the emergent plant parasitic nematode, *Meloidogyne enterolobii* (Yang & Eisenback, 1983) by inoculating 246 segregating lines with 10,000 eggs each under greenhouse conditions. 'Tanzania', the female parent, was highly resistant, while 'Beauregard' was highly susceptible. Our bioassays exhibited strong skewing toward resistance for three measures of resistance: reproductive factor (RF), eggs per gram of root tissue (EGRT), and gall ratings. A 1:1 segregation for resistance suggested a simplex major allele conferred *M. enterolobii.* resistance. Using QTLpoly and a random-effect multiple interval mapping (REMIM) model, we identified a single major QTL peak (57.5cM) on linkage group 4 that explained 70% of variation in resistance to *M. enterolobii.* This study provides new understanding of the genetic basis of *M. enterolobii* resistance in sweetpotato and represents a major step towards the identification of selectable markers for resistance breeding for domestic and international breeding programs.

Key Message: Using a segregating mapping population for *Meloidogyne enterolobii* resistance in sweetpotato, we report a major QTL that has potential for marker-assisted selection in sweetpotato breeding for accelerated genetic gains.

Introduction

Meloidogyne spp., the root-knot nematodes (RKN), are globally distributed plant parasitic roundworms with worldwide economic impact and a host range that includes most higher plants (Moens et al., 2009). *Meloidogyne enterolobii* (Yang & Eisenback, 1983) is among the most damaging species in its genus. *Meloidogyne enterolobii* has a host range of at least 121 plants, extreme pathogenicity, and the ability to develop and reproduce on crops and weeds known to carry resistance genes for other *Meloidogyne* species (Castagnone-Sereno, 2012; Moens et al., 2009; OEPP/EPPO, 2011; Rutter et al., 2018). Total crop failure due to quality and yield loss has been attributed to *M. enterolobii* in sweetpotato (*Ipomoea batatas* (L.) Lam.) (Kirkpatrick et al., 2019; Ye, 2018). North Carolina (NC) produced approximately 65% of the US sweetpotato crop in 2019 (USDA/NASS, 2020), and in NC the two RKN of most concern are *Meloidogyne incognita* (Kofoid & White) Chitwood, the southern RKN, and the recently introduced *M. enterolobii*, the guava RKN.

Resistance breeding may be the most effective, safe (i.e., not requiring toxic nematicides), sustainable, and economical solution to managing *Meloidogyne* in sweetpotato (Cervantes-Flores et al., 2008; Clark & Moyer, 1988). Plant breeders have addressed root-knot nematode problems in the past by releasing resistant crop varieties, including *Meloidogyne* spp. resistant alfalfa (Hunt et al., 1972), *M. incognita*-resistant tomato (Bhavana et al., 2019; Singh et al., 1974), the *M. incognita*-resistant variety ‘Covington’ sweetpotato (Yencho et al., 2008), and

others. Each dollar put towards research and development of nematode resistant crop varieties has been estimated to pay off several hundredfold (Boerma & Hussey, 1992). Introducing new genes into breeding germplasm and the development of new varieties, however, is a time-consuming and expensive process.

'Covington', the predominant sweetpotato variety (~90%) grown in NC (Baselga et al., 2020), has strong resistance to *M. incognita* yet is susceptible to *M. enterolobii* (Rutter et al., 2018; Schwarz et al., 2021). Currently, all commercial sweetpotato varieties grown in NC have tested susceptible to *M. enterolobii*, including 'Covington', 'Beauregard', 'Hernandez', 'Averre', 'Bonita', 'Bayou Belle', and 'Bellevue' (Rutter et al., 2021; Schwarz et al., 2021). *Meloidogyne enterolobii* is now established in NC (Ye et al., 2013), and growers need a resistant and locally-adapted variety of orange-fleshed moist sweetpotato to stay competitive in the global marketplace.

Of the commercial varieties grown in NC, only the specialty variety 'Murasaki-29', a starchy, cream-fleshed, purple-skinned clone, is *M. enterolobii* resistant (Schwarz et al., 2021). 'Tanzania', a starchy, high dry matter Ugandan landrace (Mwanga et al., 2001) with poor adaptation to the growing regions of NC, also tested resistant to *M. enterolobii* in independent trials (Rutter et al., 2021; Schwarz et al., 2021). Breeding with such resistant varieties is a challenge, especially when crossing exotic and elite lines whose segregating progenies exhibit significant linkage drag in addition to providing resistance. Crossing two highly heterozygous polyploid sweetpotato varieties results in segregation at the F1 level (Jones et al., 1986), and a population suitable for linkage mapping and QTL analysis can be made in a single cross. While generating segregating sweetpotato populations is relatively simple, breeding sweetpotato is not. Many horticultural traits in sweetpotato are quantitative in nature, including dry matter, starch,

and β-carotene content (Cervantes-Flores et al., 2011), and others. Further, the hexaploid nature of sweetpotato and allele dosage effects can lead to incredibly complex segregation ratios, such that it is often easier to consider heritability of a trait in sweetpotato than the Mendelian segregation patterns (Jones et al., 1986). The sweetpotato breeding program at NCSU routinely evaluates 30-45 or more traits. Therefore, introgressing a simplex trait, such as the dominant *M. incognita* resistance locus hypothesized by Oloka et al. (2021), does not lead to immediate variety release. Elite varieties are so classified because they excel in most traits, yet a single cross between elite lines will yield mostly progenies that fall short, and sweetpotato will not tolerate backcrossing. The next major variety must excel in all traits across multiple environments and possess the *M. enterolobii* resistance that does not yet exist in elite breeding germplasm.

Cervantes-Flores et al. (2008b) reported on the development of the 'Tanzania' x 'Beauregard' (TB) mapping population and, using an amplified fragment length polymorphism (AFLP) based linkage map (Cervantes-Flores et al., 2008a), conducted QTL analyses for several traits including *M. incognita* race 3 resistance. 'Beauregard', once the major US variety, is susceptible to *M. enterolobii*, *M. incognita* races 1, 2, 3 and 4, and *M. javanica* (Cervantes-Flores et al., 2002), while 'Tanzania' is resistant to *M. enterolobii* (Rutter et al., 2021; Schwarz et al., 2021), all four races of *M. incognita*, and *M. javanica* (Cervantes-Flores et al., 2002). Mollinari et al. (2020) constructed an ultra-dense integrated multi-locus SNP-based linkage map for TB using comparative SNP information from the *Ipomoea trifida* (Kunth.) G. Don reference genome published by Wu et al. (2018), and Oloka et al. (2021) used this map for QTL analysis of TB for *M. incognita* race 3 resistance. Within TB, *M. incognita* resistance was highly skewed towards 'Tanzania', the resistant parent, and QTL analysis revealed one major QTL on linkage group 7

explaining 58.3% of variation for resistance (Oloka et al., 2021). *Meloidogyne enterolobii* was not known in the United States at the time of the TB population's development, but 2021 resistance screens by Schwarz et al. (2021) revealed 'Tanzania' to be resistant and 'Beauregard' to be susceptible to *M. enterolobii*. Schwarz et al. (2021) also screened eight TB progeny and found five to be resistant and three to be susceptible. This warranted a closer look at the TB population and its segregation for *M. enterolobii* resistance.

Here, we describe QTL analysis for *M. enterolobii* resistance using biallelic SNP marker data from the TB sweetpotato mapping population, and phenotypic data from improved *M. enterolobii* inoculation-based greenhouse bioassays. This study was made possible by new genomic tools and resources, including the high-quality genome assembly of *I. trifida* (NCNSP306) (Wu et al., 2018), the heterozygous polyploid-optimized genotyping-by-sequencing protocol GBSpoly (Wadl et al., 2018), and R packages MAPpoly (Mollinari & Garcia, 2019) and QTLpoly (da Silva Pereira et al., 2020).

Materials and Methods

Plant Materials

The biparental 'Tanzania' x 'Beauregard' (TB) mapping population has been maintained in vegetative state in virus-free greenhouse conditions (regular watering and fertilizer, 25-45ºC) in 72-cell Landmark™ seedling trays (Stuewe & Sons, Corvallis, OR) in Fafard P4 soil mix (Fafard, Agawam, MA) at North Carolina State University Method Road complex since its inception. Quarterly, vegetative cuttings were made and rejuvenated in fresh media. The following resistant and susceptible checks were also included in all experiments: 'Covington' and 'Beauregard' as *M. enterolobii* susceptible checks; and 'Jewel' and 'Tanzania' as resistant

checks (Schwarz et al., 2021). These check lines were included as inoculated (positive) and uninoculated (negative) controls. For each replicate, four positive and two negative controls were included for ‘Covington’, ‘Beauregard’, ‘Tanzania’, and ‘Jewel’. Two replicates were generated from vine cuttings of each genotype, including checks and TB progeny, for a total of 516 plant samples.

Nematode Preparation

The *M. enterolobii* population used for this trial was isolated from a soybean/sweetpotato rotation field in Johnston County, NC in 2017 and has been maintained on ‘Rutgers’ tomato plants (*Solanum lycopersicum* L.) (Schwarz et al., 2021). Nematode egg extraction methods were described by Schwarz et al. (2021) which were based on modified protocols by Hussey and Barker (1973). Briefly, a 10% v/v Clorox bleach at 7.4% sodium hypochlorite solution was used to break down egg matrices on inoculated stock tomato plants, and this solution was poured through a stacked series of brass sieves (#60, #120, #500, from top to bottom) to capture *M. enterolobii* eggs. The concentration of eggs in stock solution samples was estimated by counting 1 mL aliquots at 40× using a Nikon TMS Inverted Phase Contrast microscope, and total eggs in solution was extrapolated.

Sweetpotato Planting and Inoculation

Following procedures described by Schwarz et al. (2021), 12-15cm sweetpotato vine cuttings were collected from the 246 TB clones and check lines. Cuttings were planted in 15cm terra cotta pots in a 1:1 mix of sand and soil (88.9% sand, 8.2% silt, 2.8% clay) (Schwarz et al., 2021). Pots were arranged in a completely randomized design on galvanized steel mesh

greenhouse benches which prevented standing water from contaminating neighbors. Greenhouse conditions were 25-28ºC with no supplemental lighting. Inoculation with *M. enterolobii* (10,000 eggs per plant) in 15 ml of water at a depth of 2cm occurred 14 days post-planting. Nematode eggs were inoculated within 48 hours of extraction from stock culture plants. Light watering occurred as needed, with care not to overwater which might flush nematodes from the media.

Egg Extractions

Evaluations began at 60 days post-inoculation. Sweetpotato plants were cut at crown-level and then rinsed of soil in wash buckets. After washing, roots were bundled in dry paper towels to absorb excess moisture and stored in a 20°C refrigerator overnight. The following day, paper towels were removed, and each root mass was weighed. Root masses were soaked at room temperature approximately 15 to 30 minutes in red food dye (12.5% by volume) (McCormick and Co., Baltimore, MD) by which the egg masses were stained, achieving a similar effect as phloxine B while being non-toxic (Thies et al., 2002). Gall ratings were performed visually by a single researcher based on the percent of total root volume with galls present (Schwarz et al., 2021): 1 galled root in 100 root was 1% galling; 20 galled roots in 200 total was 10% galling, etc.

Egg extractions from each sample followed the same extraction protocol described above (Hussey & Barker, 1973). A 10% v/v Clorox bleach at 7.4% sodium hypochlorite solution was poured over individual samples in 1-liter flasks and samples were soaked for 30-60 seconds. Wearing gloves, fine roots and storage roots were massaged in the bleach solution to dissolve the gelatinous matrix which adheres the eggs to the roots. Samples were poured through three stacked sieves (#60, #120, #500, from top to bottom) and carefully rinsed under running tap

water to concentrate eggs in the bottom sieve. The top two sieves were then removed, and contents discarded; contents of the bottom sieve (i.e., *M. enterolobii* eggs) were washed to one side using tap water, and extracted eggs were poured into 50 ml Falcon tubes. These tubes were adjusted with tap water to 50 ml total volume, which reduced egg concentration to ease quantification. Falcon tubes were stored in a 20°C refrigerator for up to one week prior to egg counting.

Meloidogyne enterolobii Quantification

Nematode eggs were quantified using four subsamples of 5mL from each 50mL egg extraction solution. After gentle inversion of the Falcon tube (not a swirling motion, which can concentrate eggs in the center and bias egg counts) a 5mL aliquot from each extraction was dispensed into four separate wells of a six-well tissue culture plate. Tissue culture plates were manually etched on the underside with a ¼" (0.635cm) grid to ease counting. Egg counting was performed using an inverted Nikon TMS Inverted Phase Contrast microscope (4-40× magnification). The mean of the four subsamples was extrapolated to calculate the total number of eggs in the 50 ml extraction sample. Total eggs in solution represents the same value as total eggs per plant sample and final nematode egg population. As needed, samples were diluted 1:50 or 1:100 to facilitate counting, with care to factor this dilution in while calculating total eggs. Total eggs were used to predict two measures of resistance. Reproductive factor (RF) measures resistance as the change in RKN populations with the ratio of final nematode eggs to initial inoculum (Oostenbrink, 1966; Seinhorst, 1965; Trudgill & Phillips, 1997). An RF greater than one suggests the nematode can proliferate on the host, classifying it as susceptible, while RF below one suggests resistance. The second measure, eggs per gram of root tissue (EGRT), was

total eggs divided by root mass to standardize egg production across root systems of variable mass, in which larger, heavier root systems may have supported higher nematode populations.

QTL Analysis

Genotyping of the TB population was conducted using GBSPoly (Wadl et al., 2018) as described by Amankwaah et al. (2019). Briefly, GBSPoly is a genotyping-by-sequencing protocol developed for heterozygous polyploids like sweetpotato. Library preparation and sequencing were done at the North Carolina State University Genomic Sciences Laboratory as described by Oloka et al. (2021), and reads were aligned with the *I. trifida* (NCNSP306) reference genome (Wu et al., 2018). Dosage calling followed procedures described by Oloka et al. (2021). The R package MAPpoly (Mollinari & Garcia, 2019) was used for linkage map construction of the TB population as described by Mollinari et al. (2020). A version of this map was used for our study, with the following modifications: clones with >23% missing marker information were removed from the linkage map, and markers which were absent in >25% of the TB population were not considered. Clone TB119 was identified as a self of 'Tanzania' and was also removed.

QTL analysis was performed using both the fixed-effect interval mapping (FEIM) and random-effect multiple interval mapping (REMIM) models for both replicates of the three traits of interest (RF, EGRT, gall rating), as well as the means for these three traits. For all analyses, REMIM window size was set at 20 with a forward search threshold of 0.25 and a backward elimination threshold of 0.05 based on significance thresholds established by the score-based resampling method in QTLpoly to assess genome-wide QTL significance (da Silva Pereira et al., 2020). For all instances of the FEIM model 1000 simulations were performed with a window

size of 20. For both models, constant *d* was set at 1.5 to approximate a 95% support interval by *LOD-d* for the FEIM model, and *LOP-d* for the REMIM model as described by da Silva Pereira et al. (2020).

Using the QTL positions estimated by QTLpoly, we performed a BLAST search within QTL support intervals using the *I. trifida* (NCNSP306) assembly to identify candidate genes involved in RKN resistance (available at http://sweetpotato.uga.edu/). Graphics from QTL analyses and for the modified linkage map were generated using R-package VIEWpoly (v. 0.1.1) (Taniguti et al., 2022) and correlations were performed using ggplot2 (v. 3.3.3) (Wickham, 2016).

Results

Frequency histograms for RF, gall ratings, and EGRT all demonstrated skewing toward resistance (Figure 1), suggesting a 1:1 segregation pattern for resistance to *M. enterolobii* within TB. By average RF score, 126 TB progenies were classified as resistant (RF<1, 51.2%), while 120 were susceptible (RF>1, 48.8%) (Table 1). Thresholds for resistance by EGRT have not been defined, however, RF score and EGRT were positively correlated (r=.948; $P < 0.001$), and an RF of 1 corresponded to roughly 158 EGRT.

Uninoculated controls ‘Tanzania’, ‘Beauregard’, and ‘Covington’ had gall ratings of 0%, and no eggs were observed from extracted samples in either of the two replicates. ‘Jewel’ (uninoculated) had gall ratings of 1% in both negative control replicates, and while one replicate yielded an egg count of zero, the other replicate appeared to be contaminated with 9.0 EGRT, suggesting a low amount of splash dispersal in the greenhouse during the trial. Positive resistant checks behaved as expected (Figure 2). ‘Tanzania’ (inoculated) was rated as highly resistant,

with average RF, EGRT and gall rating of 0.026, 7.5, and 0.0%, respectively. 'Jewel' (inoculated) was also resistant, with RF 0.027 and 6.0 EGRT and 2.3% galling. The susceptible checks were susceptible: 'Covington' (inoculated) RF=6.843 with 2190.8 EGRT and 12.5% galling, and 'Beauregard' (inoculated) RF=13.691 with 3912.5 EGRT and 14.5% galling. A list of mean resistance ratings for check lines and TB progenies is available in Appendix 1.

Gall ratings were weakly correlated with *M. enterolobii* EGRT within the TB population ($r = 0.581$, $P < 0.001$), and gall ratings were also weakly correlated with RF score ($r = 0.632$, $P < 0.001$) (Figure 1). Correlations between the two replicates of all genotypes were weak for both RF score ($r = 0.534$, $P < 0.001$) and EGRT ($r = 0 .529$, $P < 0.001$). Subsamples, representing the four individual 5mL aliquots of extracted egg solution from each plant, were strongly correlated ($r = 0.99$, $P < 0.001$) (Figure 3).

Transgressive segregation for resistance and susceptibility was observed, where 42 lines (~17%) averaged a higher RF than 'Beauregard', and 39 lines (~16%) averaged a lower RF than 'Tanzania'. These values were much the same for average EGRT, with 42 lines measuring higher than 'Beauregard', and 35 lines (~14%) lower than 'Tanzania'.

The 1:1 segregation pattern in TB for resistance to *M. enterolobii* about RF=1 was strong evidence that resistance was a simplex dominant trait in this population.

QTL Analysis

Using the modified TB linkage map developed by Mollinari et al. (2020), the score-based REMIM model in QTLpoly revealed one major QTL peak for *M. enterolobii* resistance within the TB population on LG 4 (57.5cM) (Figure 4) explaining 70% of variation for resistance to *M. enterolobii* (Table 2), suggesting the presence of a single dominant gene controlling for *M.*

enterolobii resistance. For the trait means using FEIM, RF score produced a single major QTL peak at 57.5cM (LOD=38.51, adjusted R^2=0.555), EGRT at 57.5cM (LOD=37.94, adjusted R^2=0.550), and gall rating at 58.3cM (LOD=33.69, adjusted R^2=0.505). The REMIM model trait means also each produced a single major QTL in approximately the same position: RF at 57.5cM (*P* value <2.22e-16, h^2=0.7), EGRT at 57.5cM (*P* value <2.22e-16, h^2=0.7), and gall rating at 59.0cM (*P* value <2.22e-16, h^2=0.67) (Table 2).

The QTL peak (57.5cM) was in the same position for RF and EGRT for both the FEIM and REMIM models in both replicates 1 and 2 and their means. The REMIM 95% support interval was 41.68-71.04cM, spanning approximately 10.0Mb. The FEIM 95% support interval was 56.29-58.3cM, spanning approximately 1.9Mb. QTL for mean gall ratings peaked at 57.5cM for REMIM, and 58.3cM for FEIM (Figure 5). Support intervals for gall ratings were broader than for RF and EGRT in both models (Table 2). Support intervals spanned from 1.9Mb (FEIM) to 10.0Mb (REMIM), or 0.06% to 0.3% of the 3.3Gb sweetpotato genome.

Additive effects of parental alleles (Figure 6) revealed that allele *h* in 'Tanzania' negatively affected population means for RF and EGRT at the peak QTL position 57.5cM, while 'Beauregard' alleles contributed little to resistance (Figure 6). Negative effects indicate increased resistance, while positive effects would indicate increased susceptibility. Population means (μ) for resistance traits were: $\mu_{RF} = 6.06$, $\mu_{EGRT} = 1{,}767$ eggs, and $\mu_{gallrating} = 3.96\%$. Per the trait means REMIM model, estimated effects predict that the presence of allele *h* in TB progeny is associated with reduced RF (-5.53), EGRT (-1,593 eggs), and gall ratings (-3.49%). Allele *h* at this locus is evidence that a resistance gene comes from 'Tanzania' with a dosage of one.

Candidate Gene Search

A BLAST search within the support interval (Table 2) revealed three recognized resistance genes in *I. trifida* near the peak QTL position 57.5cM (7,039,636 bp). These were NB-ARC domain-containing disease resistance proteins: ift04g10180.t1, ift04g10190.t1, ift04g10200.t1. The NB-ARC (nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4) domains are central to most R proteins, which recognize pathogens and trigger an innate immune response (Tameling et al., 2006; van Ooijen et al., 2008).

Discussion

Bioassay

Quantifying sweetpotato resistance to *M. enterolobii* is currently expensive and labor-intensive, and standardized resistance thresholds for *M. enterolobii* do not yet exist. Resistance in sweetpotato is here defined as a diminished capacity for RKN to reproduce on the host. While RF less than one is, by this definition, resistant, maintaining nematode populations at or near the initial load is not satisfactory. Ideally, a next-generation sweetpotato would be highly resistant as to be considered a non-host (RF<0.1), as defined by Hamidi & Hajihassani (2020). The TB population has demonstrated that this level of resistance is attainable through a single cross with a resistant parent. 'Tanzania' averaged RF=0.03, and 87/246 clones (35.4%) of the TB progeny could be classed as non-hosts by this definition of RF<0.1. Our second measure of resistance, EGRT, may be confounded by the fact that sweetpotato forms storage roots, though not all genotypes did in our bioassay. Storage roots have greater mass and lesser surface area than fibrous roots; it is not known if *M. enterolobii* interacts with these tissues differently, but EGRT is affected. A third measure, gall ratings are sometimes used for *Meloidogyne* spp., however, gall

ratings are poorly correlated with egg counts in the case of *M. enterolobii.* For example, in a diverse set of sweetpotato germplasm, Schwarz et. al (2021) saw no *M. enterolobii* gall rating above 20%, despite heavy egg production in some lines. Kiewnick et al. (2021) also observed an inconsistent relationship between gall ratings and egg counts for *M. enterolobii* in tomato. Carmona et al. (2020) recommended that RF be used as the primary measure of resistance in *M. enterolobii*, while gall ratings can facilitate interpretation of RF. In this experiment we found samples with high egg counts and low (or zero) gall ratings. For example, in replicate 1, TB 210 had a gall rating of 0% yet 3,793 EGRT, while TB 135 in replicate 1, had a gall rating of 50% and 1,700 EGRT. Certain environmental conditions have been observed to favor many eggs yet few galls, or many galls with few eggs in *M. incognita* (Shepherd, 1979). *Meloidogyne enterolobii* may be more sensitive than *M. incognita* to these effects, and this relationship would be worth exploring. By all traits evaluated, 'Tanzania' was highly *M. enterolobii* resistant while 'Beauregard' was highly susceptible, which is consistent with results from Schwarz et al. (2021). Our results also agree with resistance ratings for the eight TB progeny screened by Schwarz et al. (2021): TB019, TB056, TB068, TB146, TB257 were resistant, while TB085, TB131, TB252 were susceptible.

Meloidogyne enterolobii is under internal quarantine in NC and is further included as a European and Mediterranean Plant Protection Organization (EPPO) A1 pest, meaning it is not currently present in most represented nations. These quarantines represent a barrier to resistance evaluations in field settings. The inoculation-based greenhouse bioassay provided consistent inoculation to all genotypes tested, but there were some drawbacks. Due to their small size, *M. enterolobii* eggs are particularly prone to being flushed from our sand-based media by an overwatering event, and great care was taken to avoid this. Contamination between samples (i.e.,

splashing) may also confound results, though this would largely be an issue for negative controls, as small amounts of added inoculum should not drastically affect resistance classes of inoculated plants. Correlations between replicates were weak for RF, yet highly significant ($r = 0.534$, $P < 0.001$). Eggs in some plants may have had differential hatch rates between replicates, and root growth differences could also explain the weak correlation. Nevertheless, 233 out of the 246 TB genotypes assayed (~95%) were in agreement between replicates about RF=1.

The bioassay for *M. enterolobii* requires permitted and quarantined greenhouse space, specially trained staff, and takes at least 60 days. We estimate that to screen 500 sweetpotato lines in 3 replicates for *M. enterolobii* resistance would require between 200-500 hours of labor, plus supply costs and specialized greenhouse space. It is not feasible to routinely screen large sets of sweetpotato for *M. enterolobii* resistance in this fashion, and in a breeding program high throughput is increasingly necessary.

Plant resistance to RKN is typically race-specific, so it is imperative to identify the nematode race used in resistance screening (Ukoskit et al., 1997). Currently it is not clear if distinct races or pathotypes exist for *M. enterolobii.* For example, *M. enterolobii* populations on a diversity of crops from sixteen nations exhibited very low marker diversity using several different marker types (AFLP, ISSR and RAPD), leading to the conclusion that *M. enterolobii* was “genetically homogeneous” (Tigano et al., 2010). A phylogenetic analysis and sequencing study of five African *M. enterolobii* populations found no genetic differences between groups (Onkendi & Moleleki, 2013). Four populations of *M. enterolobii* isolated in NC were found to have no variation in virulence (Schwarz et al., 2020). It may be that diversification of this pest has yet to occur. A recent USDA report found some variation in sweetpotato cultivar response to different *M. enterolobii* isolates from the Carolinas (Rutter et al., 2021), but there is insufficient

evidence to conclusively support the presence of multiple races of *M. enterolobii* at this time. *Meloidogyne enterolobii* reproduces via obligate mitotic parthenogenesis, and extremely low diversity would be expected in an asexually reproducing species, however *M. incognita*, which does have several races, reproduces in the same fashion (Tigano et al., 2010; Yang & Eisenback, 1983). Because of the low polymorphism between *M. enterolobii* populations worldwide, it has been be hypothesized that host resistance against one *M. enterolobii* population may be effective against many (Tigano et al., 2010).

QTL Analysis

The screening protocols developed by Schwarz et al. (2021) and our use of multiple parameters to measure resistance provided high-quality phenotypic data on which to perform QTL analyses. The main sources of experimental error in QTL analysis are in phenotype and genotype data collection (Collard et al., 2005). Here, we report a single major QTL on linkage group 4 detected by multiple models for multiple resistance measures. The REMIM model produced a QTL explaining 70% of the variation in *M. enterolobii* RF and EGRT in the TB population. There is no definitive population size required for a given statistical power threshold in QTL analysis, as power depends on recombination fractions, which further depend on ploidy, species, and chromosomal assortment (Ripol et al., 1999). Ukoskit et al. (1997) described a RAPD marker linked to *M. incognita* in sweetpotato with a recombination fraction of 0.2421 ± 0.057 in 71 progenies. A population size of 200 individuals is sufficient for detecting QTL with large effects, especially in high density maps (Hackett et al., 2014). For a map of this marker density (2,120.97 cM, with 6.99 SNPs/cM) (Amankwaah, 2019), the TB population size of 246

should correspond with a statistical power greater than 80% for QTL detection and the capture of recombination events (Ripol et al., 1999).

The fixed-effect interval mapping (FEIM) model tests the null hypothesis that the additive allelic effects are zero using likelihood ratio tests, versus the alternate hypothesis that there is one QTL present (da Silva Pereira et al., 2020). Fixed effect models are appropriate for single-QTL scenarios, however they offer reduced detection power as compared to multiple effect models (da Silva Pereira et al., 2020). Oloka et al. (2021) used a random-effect multiple interval mapping (REMIM) model for their QTL analysis. REMIM is the better model for traits explained by multiple QTL, or linked QTL, and this model produces consistently better results than FEIM (da Silva Pereira et al., 2020) by using forward-backward significance thresholds established by score-based genome-wide resampling (Zou et al., 2004). As was the case here, REMIM was also the better model for a single-QTL scenario.

Both FEIM and REMIM models estimated a single major QTL in the same peak marker position for mean RF score and mean EGRT, and in a nearby position for mean gall ratings. We also tested each replicate of each trait individually and found that both REMIM and FEIM models and all replicates of RF and EGRT estimated peak QTL position at 57.5cM (Table 2). The 95% support intervals for these QTL was estimated using the peak LOP (or LOD, for FEIM) score minus constant *d*, for which we used the widely adopted value d=1.5 (da Silva Pereira et al., 2020; Li, 2011; Wu et al., 2021). Confidence could be increased to 98%, but the tradeoff is a much broader support interval and therefore less precise position estimates for trait-associated markers.

In summary, we report a single major QTL peak at 57.5cM on LG 4 of *I. batatas* explaining 70% of variation in resistance to *M. enterolobii* within the 'Tanzania' x 'Beauregard'

mapping population. We observed a 1:1 segregation pattern for resistance based on three parameters (RF, EGRT, and gall ratings) and a single major QTL was detected in approximately the same position by all three measures. This segregation pattern, the presence of a single major QTL, and the allele *h* detected on 'Tanzania' all provide strong evidence that *M. enterolobii* is controlled by a simplex dominant allele. Detection of this QTL or markers therein within other populations and germplasm is necessary for validation, as it is possible that there are different genetic bases for resistance. 'Tanzania' may have acquired its resistance to *M. enterolobii* through a different evolutionary pathway than other resistant lines. The next steps of this research should include identification of markers within this QTL region and testing their accuracy at predicting resistance. These results represent major progress towards the first application of marker-assisted selection in sweetpotato breeding, which supports the sweetpotato breeding and research community toward the release of a Southeastern US-adapted and *M. enterolobii* resistant sweetpotato variety.

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REFERENCES

Amankwaah, V. A. (2019). *Phenotyping and Genetic Studies of Storage Root Chemistry Traits in Sweetpotato* [Ph.D., North Carolina State University]. http://www.proquest.com/docview/2385636619/abstract/B1E1FF11D3044EC7PQ/1

Baselga, F. M. de E., Schultheis, J. R., Boyette, M. D., Quesada-Ocampo, L. M., Starke, K. D., & Monks, D. W. (2020). Vine Removal Prior to Harvest, and Curing Duration and Temperature Affect the Incidence and Severity of Internal Necrosis in 'Covington' Sweetpotato. *HortTechnology*, *30*(5), 544–551. https://doi.org/10.21273/HORTTECH04408-19

Bhavana, P., Singh, A. K., Kumar, R., Prajapati, G. K., Thamilarasi, K., Manickam, R., Maurya, S., & Choudhary, J. S. (2019). Identification of Resistance in Tomato Against Root Knot Nematode (*Meloidogyne incognita*) and Comparison of Molecular Markers for Mi Gene. *Australasian Plant Pathology*, *48*(2), 93–100. https://doi.org/10.1007/s13313-018-0602-8

Boerma, H. R., & Hussey, R. S. (1992). Breeding Plants for Resistance to Nematodes. *Journal of Nematology*, *24*(2), 242–252.

Carmona, P. A. O., Pinheiro, J. B., Amaro, G. B., Silva, G. O. da, Peixoto, J. R., & Cares, J. E. (2020). Resistance Sources to Root-knot Nematodes *Meloidogyne javanica*, *M. incognita* and *M. enterolobii* in Sweet Potato. *Horticultura Brasileira*, *38*, 126–133. https://doi.org/10.1590/S0102-053620200203

Castagnone-Sereno, P. (2012). *Meloidogyne enterolobii* (= *M. mayaguensis*): Profile of an Emerging, Highly Pathogenic, Root-knot Nematode Species. *Nematology*, *14*(2), 133–138. https://doi.org/10.1163/156854111X601650

Cervantes-Flores, J. C., Sosinski, B., Pecota, K. V., Mwanga, R. O. M., Catignani, G. L., Truong, V. D., Watkins, R. H., Ulmer, M. R., & Yencho, G. C. (2011). Identification of Quantitative Trait Loci for Dry-matter, Starch, and β-carotene Content in Sweetpotato. *Molecular Breeding*, *28*(2), 201–216. https://doi.org/10.1007/s11032-010-9474-5

Cervantes-Flores, J. C., Yencho, G. C., & Davis, E. L. (2002). Host Reactions of Sweetpotato Genotypes to Root-knot Nematodes and Variation in Virulence of *Meloidogyne incognita* Populations. *HortScience*, *37*(7), 1112–1116. https://doi.org/10.21273/HORTSCI.37.7.1112

Cervantes-Flores, J. C., Yencho, G. C., Kriegner, A., Pecota, K. V., Faulk, M. A., Mwanga, R. O. M., & Sosinski, B. R. (2008a). Development of a Genetic Linkage Map and Identification of Homologous Linkage Groups in Sweetpotato Using Multiple-dose AFLP Markers. *Molecular Breeding*, *21*(4), 511–532. https://doi.org/10.1007/s11032-007-9150-6

Cervantes-Flores, J. C., Yencho, G. C., Pecota, K. V., Sosinski, B., & Mwanga, R. O. M. (2008b). Detection of Quantitative Trait Loci and Inheritance of Root-knot Nematode Resistance in Sweetpotato. *Journal of the American Society for Horticultural Science*, *133*(6), 844–851. https://doi.org/10.21273/JASHS.133.6.844

Clark, C. A., & Moyer, J. W. (1988). *Compendium of Sweet Potato Diseases*. APS Press.

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An Introduction to Markers, Quantitative Trait Loci (QTL) Mapping and Marker-assisted Selection for Crop Improvement: The Basic Concepts. *Euphytica*, *142*(1–2), 169–196. https://doi.org/10.1007/s10681-005-1681-5

da Silva Pereira, G., Gemenet, D. C., Mollinari, M., Olukolu, B. A., Wood, J. C., Diaz, F., Mosquera, V., Gruneberg, W. J., Khan, A., Buell, C. R., Yencho, G. C., & Zeng, Z.-B. (2020). Multiple QTL Mapping in Autopolyploids: A Random-Effect Model Approach with Application in a Hexaploid Sweetpotato Full-Sib Population. *Genetics*, *215*(3), 579–595. https://doi.org/10.1534/genetics.120.303080

Hackett, C. A., Bradshaw, J. E., & Bryan, G. J. (2014). QTL Mapping in Autotetraploids Using SNP Dosage Information. *Theoretical and Applied Genetics*, *127*(9), 1885–1904. https://doi.org/10.1007/s00122-014-2347-2

Hamidi, N., & Hajihassani, A. (2020). Differences in Parasitism of Root-knot Nematodes (*Meloidogyne* spp.) on Oilseed Radish and Oat. *Journal of Nematology*, *52*, e2020-43. https://doi.org/10.21307/jofnem-2020-043

Hunt, O. J., Faulkner, L. R., & Peaden, R. N. (1972). Breeding for Nematode Resistance. In *Alfalfa Science and Technology* (pp. 355–370). John Wiley & Sons, Ltd. https://doi.org/10.2134/agronmonogr15.c16

Hussey, R. S., & Barker, K. R. (1973). Comparison of Methods of Collecting Inocula of *Meloidogyne* spp., Including a New Technique. *Plant Disease Reporter*.

Jones, A., Dukes, P. D., & Schalk, J. M. (1986). Sweet Potato Breeding. In *Breeding Vegetable Crops*. AVI Publishing Co.

Kiewnick, S., Dessimoz, M., & Franck, L. (2009). Effects of The Mi-1 and The N Root-knot Resistance Gene on Infection and Reproduction of *Meloidogyne enterolobii* on Tomato and Pepper Cultivars. *Journal of Nematology*, *41*, 134–139.

Kirkpatrick, T., Lee, J., & Faske, T. (2019). The Guava Root-knot Nematode (*Meloidogyne enterolobii*), a Potential Threat to Arkansas Sweet Potatoes and Other Crops. *University of Arkansas Cooperative Extension Service Printing Services*, 2.

Li, H. (2011). A Quick Method to Calculate QTL Confidence Interval. *Journal of Genetics*, *90*(2), 6.

Moens, M., Perry, R. N., & Starr, J. L. (2009). *Meloidogyne* Species—A Diverse Group of Novel and Important Plant Parasites. In R. N. Perry, M. Moens, & J. L. Starr (Eds.), *Root-knot nematodes* (pp. 1–17). CABI. https://doi.org/10.1079/9781845934927.0001

Mollinari, M., & Garcia, A. A. F. (2019). Linkage Analysis and Haplotype Phasing in Experimental Autopolyploid Populations with High Ploidy Level Using Hidden Markov Models. *G3 Genes|Genomes|Genetics*, *9*(10), 3297–3314. https://doi.org/10.1534/g3.119.400378

Mollinari, M., Olukolu, B. A., Pereira, G. da S., Khan, A., Gemenet, D., Yencho, G. C., & Zeng, Z.-B. (2020). Unraveling the Hexaploid Sweetpotato Inheritance Using Ultra-Dense Multilocus Mapping. *G3 Genes|Genomes|Genetics*, *10*(1), 281–292. https://doi.org/10.1534/g3.119.400620

Mwanga, R. O. M., Odongo, B., p'Obwoya, C. O., Gibson, R. W., Smit, N. E. J. M., & Carey, E. E. (2001). Release of Five Sweetpotato Cultivars in Uganda. *HortScience*, *36*(2), 385–386. https://doi.org/10.21273/HORTSCI.36.2.385

OEPP/EPPO. (2011). *Meloidogyne enterolobii*. *EPPO Bulletin*, *41*(3), 329–339. https://doi.org/10.1111/j.1365-2338.2011.02497.x

Oloka, B. M., da Silva Pereira, G., Amankwaah, V. A., Mollinari, M., Pecota, K. V., Yada, B., Olukolu, B. A., Zeng, Z.-B., & Craig Yencho, G. (2021). Discovery of a Major QTL for

Root-knot Nematode (*Meloidogyne incognita*) Resistance in Cultivated Sweetpotato (*Ipomoea batatas*). *Theoretical and Applied Genetics*, *134*(7), 1945–1955. https://doi.org/10.1007/s00122-021-03797-z

Onkendi, E. M., & Moleleki, L. N. (2013). Detection of *Meloidogyne enterolobii* in Potatoes in South Africa and Phylogenetic Analysis Based on Intergenic Region and the Mitochondrial DNA Sequences. *European Journal of Plant Pathology*, *136*(1), 1–5. https://doi.org/10.1007/s10658-012-0142-y

Oostenbrink, M. (1966). Major Characteristics of the Relation Between Nematodes and Plants. *Major characteristics of the relation between nematodes and plants.*, *66–4*. http://www.cabdirect.org/cabdirect/abstract/19670801714

Ripol, M. I., Churchill, G. A., da Silva, J. A. G., & Sorrells, M. (1999). Statistical Aspects of Genetic Mapping in Autopolyploids. *Gene*, *235*(1), 31–41. https://doi.org/10.1016/S0378-1119(99)00218-8

Rutter, W. B., Skantar, A. M., Handoo, Z. A., Mueller, J. D., Aultman, S. P., & Agudelo, P. (2018). *Meloidogyne enterolobii* Found Infecting Root-Knot Nematode Resistant Sweetpotato in South Carolina, United States. *Plant Disease*, *103*(4), 775–775. https://doi.org/10.1094/PDIS-08-18-1388-PDN

Rutter, W., Wadl, P., Mueller, J. D., & Agudelo, P. (2021). Identification of Sweetpotato Germplasm Resistant to Pathotypically Distinct Isolates of *Meloidogyne enterolobii* from the Carolinas. *Plant Disease*, PDIS-02-20-0379-RE. https://doi.org/10.1094/PDIS-02-20-0379-RE

Schwarz, T., Li, C., Ye, W., & Davis, E. (2020). Distribution of *Meloidogyne enterolobii* in Eastern North Carolina and Comparison of Four Isolates. *Plant Health Progress*, *21*(2), 91–96. https://doi.org/10.1094/PHP-12-19-0093-RS

Schwarz, T. R., Li, C., Yencho, G. C., Pecota, K. V., Heim, C. R., & Davis, E. L. (2021). Screening Sweetpotato Genotypes for Resistance to a North Carolina Isolate of *Meloidogyne enterolobii*. *Plant Disease*, *105*(4), 1101–1107. https://doi.org/10.1094/PDIS-02-20-0389-RE

Seinhorst, J. W. (1965). The Relation Between Nematode Density and Damage To Plants. *Nematologica*, *11*(1), 137–154. https://doi.org/10.1163/187529265X00582

Shepherd, R. L. (1979). A Quantitative Technique for Evaluating Cotton for Root-Knot Nematode Resistance. *Phytopathology*, *69*(4), 427. https://doi.org/10.1094/Phyto-69-427

Singh, B., Bhatti, D. S., & Singh, K. (1974). Resistance to Root-knot Nematodes (*Meloidogyne* spp.) in Vegetable Crops. *PANS Pest Articles & News Summaries*, *20*(1), 58–67. https://doi.org/10.1080/09670877409412336

Tameling, W. I. L., Vossen, J. H., Albrecht, M., Lengauer, T., Berden, J. A., Haring, M. A., Cornelissen, B. J. C., & Takken, F. L. W. (2006). Mutations in the NB-ARC Domain of I-2 That Impair ATP Hydrolysis Cause Autoactivation. *Plant Physiology*, *140*(4), 1233–1245. https://doi.org/10.1104/pp.105.073510

Taniguti, C. H., Gesteira, G., Lau, J., da Silva Pereira, G., Zeng, Z.-B., Byrne, D., Riera-Lizarazu, O., Mollinari, M. (Submitted 2022, Feb. 21). VIEWpoly: a Visualization Tool to Integrate and Explore Results of Polyploid Genetic Analysis [unpublished manuscript]. *JOSS*.

Thies, J. A., Merrill, S. B., & Corley, E. L. (2002). Red Food Coloring Stain: New, Safer Procedures for Staining Nematodes in Roots and Egg Masses on Root Surfaces. *Journal of Nematology*, *34*(2), 179–181.

Tigano, M., Siqueira, K. D., Castagnone-Sereno, P., Mulet, K., Queiroz, P., Santos, M. D., Teixeira, C., Almeida, M., Silva, J., & Carneiro, R. (2010). Genetic Diversity of the Root-knot Nematode *Meloidogyne enterolobii* and Development of a SCAR Marker for This Guava-damaging Species. *Plant Pathology*, *59*(6), 1054–1061. https://doi.org/10.1111/j.1365-3059.2010.02350.x

Trudgill, D., & Phillips, M. (1997). Nematode Population Dynamics, Threshold Levels and Estimation of Crop Losses. In *Plant Nematode Problems and their Control in the Near East Region*. Food and Agriculture Organization of the United Nations. https://www.fao.org/3/v9978e/v9978e07.htm

Ukoskit, K., Thompson, P. G., Watson, C. E., & Lawrence, G. W. (1997). *Identifying a Randomly Amplified Polymorphic DNA (RAPD) Marker Linked to a Gene for Root-knot Nematode Resistance in Sweetpotato*. https://doi.org/10.21273/jashs.122.6.818

USDA/NASS. (2020, June 9). *2019 State Agriculture Overview for North Carolina*. https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH%20CAROLINA

van Ooijen, G., Mayr, G., Kasiem, M. M. A., Albrecht, M., Cornelissen, B. J. C., & Takken, F. L. W. (2008). Structure-function Analysis of the NB-ARC Domain of Plant Disease Resistance Proteins. *Journal of Experimental Botany*, *59*(6), 1383–1397. https://doi.org/10.1093/jxb/ern045

Wadl, P. A., Olukolu, B. A., Branham, S. E., Jarret, R. L., Yencho, G. C., & Jackson, D. M. (2018). Genetic Diversity and Population Structure of the USDA Sweetpotato (*Ipomoea batatas*) Germplasm Collections Using GBSpoly. *Frontiers in Plant Science*, *9*, 1166. https://doi.org/10.3389/fpls.2018.01166

Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. https://ggplot2.tidyverse.org

Wu, P.-Y., Yang, M.-H., & Kao, C.-H. (2021). A Statistical Framework for QTL Hotspot Detection. *G3 Genes|Genomes|Genetics*, *11*(4), jkab056. https://doi.org/10.1093/g3journal/jkab056

Wu, S., Lau, K. H., Cao, Q., Hamilton, J. P., Sun, H., Zhou, C., Eserman, L., Gemenet, D. C., Olukolu, B. A., Wang, H., Crisovan, E., Godden, G. T., Jiao, C., Wang, X., Kitavi, M., Manrique-Carpintero, N., Vaillancourt, B., Wiegert-Rininger, K., Yang, X., Bao, K., Schaff, J., Kreuze, J., Gruneberg, W., Khan, A., Ghislain, M., Ma, D., Jiang, J., Wmanga, R., Leebens-Mack, J., Coin, L., Yencho, G. C., Buell, C. R., Fei, Z. (2018). Genome Sequences of Two Diploid Wild Relatives of Cultivated Sweetpotato Reveal Targets for Genetic Improvement. *Nature Communications*, *9*(1), 4580. https://doi.org/10.1038/s41467-018-06983-8

Yang, B., & Eisenback, J. D. (1983). *Meloidogyne enterolobii* n. Sp. (Meloidogynidae), a Root-knot Nematode Parasitizing Pacara Earpod Tree in China. *Journal of Nematology*, *15*(3), 381–391.

Ye, W. (2018). Nematodes of Agricultural Importance in North and South Carolina. In S. A. Subbotin & J. J. Chitambar (Eds.), *Plant Parasitic Nematodes in Sustainable Agriculture*

of North America (pp. 247–276). Springer International Publishing. https://doi.org/10.1007/978-3-319-99588-5_10

Ye, W. M., Koenning, S. R., Zhuo, K., & Liao, J. L. (2013). First Report of *Meloidogyne enterolobii* on Cotton and Soybean in North Carolina, United States. *Plant Disease*, *97*(9), 1262. https://doi.org/10.1094/PDIS-03-13-0228-PDN

Yencho, G. C., Pecota, K. V., Schultheis, J. R., VanEsbroeck, Z.-P., Holmes, G. J., Little, B. E., Thornton, A. C., & Truong, V.-D. (2008). 'Covington' Sweetpotato. *HortScience*, *43*(6), 1911–1914. https://doi.org/10.21273/HORTSCI.43.6.1911

Zou, F., Fine, J. P., Hu, J., & Lin, D. Y. (2004). An Efficient Resampling Method for Assessing Genome-Wide Statistical Significance in Mapping Quantitative Trait Loci. *Genetics*, *168*(4), 2307–2316. https://doi.org/10.1534/genetics.104.031427

CHAPTER 2: FIGURES

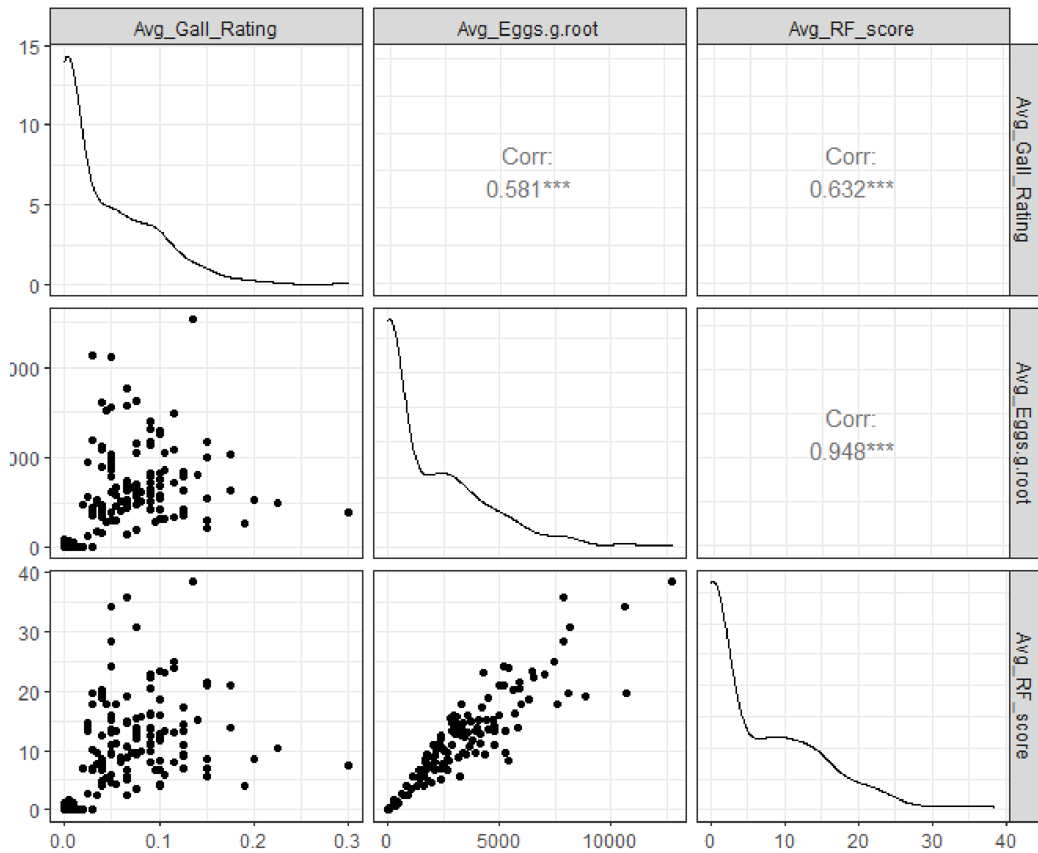

Figure 1. Pairwise scatter plots, histograms, and Pearson correlations of average gall ratings, eggs per gram of root tissue, and reproductive factor (RF, or total final eggs/10,000 initial inoculated eggs). Values in the top right triangle show Pearson correlation coefficient (r) between parameters; coefficient of determination (R^2) is the square of this value.

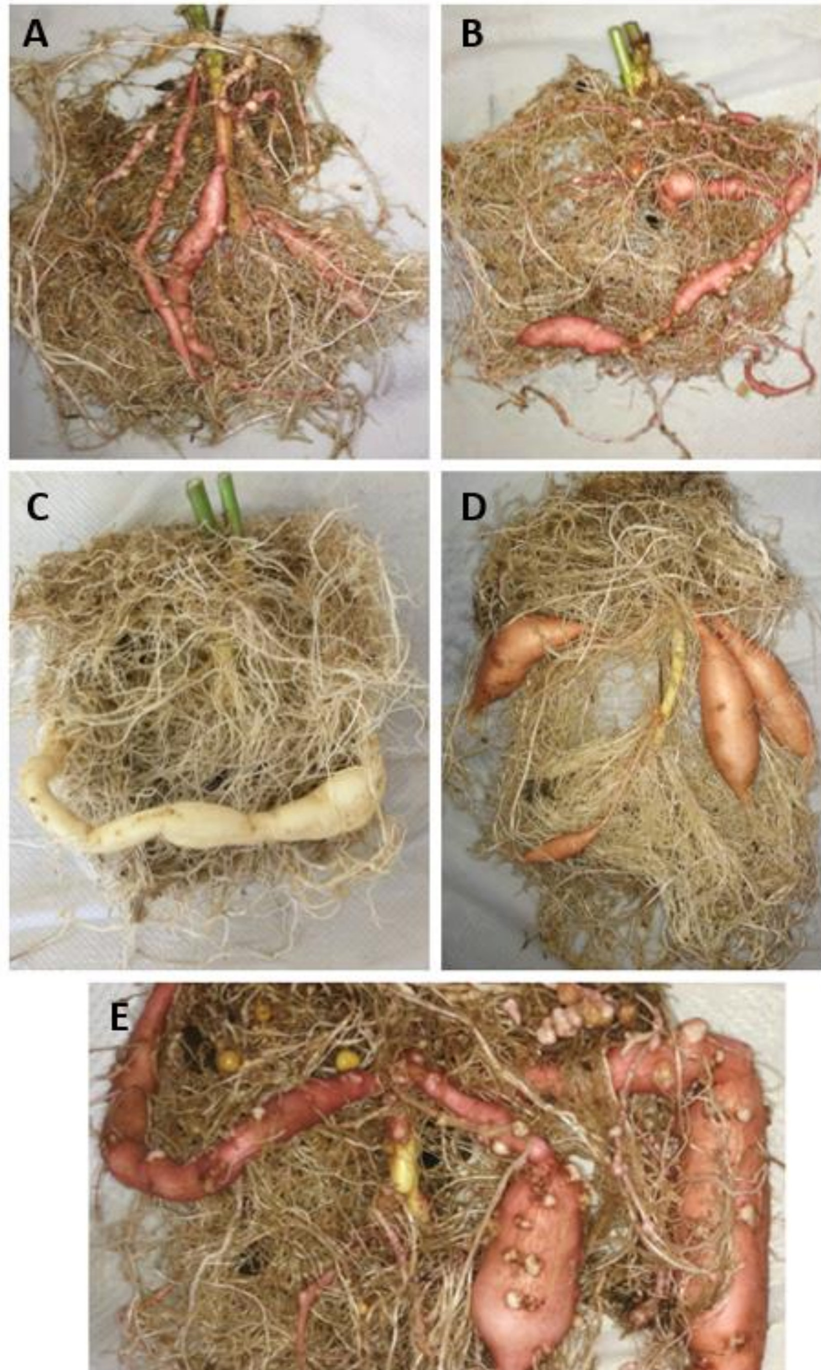

Figure 2. Images of positive controls: sweetpotato plants inoculated with 10,000 *Meloidogyne enterolobii* eggs, 60 days post-inoculation. Contrast adjusted to better display galling. **A** ‘Covington’ (susceptible). **B** ‘Beauregard’ (susceptible). **C** ‘Tanzania’ (resistant). **D** ‘Jewel’ (resistant). **E** Heavily galled ‘Beauregard’ (susceptible).

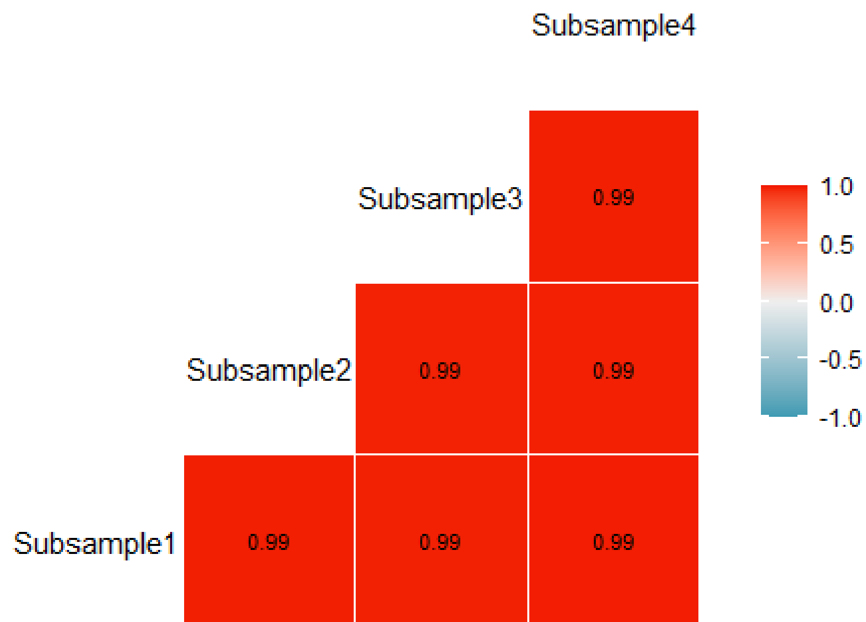

Figure 3. Within each sample, four egg count subsample measurements were highly consistent and had a significant positive relationship (*r* = 0.99) ($P < 0.001$). Each subsample was a 5ml aliquot from a 50ml egg extraction solution, which represents total eggs extracted from each plant in the experiment. All genotypes are represented, including all 246 TB lines, and inoculated and uninoculated control plants. Inoculated plants were administered 10,000 *Meloidogyne enterolobii* eggs 14 days after sweetpotato vine cuttings were planted.

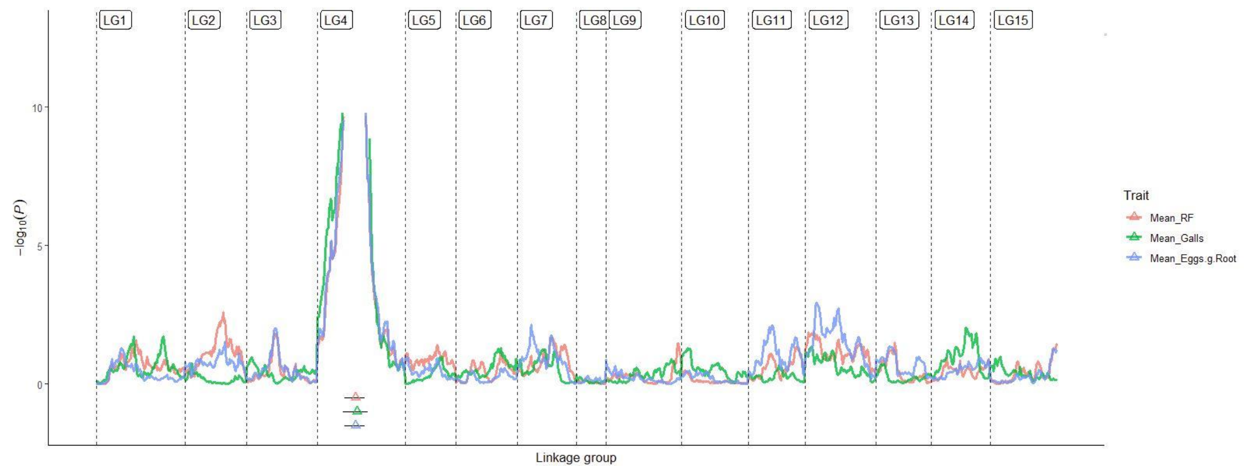

Figure 4. Manhattan plot of the entire sweetpotato genome, depicting the QTL profile for reproductive factor (RF), gall rating, and eggs per gram of root tissue of *Meloidogyne enterolobii* in inoculated sweetpotato vine cuttings of the TB population. A QTL on chromosome 4 was detected by all three measures of resistance, providing strong evidence that a single major gene controlling for *M. enterolobii* resistance exists within this region.

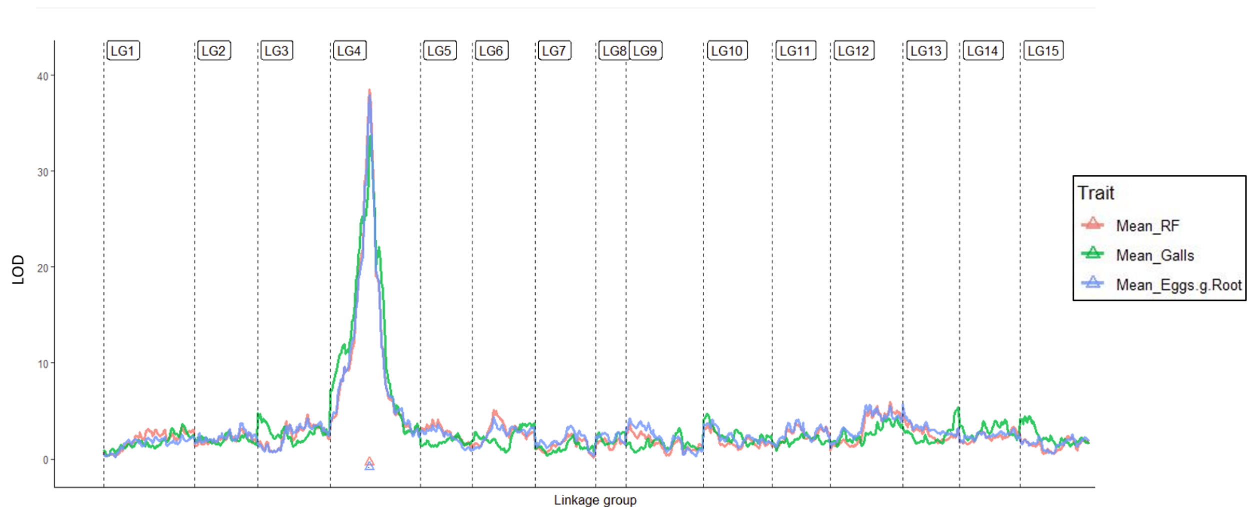

Figure 5. QTL profiles for the fixed effect interval mapping (FEIM) model for reproductive factor (RF), gall rating, and eggs per gram of root tissue of *Meloidogyne enterolobii* in inoculated sweetpotato vine cuttings of the TB population. A QTL on chromosome 4 was detected by all three measures of resistance, providing strong evidence that a single major gene controlling for *M. enterolobii* resistance exists within this region. This QTL was detected by a random effects multiple interval mapping (REMIM) approach as well, with identical peak QTL positions for RF and eggs per gram of root tissue at 57.5cM.

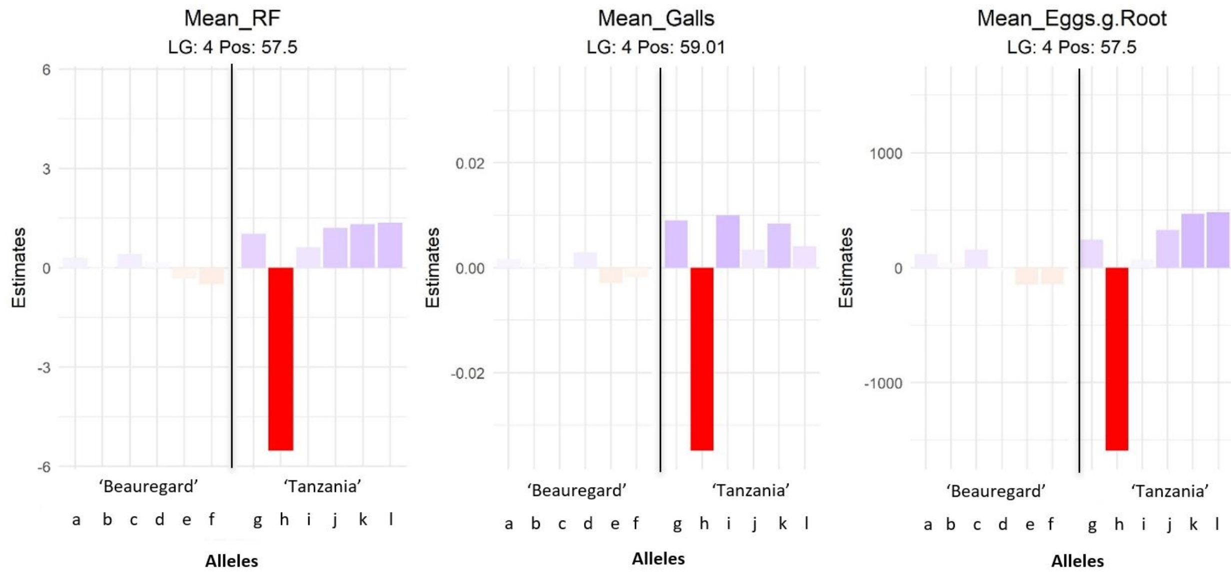

Figure 6. Additive allelic effects 'Tanzania' and 'Beauregard'. Allele *h* in 'Tanzania' negatively affected population means (i.e., increased resistance) for RF and EGRT at the peak QTL position 57.5cM, while 'Beauregard' alleles contributed little to resistance. Population means (μ) for resistance traits were: $\mu_{\mathrm{RF}} = 6.06$, $\mu_{\mathrm{EGRT}} = 1{,}767$ eggs, and $\mu_{\mathrm{gallrating}} = 3.96\%$. Allele *h* dropped population means by: RF (-5.53), EGRT (-1,593 eggs), and gall ratings (-3.49%). A resistance gene likely comes from 'Tanzania' with a dosage of one.

CHAPTER 2: TABLES

Table 1. TB progenies and their reproductive factors (RF). Population segregates approximately 1:1 for resistant:susceptible based on RF scores (RF<1 = resistant, RF >1 = susceptible). Resistance to *M. enterolobii* appears to be highly heritable, and a 1:1 segregation pattern suggests a simplex dominant trait, likely conferred by parent 'Tanzania'.

Average Reproductive Factor:	Total eggs	Number of TB progenies	% of TB population
RF>1	>10,000	120	48.8%
RF<1	<10,000	126	51.2%
RF<.5	<5,000	122	49.6%
RF<.25	<2,500	112	45.5%
RF<.1	<1000	83	34.4%
RF<.02	<200	32	13.0%

Table 2. Summary statistics of REMIM and FEIM model QTL analyses for three different measures of *M. enterolobii* resistance in sweetpotato. For the REMIM model, RF and EGRT had the same support interval and peak QTL position, while gall counts analysis produced a broader support interval. h2 represents the heritability of this QTL, or the percentage of variation for this resistance explained by genetic factors within this support interval. For RF and EGRT, 70% of variation for resistance is explained by genetic factors between 41.68-71.04cM. The FEIM model that provides less statistical power and explains less variation for resistance in approximately the same peak QTL positions.

REMIM Trait Means	**Linkage group**	**Position**	**Lower interval**	**Upper interval**	**p-value**	**h2**
RF	4	57.5 cM 7,069,636 bp	41.68 cM 4,610,261 bp	71.04 cM 14,636,794 bp	<2.22e-16	0.7
Gall Count	4	59.01 cM 9,439,774 bp	39.85 cM 4,501,393 bp	76.18 cM 23,719,658 bp	<2.22e-16	0.67
Eggs/g.Root	4	57.5 cM 7,069,636 bp	41.68 cM 4,610,261 bp	71.04 cM 14,636,794 bp	<2.22e-16	0.7

FEIM Trait Means	**Linkage group**	**Position**	**Lower interval**	**Upper interval**	**LOD**	**Adjusted *R2***
RF	4	57.5 cM 7,069,636 bp	56.29 cM 7,568,324 bp	58.3 cM 9,429,705 bp	38.51	0.555
Gall Count	4	58.3 cM 9,429,705 bp	57.5 cM 7,069,636 bp	60.43 cM 9,704,637 bp	33.69	0.505
Eggs/g.Root	4	57.5 cM 7,069,636 bp	56.29 cM 7,568,324 bp	58.3 cM 9,429,705 bp	37.94	0.550

Chapter 3: Design and validation of a KASP-based assay to screen for root-knot nematode resistance in sweetpotato

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Keywords: sweetpotato, *Meloidogyne enterolobii*, *Meloidogyne incognita*, KASP

Abstract

Meloidogyne enterolobii (Yang & Eisenback, 1983), the guava root-knot nematode, is an emergent pest in the United States for which there is little resistance in domestic sweetpotato (*Ipomoea batatas* (L.) Lam.) germplasm. *Meloidogyne incognita* (Kofoid & White) Chitwood is a less damaging relative of significant economic concern. Previous quantitative trait loci (QTL) studies described in Chapter 2 revealed the presence of a QTL on LG 4 of the 'Tanzania' x 'Beauregard' sweetpotato mapping population explaining 70% of the variation in *M. enterolobii* resistance. *Meloidogyne enterolobii* resistance fit a 1:1 segregation pattern, suggesting simplex dominant inheritance. In 2021 Oloka et al. detected a QTL for *M. incognita* on LG 7 explaining 58.3% of variation in gall counts. We compared whole genome sequence (WGS) data for 'Tanzania' and 'Beauregard' with the *Ipomoea trifida* (Kunth.) G. Don (NCNSP306) reference genome and identified 2,444 biallelic SNP markers within the QTL on linkage groups 4 and 7. Using LGC Biosearch Technologies' KASP by Design (KBD) service, we selected 24 putative PCR-compatible SNPs flanking (or at) QTL peaks for KASP marker design. Sweetpotato lines of known nematode resistance, including 122 TB progeny and 82 other diverse germplasm, were genotyped using LGC Biosearch Technologies™ KASP by Design service. Sixteen KASP

assays were developed from the 24 sequences submitted, and 11 were polymorphic. Genotype and resistance data were compared using a chi-square goodness of fit test. We report two markers for *M. enterolobii* resistance (LG4: 7038930 & 7039636) with 96.8% accuracy, and two markers for *M. incognita* resistance (LG7: 1699509 & 1700440) with 95.2% and 86.4% accuracy, respectively ($P < 0.001$). Validation in additional breeding lines may establish these markers as suitable for marker-assisted breeding in sweetpotato.

Introduction

Sweetpotato, *Ipomoea batatas* (L.) Lam. (2n=6X=90), has a large genome size of approximately 3.3Gb (Arumuganathan & Earle, 1991) and marker-assisted breeding (MAB) in this crop has yet to be implemented. New opportunities to pursue MAB have emerged with the advent of high-quality reference genomes of relatives of *I. batatas*, especially the diploid *Ipomoea trifida* (Kunth.) G. Don reference genome (Wu et al., 2018) which has 96.5% collinearity to *I. batatas* (Mollinari et al., 2020).

The root-knot nematodes (RKN) *Meloidogyne enterolobii* (Yang & Eisenback, 1983) and *Meloidogyne incognita* (Kofoid & White) Chitwood are important pests of sweetpotato. Prior research has indicated that resistance to *M. enterolobii* and *M. incognita* appears to be mediated by two distinct dominant loci (Fraher et al., 2022 [see Chapter 2]; Oloka et al., 2021); therefore, these traits represent feasible targets for MAB in sweetpotato. A pest with tropical origins, *M. enterolobii*, also known as the guava RKN, was first detected in North Carolina (NC) in 2011 on soybean and cotton (Ye et al., 2013). There are at least 121 hosts for *M. enterolobii* (EPPO, 2021), many of which are major crops or weeds in NC. As of 2018 there has been an internal quarantine on *M. enterolobii* infested sweetpotatoes in NC, prohibiting interstate transport

(Wilson, 2018). Roughly 65% of sweetpotatoes in the United States were produced in NC in 2019 (USDA/NASS, 2020), and the United States is the largest exporter of sweetpotatoes in the world (FAO, 2020).

Resistance to *M. incognita* has long been a target for sweetpotato breeders, and major varieties like ‘Covington’ carry robust resistance to *M. incognita* race 3 (Yencho et al., 2008). Hereafter, *M. incognita* refers to race 3 unless otherwise specified. Phylogenetic analyses and bioassays do not support the existence of multiple races for *M. enterolobii* at this time (Onkendi & Moleleki, 2013; Schwarz et al., 2020; Tigano et al., 2010).

Inoculation-based greenhouse bioassays have been used to quantify cultivar resistance to RKN by measuring gall ratings, reproductive factor (RF), or eggs per gram of root tissue (EGRT) (Schwarz et al., 2021). For *M. enterolobii*, quarantined greenhouse space and specially trained staff are required to conduct these complex bioassays, preventing high throughput phenotyping. All major sweetpotato varieties grown in NC have tested susceptible to *M. enterolobii*, including ‘Covington’, ‘Beauregard’, ‘Bonita’, ‘Bayou Belle’, and ‘Bellevue’ (Rutter et al., 2021; Schwarz et al., 2021). Some older varieties possess *M. enterolobii* resistance, including ‘Carver’, ‘Caromex’, ‘Centennial’, ‘Pelican Processor’, ‘Jewel’, as do some USDA releases from the 1980s including ‘Regal’, ‘Resisto’, ‘Red Resisto’, and ‘Southern Delite’ (Rutter et al., 2021; Schwarz et al., 2021). Of the commercial varieties grown in NC, only the specialty variety ‘Murasaki-29’ (purple skin with starchy, cream-colored flesh) is resistant (Schwarz et al., 2021). ‘Tanzania’, a starchy high dry matter Ugandan landrace, also tested resistant (Rutter et al., 2021; Schwarz et al., 2021), yet is poorly adapted to the US. Effort is made to breed with elite orange-fleshed moist sweetpotatoes, which are the preferred type in the

US (Leksrisompong et al., 2012), yet resistance to *M. enterolobii* is rare in such materials, and difficult to phenotype in promising new lines.

Single nucleotide polymorphism (SNP) markers are abundant in the genomes of all organisms, they are relatively cheap and easy to genotype, and they are less prone to mutation than other genetic variations (He et al., 2014). High locus-specificity and low error rates make SNP markers appealing targets for MAB (Semagn et al., 2014). Kompetitive allele specific PCR (KASP) assays are increasingly popular for their robust and flexible ability to detect SNPs (Semagn et al., 2014). These genotyping assays are rapid, economical, relatively accessible to breeders, and suitable for high-throughput applications (Alvarez-Fernandez et al., 2021; Meade et al., 2020). KASP assays have been developed for other polyploid crops, including prairie cordgrass (Graves et al., 2016), wheat (Rasheed et al., 2016), potato (Uitdewilligen et al., 2013), peanut (Leal-Bertioli et al., 2015), and cotton (Byers et al., 2012).

The biparental sweetpotato mapping population 'Tanzania' x 'Beauregard' (TB), a wide cross segregating for many traits, has been a target for quantitative trait loci (QTL) analyses. To date, multiple QTL for dry matter, starch, and β-carotene (Cervantes-Flores et al., 2011), as well as α- and β-amylase, fructose, glucose, sucrose, and total sugar (Amankwaah et al., 2019) have been reported in these materials. Recently, Oloka et al. (2021) described a single major QTL on linkage group (LG) 7 of TB explaining 58.3% of variation in *M. incognita* gall counts. In Chapter 2 we described a single major QTL within TB on LG 4 that explained 70% of variation in *M. enterolobii* RF and EGRT. The 1:1 segregation ratio for *M. enterolobii* resistance in TB fit a simplex dominant pattern, regardless of polyploidy (Bourke et al., 2018; Cervantes-Flores et al., 2008; Martin & Jones, 1986; Monden & Tahara, 2017).

This research was conducted to: 1) identify biallelic SNP markers within the two major QTL for *M. enterolobii* and *M. incognita*; 2) design KASP primers for alleles linked to *M. enterolobii* and *M. incognita* resistance; and 3) determine if these markers were diagnostic for *M. enterolobii* and *M. incognita* resistance in TB and other diverse breeding germplasm. Successful completion of these goals could enable MAB in sweetpotato for this critical resistance trait.

Materials and Methods

Plant Materials. A total of 204 sweetpotato clones were included in this study. A panel from the biparental mapping population TB ('Tanzania' x 'Beauregard') consisting of 122 individuals was included, representing a range of resistance levels for both *M. enterolobii* and *M. incognita.* Evaluations for *M. incognita* resistance in TB were previously described by Oloka et al. (2021), while *M. enterolobii* resistance evaluations in TB were described by Fraher et al. (2022) (see Chapter 2). *Meloidogyne enterolobii* resistance is here defined as RF<1 (Rutter et al., 2021; Schwarz et al., 2021), while *M. incognita* resistance is less than or equal to 10 galls (Nakayama et al., 2012; Sasai et al., 2019; Tabuchi et al., 2017). From diverse breeding germplasm, an additional 82 lines were included, 42 of which had been phenotyped for *M. incognita* as part of routine NCSU bioassays. Within these 42 lines, 29 were considered *M. incognita* resistant (equal to or fewer than 10 galls), while 13 were susceptible. Additionally, 78 of these 82 lines had been characterized for *M. enterolobii* resistance, with 49 resistant and 29 susceptible about RF=1. *Meloidogyne enterolobii* resistant lines were diverse and included: 'Caromex', 'Carver', 'Centennial', 'Gem', 'Jewel', 'Murasaki-29', 'Nancy Hall', 'Norton', 'Okinawa No. 100', 'Pelican Processor', 'Red Resisto', 'Resisto', 'Southern Delite', 'Tanzania', 'Tib11', 'Wagabolige', 'Whitestar', and other public university breeding lines. Susceptible lines included:

‘Averre’, ‘Bayou Belle’, ‘Beauregard’, ‘Bellevue’, ‘Bienville’, ‘Bonita’, ‘Covington’, ‘Evangeline’, ‘Excel’, ‘Hernandez’, ‘Patriot’, ‘Ruddy’, ‘Tib4’, and other public university breeding lines. A full list of genotyped lines and their RKN resistance data is included in Appendix 2.

DNA Extraction and Quality. Sweetpotato leaf tissue samples were collected from plants maintained at NCSU greenhouses. From each genotype, a 1cm x 2cm (~1 gram) leaf edge sample was cut from the first fully expanded leaf, folded several times, placed in microtubes with preloaded grinding beads and immediately stored on dry ice and stored at -80°C. DNA extractions followed modified CTAB protocols (Doyle & Doyle, 1990). DNA quantity was assessed using absorbance readings from BioTek Take3 plate on a Synergy HT plate reader (*Synergy HT Operator’s Manual*, 2008), and gel electrophoresis was used as a visual test to verify approximate DNA quantity and quality. An additional quantification was performed using the PicoGreen assay with black Greiner 96-well flat bottom plates (Brescia & Banks, 2010). Low quality DNA samples were re-extracted from new leaf tissue. DNA ranged from 5-100 ng/μL; higher concentrations were diluted with dH2O to 100ng/uL. DNA was transferred to full-skirt 96-well plates in 50ul aliquots in two replicates. Plates were sent to LGC Biosearch Technologies™ (Hoddesdon, UK) for KASP marker development as part of their KASP by Design (KBD) service (product code KBS-2300-001).

SNP Variant Calling for KASP Assay Development. SNP variants between ‘Tanzania’ and ‘Beauregard’ for the development of KASP markers were determined by the following protocols. To generate whole genome shotgun reads of the paternal parent ‘Beauregard’, DNA was

extracted from young leaves using the DNeasy Plant Mini Kit (QIAGEN). PCR-free paired-end DNA libraries were constructed using the Illumina Genomic DNA Sample Preparation kit following the manufacturer's instructions (Illumina) and sequenced on an Illumina HiSeq 4000 system. Raw 'Beauregard' genome sequencing reads have been deposited in the NCBI BioProject database under the accession number PRJNA799062. 'Tanzania' 10X Genomics Chromium reads were described previously by Wu et al. (2018). The *I. trifida* (NCNSP306) (v3 assembly, http://sweetpotato.uga.edu/) was used as the reference genome for this project. *Ipomoea batatas* aligns closely with *I. trifida* at 96.5% collinearity with an average marker density of one SNP/14.2kb (Mollinari et al., 2020).

'Beauregard' reads were trimmed using Cutadapt (v2.10; Martin, 2011) to a minimum quality of 10 and a minimum read length of 100 nt. The trimmed reads were aligned with *I. trifida* (Wu et al., 2018) using BWA-MEM (v0.7.17-r1188; Li, 2013) to produce binary alignment (BAM) files. SAMtools (v1.10; Li et al., 2009) was used to sort BAM files, and duplicate reads were marked using the bammarkduplicates tool from biobambam (v1.14; Tischler & Leonard, 2014). 'Tanzania' reads were aligned with *I. trifida* using the LongRanger (v2.2.2; Marks et al., 2019) pipeline from 10X Genomics; duplicates in 'Tanzania' were marked with biobambam (Tischler & Leonard, 2014).

The final BAM files for both parents were variant-called using Freebayes (v1.3.5; Garrison & Marth, 2012) with a ploidy level of 6 (-p 6), max read depth of 1000 (-g 1000), minimum MAPQ of 30 (-m 30), minimum base quality of 20 (-q 20), and the QTL support interval as region read span (-r, LG4_6,788,877:7,226,599 for *M. enterolobii*, and LG7_4,668,738:55,468 for *M. incognita*). Variants were filtered by removing indels, structural variants, or low-quality SNPs. The remaining SNPs were then filtered to retain only biallelic

SNPs with simplex (0/0/0/0/0/1) or pentaplex (0/1/1/1/1/1) genotype calls to capture any potential allele present in 'Tanzania' which conferred resistance (simplex case), or a single allele missing from 'Beauregard' which resulted in susceptibility (pentaplex case).

Marker Identification and KASP Pilot Study. To select SNPs, a pileup vector was created from read alignments between both parents using pysam (v0.16.0.1) (Pysam-developers, 2022). Low quality alignments (MAPQ <30), duplicates, and mirror SNPs were skipped. Alleles within the pileup vector were counted, and SNPs with read depth <25 were skipped. Variant allele fraction was set at >0.95 to capture only inherited variants; values below this were skipped to prevent detection of somatic mutants. We also skipped SNPs where the major allele was not biallelic (i.e., positions which either had the reference allele, or one non-reference variant). Biallelic sites were nulliplex/simplex, or pentaplex/hexaplex; these positions were recorded as selectable SNP positions.

A pool of 2,444 biallelic SNPs that differed between the reference genome and parental genotypes were detected by the described pipeline: 'Beauregard' had 562 SNPs on LG4 and 1,736 on LG7, while 'Tanzania' had 17 on LG4 and 129 on LG7. We selected a total of 300 SNPs closest to the major QTLs for *M. enterolobii* and *M. incognita*, and LGC Biosearch Technologies™ used their Kraken™ software to identify SNPs likely to be compatible with their assays as part of the KBD service. From this pool of SNPs, we chose the 12 nearest peak-flanking markers for each QTL, including markers within QTL peaks where possible, for a total of 24 positions.

From stock DNA plates, the aforementioned samples were transferred in two 50uL replicates to 96-well plates for genotyping at these 24 SNP positions using KBD. Plates also

included blanks (no template control), one per 96-well plate in a different position on each plate. These DNA plates were mailed on ice to LGC Biosearch Technologies™ (Hoddesdon, UK) for genotyping, and results were returned in a .CSV file including a list of genotypes which amplified during PCR and their variant calls by the KASP markers, with accompanying software SNPViewer (v.4.1.2.21968) for viewing cluster plots.

Trait data from Oloka et al. (2021) and Chapter 2 were compared with KASP genotyping results to determine marker accuracy. Chi-square goodness of fit tests with one variable (resistant or susceptible, df=1) tested the null hypothesis that each marker was equally predictive of resistant and susceptible genotypes (1:1 segregation). The number of samples (n) represented all samples which amplified at that marker position.

Results

Of the 24 KASP markers evaluated 16 were PCR-compatible and 11 of these 16 were polymorphic as determined from SNPViewer, while 5 markers were monomorphic (Figure 1). Genotype calls for both DNA replicates agreed in 96.4% of cases (all genotypes, 24 markers), including situations where one replicate did not amplify while the other did. Within the evaluated germplasm of 122 TB lines and 82 diverse genotypes, the overall call rate was 82%. Where one replicate had missing marker data, a single replicate was considered. Some TB lines had one replicate amplify while the other did not at some marker positions, while all other marker positions were in concordance. For these cases, genotype calls between replicates were merged to create a more complete genotype for these individuals. This was done only for TB016 and TB216.

Eight of the polymorphic markers were on LG 4 where the major QTL for *M. enterolobii* was detected, and three were on LG 7 where the *M. incognita* QTL was detected (Table 1). Chi-square goodness of fit tests revealed four markers, two on LG 4 and two on LG 7, that predicted resistance (or susceptibility) with greater than 80% accuracy (P<0.001) (Table 2). For *M. enterolobii*, LG4_7038930 (A:G) predicted resistance in 96.8% of resistant sweetpotato lines. Marker LG4_7039636 (C:T) had the same predictive power (96.8%) for *M. enterolobii* resistance in resistant lines. Two other markers predicted *M. enterolobii* resistance with 100% accuracy in only two resistant genotypes, thereby lacking statistical significance.

Resistance to *M. incognita* was accurately predicted by two markers (Figure 3). Marker LG7_1699509 (T:C) predicted resistance to *M. incognita* in 94.0% of resistant genotypes and further predicted susceptibility (C:C) in 90.9% of susceptible genotypes. Marker LG7_1700440 predicted resistance in 85.1% (G:G) of resistant lines. Other forms of the allele (A:A, A:G) at LG7_1700440 were not significantly predictive of susceptibility (P>0.001).

Discussion

The development of KASP marker from SNPs at or flanking peak QTL positions is becoming increasingly popular. For example, Leal-Bertoli et al. (2015) designed 24 KASP assays to detect markers in QTL regions associated with rust (*Puccinia arachidis*) in peanut (2x and 4x). KASP markers have also been designed for nematode resistance in other crops: Strachan et al. (2019) observed 1:1 segregation for resistance to the potato cyst nematode (*Globodera padilla*) in an F1 potato population and used a small pool of 11 SNPs to create KASP markers that defined a 4.7Mb region (containing the *H1* locus) associated with resistance. Meade et al. (2020) described the development and validation of KASP markers for potato at the

H1 locus, as well as a partial resistance gene (*R2* locus) for late blight resistance. However, this KASP-based approach may only be appropriate for traits which are highly heritable and/or controlled by single-gene, such as many resistance loci (Mammadov et al., 2012; Meade et al., 2020). Complex traits like yield, shape, and abiotic stress tolerances are likely to be polygenic. For the NCSU sweetpotato breeding program a suitable marker would require 80% or greater accuracy for making decisions about advancing (or culling) a sweetpotato clone. Wairich et al. (2022) used this same accuracy threshold to design KASP markers for grape downy mildew resistance. These thresholds would vary for other programs and crops.

Of the germplasm evaluated, 122 out of the 204 samples initially evaluated (~60%) were TB progeny while 82 (~40%) were lines of a known resistance class from our broader germplasm, representing clones from Africa, South America, Asia, as well as the USDA and other US public breeding programs. We also included NCSU advanced breeding materials and past and present major US varieties. Genotype calls for both replicates were consistent in 96.4% of cases (all genotypes, 24 markers), including situations where one replicate did not amplify while the other did.

An industry-wide threshold for *M. enterolobii* resistance in sweetpotato has yet to be established. Using RF≤1 is a common value in the literature, and has been used to describe resistance to potato cyst nematode (Mahran et al., 2010). Schwarz et al. (2021) and Rutter et al. (2021) both used RF<1 to indicate resistance to *M. enterolobii* in sweetpotato. We compared chi-square analyses of our *M. enterolobii* markers using RF≤1 and RF≤0.25 and found lower accuracy for identifying resistance at RF≤0.25 yet a slightly improved accuracy in identifying susceptible lines. At RF≤0.25, LG7_7038930 (A:G) was 88.2% resistant and (G:G) was 82.5% susceptible; for LG7_7039636, (C:T) was 88.2% resistant and (T:T) was 80.5% susceptible

(*P*<0.001). Increasing selection pressure by lowering RF tolerance for *M. enterolobii* may be worthwhile once resistance is common in elite breeding germplasm, but currently elite sweetpotato lines with RF≤1 are rare. Shifting the NCSU sweetpotato breeding germplasm towards a mean RF≤1 is a preliminary step. Creating varieties with RF<0.1 (non-host status) (Hamidi & Hajihassani, 2020) is attainable through breeding: 'Tanzania' (RF=0.027) crossed with 'Beauregard' (RF=13.91) yielded 34.4% progenies with an *M. enterolobii* RF<0.1 (Fraher et al, 2022). We measure resistance to these two RKN species differently in part due to concerns about quarantine and spread of *M. enterolobii*. Of the root-knot nematodes, *M. incognita* is the most common in NC (Schwarz et al., 2020) and some field pressure is to be expected over a wide geographic distribution, and thus a moderate threshold for resistance (≤10 galls) has been used previously (Yencho et al, 2008; Nakayama et al., 2012), whereas *M. enterolobii* was recently introduced, is yet limited in distribution, and is much more aggressive, motivating breeders to pursue varieties with non-host status (RF<0.1) for this emergent parasite. In addition, gall rating methods as a means of determining resistance status in sweetpotato are not as reliable for *M. enterolobii* as for *M. incognita* (Schwarz et al., 2020; Fraher et al., 2022 [see Chapter 2]).

The threshold for *M. incognita* resistance we have used is likely more stringent than that used by Nakayama et al. (2012), Sasai et al. (2019), and Tabuchi et al. (2017), as these studies inoculated with 500 *M. incognita* J2 juveniles and spanned 35 days, while the *M. incognita* resistance evaluations performed by Oloka et al. (2021), as well as our routine bioassays, used 20,000 eggs and a 62 day trial. Markers LG7_1699509 and LG7_1700440 predicted resistance to *M. incognita* in TB with over 80% accuracy. Breeding for *M. enterolobii* resistance is a top priority for NC sweetpotato breeders, and *M. incognita* resistant genotypes were less represented in this study.

We hypothesize that a single copy of a *M. enterolobii* resistance gene is conferred by 'Tanzania', which would fit the 1:1 segregation pattern and additive allelic effects described in Chapter 2. However, as a hexaploid, it is possible for sweetpotato to carry multiple copies of a gene, resulting in varying degrees of dosage. Tanaka et al. (2019) enriched pelargonidin production through breeding in sweetpotato by increasing the dosage of a recessive allele, measuring trait-related exon fragments through PCR in segregating progenies. KASP assays have the potential for dosage estimation (Meade et al., 2020), and tools exist to enable such predictions (Voorrips, 2021) though it is not known if multiple copies of a resistance gene(s) exist in sweetpotato for *M. enterolobii* or *M. incognita*. It is not known what effect, if any, enrichment of such resistance genes to higher dosages may have. It is also possible that some genotypes fall into a moderately resistant or moderately susceptible category, which deviates from the observed 1:1 segregation pattern in TB about RF=1 for *M. enterolobii*.

Markers LG7_1699509 and LG7_1700440 predicted resistance to *M. incognita* in TB and our diversity panel with high accuracy, but there is a need to validate this in broader germplasm. Rasheed et al. (2016) reported a sequence for KASP marker validation in polyploid wheat that may also be suitable for sweetpotato: 1) compare KASP markers to predictive power of other marker types on a small, diverse germplasm; 2) validate KASP markers on a panel (300 geographically diverse cultivars) with different sets of phenotypic data; 3) test markers on one or several segregating populations. For both *M. enterolobii* and *M. incognita*, our next steps will include validation in a large number of genotypes representing a broader geographic diversity, and also validation in additional populations (Ramirez-Gonzalez et al., 2015). Meade et al. (2020) described a set of 149 commercial potato cultivars and elite breeding lines as an appropriate pool for KASP marker validation, but recommend greater numbers for confirmed

utility. Also, identification of haplotypes associated with resistance or susceptibility would provide a larger set of selectable markers. For example, Alekcevetch et al. (2021) used a haplotype-based approach to identify soybean accessions containing SNPs associated with *M. javanica* resistance as part of a MAB scheme.

Currently, the limited capacity to perform bioassays for RKN resistance allows implementation only on advanced breeding lines. This invariably results in advanced-generation materials being culled at late stages for RKN susceptibility. One solution would be to apply these economical KASP assays on second- or third-year material, after a round or two of selections which reduces population size. KASP primer mix costs roughly $0.01/sample, and the more expensive master mix is approximately $0.14/sample (Ayalew et al., 2019). The cost of DNA extraction is the greatest expense in our program. Sweetpotato DNA is difficult to extract due to the presence of secondary metabolites, latex, pigments and other compounds (Kim & Hamada, 2005; Varadarajan & Prakash, 1991) and recovery is low. Some genotypes included here required up to four extractions to reach minimum DNA quality and concentration (5ng/ul) required for genotyping. Optimization of high-throughput, cheap DNA extraction specifically for KASP marker analysis is a critical next step for sweetpotato. While the bioassay remains the gold-standard for establishing resistance, in the future this method could be reserved for only the most elite breeding parents and lines approaching release. Earlier detection of susceptible lines would enable more replicates of advanced lines in bioassays, granting higher confidence in resistance calls. Ultimately, these selectable markers may lead to the accelerated release of improved sweetpotato varieties with pyramided resistance to *M. enterolobii* and *M. incognita.*

REFERENCES

Alekcevetch, J. C., de Lima Passianotto, A. L., Ferreira, E. G. C., dos Santos, A. B., da Silva, D. C. G., Dias, W. P., Belzile, F., Abdelnoor, R. V., & Marcelino-Guimarães, F. C. (2021). Genome-wide Association Study for Resistance to the *Meloidogyne javanica* Causing Root-knot Nematode in Soybean. *Theoretical and Applied Genetics, 134*(3), 777–792. https://doi.org/10.1007/s00122-020-03723-9

Alvarez-Fernandez, A., Bernal, M. J., Fradejas, I., Martin Ramírez, A., Md Yusuf, N. A., Lanza, M., Hisam, S., Pérez de Ayala, A., & Rubio, J. M. (2021). KASP: A Genotyping Method to Rapid Identification of Resistance in *Plasmodium falciparum*. *Malaria Journal*, *20*(1), 16. https://doi.org/10.1186/s12936-020-03544-7

Amankwaah, V. A. (2019). *Phenotyping and Genetic Studies of Storage Root Chemistry Traits in Sweetpotato* [Ph.D., North Carolina State University]. http://www.proquest.com/docview/2385636619/abstract/B1E1FF11D3044EC7PQ/1

Arumuganathan, K., & Earle, E. D. (1991). Nuclear DNA Content of some Important Plant Species. *Plant Molecular Biology Reporter*, *9*(3), 11.

Ayalew, H., Tsang, P. W., Chu, C., Wang, J., Liu, S., Chen, C., & Ma, X.-F. (2019). Comparison of TaqMan, KASP and rhAmp SNP Genotyping Platforms in Hexaploid Wheat. *PLOS ONE*, *14*(5), e0217222. https://doi.org/10.1371/journal.pone.0217222

Bourke, P., Voorrips, R., Visser, R., & Maliepaard, C. (2018). Tools for Genetic Studies in Experimental Populations of Polyploids. *Frontiers in Plant Science*, *9*. https://doi.org/10.3389/fpls.2018.00513

Brescia, P., & Banks, P. (2010). *Micro-Volume PicoGreen® DNA Quantification using BioTek's Take3™ Multi-Volume Plate*. 3.

Byers, R. L., Harker, D. B., Yourstone, S. M., Maughan, P. J., & Udall, J. A. (2012). Development and Mapping of SNP Assays in Allotetraploid Cotton. *Theoretical and Applied Genetics*, *124*(7), 1201–1214. https://doi.org/10.1007/s00122-011-1780-8

Cervantes-Flores, J. C., Sosinski, B., Pecota, K. V., Mwanga, R. O. M., Catignani, G. L., Truong, V. D., Watkins, R. H., Ulmer, M. R., & Yencho, G. C. (2011). Identification of Quantitative Trait Loci for Dry-matter, Starch, and β-carotene Content in Sweetpotato. *Molecular Breeding*, *28*(2), 201–216. https://doi.org/10.1007/s11032-010-9474-5

Cervantes-Flores, J. C., Yencho, G. C., Kriegner, A., Pecota, K. V., Faulk, M. A., Mwanga, R. O. M., & Sosinski, B. R. (2008). Development of a Genetic Linkage Map and Identification of Homologous Linkage Groups in Sweetpotato Using Multiple-dose AFLP Markers. *Molecular Breeding*, *21*(4), 511–532. https://doi.org/10.1007/s11032-007-9150-6

Doyle, J., & Doyle, J. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*(13), 39–40.

EPPO. (2021). *Meloidogyne enterolobii*. Retrieved December 3, 2021, from https://gd.eppo.int/taxon/MELGMY/categorization

FAO. (2020, June 15). *FAOSTAT Crops*. http://www.fao.org/faostat/en/#data/QC/visualize

Garrison, E., & Marth, G. (2012). Haplotype-based Variant Detection from Short-read Sequencing. *ArXiv:1207.3907 [q-Bio]*. http://arxiv.org/abs/1207.3907

Graves, H., Rayburn, A. L., Gonzalez-Hernandez, J. L., Nah, G., Kim, D.-S., & Lee, D. K. (2016). Validating DNA Polymorphisms Using KASP Assay in Prairie Cordgrass (*Spartina pectinata* Link) Populations in the U.S. *Frontiers in Plant Science*, *6*, 1271. https://doi.org/10.3389/fpls.2015.01271

Hamidi, N., & Hajihassani, A. (2020). Differences in Parasitism of Root-knot Nematodes (*Meloidogyne* spp.) on Oilseed Radish and Oat. *Journal of Nematology*, *52*, e2020-43. https://doi.org/10.21307/jofnem-2020-043

He, C., Holme, J., & Anthony, J. (2014). SNP Genotyping: The KASP Assay. In D. Fleury & R. Whitford (Eds.), *Crop Breeding* (Vol. 1145, pp. 75–86). Springer New York. https://doi.org/10.1007/978-1-4939-0446-4_7

Kim, S.-H., & Hamada, T. (2005). Rapid and Reliable Method of Extracting DNA and RNA from Sweetpotato, *Ipomoea batatas* (L). Lam. *Biotechnology Letters*, *27*(23–24), 1841–1845. https://doi.org/10.1007/s10529-005-3891-2

Leal-Bertioli, S. C. M., Cavalcante, U., Gouvea, E. G., Ballén-Taborda, C., Shirasawa, K., Guimarães, P. M., Jackson, S. A., Bertioli, D. J., & Moretzsohn, M. C. (2015). Identification of QTLs for Rust Resistance in the Peanut Wild Species *Arachis magna* and the Development of KASP Markers for Marker-Assisted Selection. *G3 Genes|Genomes|Genetics*, *5*(7), 1403–1413. https://doi.org/10.1534/g3.115.018796

Leksrisompong, P. P., Whitson, M. E., Truong, V. D., & Drake, M. A. (2012). Sensory Attributes and Consumer Acceptance of Sweet Potato Cultivars with Varying Flesh Colors. *Journal of Sensory Studies*, *27*(1), 59–69. https://doi.org/10.1111/j.1745-459X.2011.00367.x

Li, H. (2013). Aligning Sequence Reads, Clone Sequences and Assembly Contigs with BWA-MEM. *ArXiv:1303.3997 [q-Bio]*. http://arxiv.org/abs/1303.3997

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence

Alignment/Map format and SAMtools. *Bioinformatics (Oxford, England)*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Mahran, A., Turner, S., Martin, T., Yu, Q., Miller, S., & Sun, F. (2010). The Golden Potato Cyst Nematode *Globodera rostochiensis* Pathotype Ro1 in the Saint-Amable Regulated Area in Quebec, Canada. *Plant Disease*, *94*(12), 1510–1510. https://doi.org/10.1094/PDIS-08-10-0584

Mammadov, J., Aggarwal, R., Buyyarapu, R., & Kumpatla, S. (2012, December 18). *SNP Markers and Their Impact on Plant Breeding* [Review Article]. International Journal of Plant Genomics; Hindawi. https://doi.org/10.1155/2012/728398

Marks, P., Garcia, S., Barrio, A. M., Belhocine, K., Bernate, J., Bharadwaj, R., Bjornson, K., Catalanotti, C., Delaney, J., Fehr, A., Fiddes, I. T., Galvin, B., Heaton, H., Herschleb, J., Hindson, C., Holt, E., Jabara, C. B., Jett, S., Keivanfar, N., Kyriazopoulou-Panagiotopoulou, S., Lek, M., Lin, B., Lowe, A., Mahamdallie, S., Maheshwari, S., Makarewicz, T., Marshall, J., Meschi, F., O'Keefe, C. J., Ordonez, H., Patel, P., Price, A., Royall, A., Ruark, E., Seal, S., Schnall-Levin, M., Shah, P., Stafford, D., Williams, S., Wu, I., Xu, A. W., Rahman, N., MacArthur, D., Church, D. M. (2019). Resolving the Full Spectrum of Human Genome Variation Using Linked-Reads. *Genome Research*, *29*(4), 635–645. https://doi.org/10.1101/gr.234443.118

Martin, F. W., & Jones, A. (1986). Breeding Sweet Potatoes. In *Plant Breeding Reviews* (pp. 313–345). John Wiley & Sons, Ltd.

Martin, M. (2011). Cutadapt Removes Adapter Sequences from High-throughput Sequencing Reads. *EMBnet.Journal*, *17*(1), 10–12. https://doi.org/10.14806/ej.17.1.200

Meade, F., Byrne, S., Griffin, D., Kennedy, C., Mesiti, F., & Milbourne, D. (2020). Rapid Development of KASP Markers for Disease Resistance Genes Using Pooled Whole-Genome Resequencing. *Potato Research*, *63*(1), 57–73. https://doi.org/10.1007/s11540-019-09428-x

Mollinari, M., Olukolu, B. A., Pereira, G. da S., Khan, A., Gemenet, D., Yencho, G. C., & Zeng, Z.-B. (2020). Unraveling the Hexaploid Sweetpotato Inheritance Using Ultra-Dense Multilocus Mapping. *G3 Genes|Genomes|Genetics*, *10*(1), 281–292. https://doi.org/10.1534/g3.119.400620

Monden, Y., & Tahara, M. (2017). Genetic Linkage Analysis Using DNA Markers in Sweetpotato. *Breeding Science*, *67*(1), 41–51. https://doi.org/10.1270/jsbbs.16142

Nakayama, H., Tanaka, M., Takahata, Y., Matsui, K., Iwahori, H., Sano, Z., & Yoshinaga, M. (2012). Development of AFLP-derived SCAR Markers Associated with Resistance to Two Races of Southern Root-knot Nematode in Sweetpotato. *Euphytica*, *188*(2), 175–185. https://doi.org/10.1007/s10681-012-0678-0

Oloka, B. M., da Silva Pereira, G., Amankwaah, V. A., Mollinari, M., Pecota, K. V., Yada, B., Olukolu, B. A., Zeng, Z.-B., & Craig Yencho, G. (2021). Discovery of a Major QTL for Root-knot Nematode (*Meloidogyne incognita*) Resistance in Cultivated Sweetpotato (*Ipomoea batatas*). *Theoretical and Applied Genetics*, *134*(7), 1945–1955. https://doi.org/10.1007/s00122-021-03797-z

Onkendi, E. M., & Moleleki, L. N. (2013). Detection of *Meloidogyne enterolobii* in Potatoes in South Africa and Phylogenetic Analysis Based on Intergenic Region and the Mitochondrial DNA Sequences. *European Journal of Plant Pathology*, *136*(1), 1–5. https://doi.org/10.1007/s10658-012-0142-y

Pysam. (2022). [C]. pysam-developers. https://github.com/pysam-developers/pysam (Original work published 2014)

Ramirez-Gonzalez, R. H., Uauy, C., & Caccamo, M. (2015). PolyMarker: A Fast Polyploid Primer Design Pipeline. *Bioinformatics*, *31*(12), 2038–2039. https://doi.org/10.1093/bioinformatics/btv069

Rasheed, A., Wen, W., Gao, F., Zhai, S., Jin, H., Liu, J., Guo, Q., Zhang, Y., Dreisigacker, S., Xia, X., & He, Z. (2016). Development and Validation of KASP Assays for Genes Underpinning Key Economic Traits in Bread Wheat. *Theoretical and Applied Genetics*, *129*(10), 1843–1860. https://doi.org/10.1007/s00122-016-2743-x

Rutter, W., Wadl, P., Mueller, J. D., & Agudelo, P. (2021). Identification of Sweetpotato Germplasm Resistant to Pathotypically Distinct Isolates of *Meloidogyne enterolobii* from the Carolinas. *Plant Disease*, PDIS-02-20-0379-RE. https://doi.org/10.1094/PDIS-02-20-0379-RE

Sasai, R., Tabuchi, H., Shirasawa, K., Kishimoto, K., Sato, S., Okada, Y., Kuramoto, A., Kobayashi, A., Isobe, S., Tahara, M., & Monden, Y. (2019). Development of Molecular Markers Associated with Resistance to *Meloidogyne incognita* by Performing Quantitative Trait Locus Analysis and Genome-wide Association Study in Sweetpotato. *DNA Research*, *26*(5), 399–409. https://doi.org/10.1093/dnares/dsz018

Schwarz, T., Li, C., Ye, W., & Davis, E. (2020). Distribution of *Meloidogyne enterolobii* in Eastern North Carolina and Comparison of Four Isolates. *Plant Health Progress*, *21*(2), 91–96. https://doi.org/10.1094/PHP-12-19-0093-RS

Schwarz, T. R., Li, C., Yencho, G. C., Pecota, K. V., Heim, C. R., & Davis, E. L. (2021). Screening Sweetpotato Genotypes for Resistance to a North Carolina Isolate of

Meloidogyne enterolobii. *Plant Disease*, *105*(4), 1101–1107. https://doi.org/10.1094/PDIS-02-20-0389-RE

Semagn, K., Babu, R., Hearne, S., & Olsen, M. (2014). Single Nucleotide Polymorphism Genotyping Using Kompetitive Allele Specific PCR (KASP): Overview of the Technology and Its Application in Crop Improvement. *Molecular Breeding*, *33*(1), 1–14. https://doi.org/10.1007/s11032-013-9917-x

Strachan, S. M., Armstrong, M. R., Kaur, A., Wright, K. M., Lim, T. Y., Baker, K., Jones, J., Bryan, G., Blok, V., & Hein, I. (2019). Mapping the H2 Resistance Effective Against *Globodera pallida* Pathotype Pa1 in Tetraploid Potato. *Theoretical and Applied Genetics*, *132*(4), 1283–1294. https://doi.org/10.1007/s00122-019-03278-4

Synergy HT Operator's Manual. (2008). 240.

Tabuchi, H., Kuranouchi, T., Kobayashi, A., Monden, Y., Kishimoto, K., Tahara, M., Okada, Y., & Iwahori, H. (2017). Southern Root-knot Nematode Race SP6 is Divided into Two Races. *Nematological Research (Japanese Journal of Nematology)*, *47*(2), 29–33. https://doi.org/10.3725/jjn.47.29

Tanaka, M., Sakai, T., & Takahata, Y. (2019). Allele Dosage–dependent Selection of Recessive F3'H Allele Homozygote Altered Anthocyanin Composition in Sweetpotato. *Molecular Breeding*, *39*(10), 152. https://doi.org/10.1007/s11032-019-1062-8

Tigano, M., Siqueira, K. D., Castagnone-Sereno, P., Mulet, K., Queiroz, P., Santos, M. D., Teixeira, C., Almeida, M., Silva, J., & Carneiro, R. (2010). Genetic Diversity of the Root-knot Nematode *Meloidogyne enterolobii* and Development of a SCAR Marker for This Guava-damaging Species. *Plant Pathology*, *59*(6), 1054–1061. https://doi.org/10.1111/j.1365-3059.2010.02350.x

Tischler, G., & Leonard, S. (2014). biobambam: Tools for Read Pair Collation Based Algorithms on BAM Files. *Source Code for Biology and Medicine*, *9*(1), 13. https://doi.org/10.1186/1751-0473-9-13

Uitdewilligen, J. G. A. M. L., Wolters, A.-M. A., D'hoop, B. B., Borm, T. J. A., Visser, R. G. F., & Eck, H. J. van. (2013). A Next-Generation Sequencing Method for Genotyping-by-Sequencing of Highly Heterozygous Autotetraploid Potato. *PLOS ONE*, *8*(5), e62355. https://doi.org/10.1371/journal.pone.0062355

USDA/NASS. (2020, June 9). *2019 State Agriculture Overview for North Carolina*. https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH%20CAROLINA

Varadarajan, G. S., & Prakash, C. S. (1991). A rapid and efficient method for the extraction of total DNA from the sweet potato and its related species. *Plant Molecular Biology Reporter*, *9*(1), 6–12. https://doi.org/10.1007/BF02669284

Voorrips, R. (2021, January 8). *Dosage Scoring / fitPoly Workshop*. https://www.polyploids.org/sites/default/files/2021-01/SCRI_fitPoly_workshop.html

Wairich, A., Malabarba, J., Buffon, V., Porto, D. D., Togawa, R., & Revers, L. F. (2022). Molecular Characterization of the Rpv3 Locus Towards the Development of KASP Markers for Downy Mildew Resistance in Grapevine (*Vitis* spp.). *Euphytica*, *218*(1), 5. https://doi.org/10.1007/s10681-021-02952-3

Wilson, P. (2018, October 5). *NCDA&CS Declares an Internal Quarantine for all North Carolina Counties for the Guava Root Knot Nematode*. NCDA&CS. http://www.ncagr.gov/paffairs/release/2018/10-18guavarootknotnematode.htm

Wu, S., Lau, K. H., Cao, Q., Hamilton, J. P., Sun, H., Zhou, C., Eserman, L., Gemenet, D. C., Olukolu, B. A., Wang, H., Crisovan, E., Godden, G. T., Jiao, C., Wang, X., Kitavi, M., Manrique-Carpintero, N., Vaillancourt, B., Wiegert-Rininger, K., Yang, X., Bao, K., Schaff, J., Kreuze, J., Gruneberg, W., Khan, A., Ghislain, M., Ma, D., Jiang, J., Wmanga, R., Leebens-Mack, J., Coin, L., Yencho, G. C., Buell, C. R., Fei, Z. (2018). Genome Sequences of Two Diploid Wild Relatives of Cultivated Sweetpotato Reveal Targets for Genetic Improvement. *Nature Communications*, *9*(1), 4580. https://doi.org/10.1038/s41467-018-06983-8

Yang, B., & Eisenback, J. D. (1983). *Meloidogyne enterolobii* n. Sp. (Meloidogynidae), a Root-knot Nematode Parasitizing Pacara Earpod Tree in China. *Journal of Nematology*, *15*(3), 381–391.

Ye, W. M., Koenning, S. R., Zhuo, K., & Liao, J. L. (2013). First Report of *Meloidogyne enterolobii* on Cotton and Soybean in North Carolina, United States. *Plant Disease*, *97*(9), 1262. https://doi.org/10.1094/PDIS-03-13-0228-PDN

Yencho, G. C., Pecota, K. V., Schultheis, J. R., VanEsbroeck, Z.-P., Holmes, G. J., Little, B. E., Thornton, A. C., & Truong, V.-D. (2008). ‘Covington’ Sweetpotato. *HortScience*, *43*(6), 1911–1914. https://doi.org/10.21273/HORTSCI.43.6.1911

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CHAPTER 3: FIGURES & TABLES

Table 1. The 24 putative KASP markers submitted to LGC, narrowed from a total of 2,444 SNPs detected within QTL support intervals for *M. enterolobii* and *M. incognita* resistance in sweetpotato. For each QTL 12 flanking markers were submitted, and of these 24 markers 16 amplified. Polymorphic markers are starred, and represent potential markers for RKN resistance.

		Parental Alleles		Parental Genotypes		Tanzania Read Counts					
LG	Marker Position (BP)	Tanzania	Beauregard	Tanzania	Beauregard	A	T	C	G	Read Depth	Context Sequence
4	7038839*	G	A	0/0/0/0/0/1	0/0/0/0/0/0	0	0	0	389	389	TGCTACAATGTTTAG[G/A]GATAGTTTTCATTC
4	7038885*	G	A	0/0/0/0/0/1	0/0/0/0/0/0	0	0	0	390	390	TCCAAGGCTTGAGAT[G/A]AGGGGGAAAAAGGC
4	7038930*	G	A	0/0/0/0/0/1	0/0/0/0/0/0	0	1	0	389	390	TAAACAATTAAAGCA[G/A]AAATTTCTGTTGTC
4	7039186*	T	G	0/0/0/0/0/1	0/0/0/0/0/0	0	428	1	0	429	AAGGGACCATATTTT[T/G]CTTTTATCAGTTTC
4	7039355*	T	A	0/0/0/0/0/1	0/0/0/0/0/0	0	449	1	1	451	CAACAAGTAATTTGG[T/A]GCAGTAAGGAAAAT
4	7039398*	G	T	0/0/0/0/0/1	0/0/0/0/0/0	0	0	0	461	461	GGTATGGTATACATC[G/T]TGGGAGGCTGGTAG
4	7039636*	T	C	0/0/0/0/0/1	0/0/0/0/0/0	0	526	0	0	526	GATCCAAACTGATTA[T/C]GTAAACCTGTCTGC
4	7039775*	T	A	0/0/0/0/0/1	0/0/0/0/0/0	0	534	0	0	534	CAGCTGGTCCCCCAA[T/A]ATGTCTTTGTGGAT
4	7040096	C	T	0/0/0/0/0/1	0/0/0/0/0/0	0	0	403	0	403	TTGAAAAGATATTGG[C/T]CCTATTTGGTAAAA
4	7040127	C	T	0/0/0/0/0/1	0/0/0/0/0/0	0	0	390	0	390	AGTTAGCCTATCAGC[C/T]AATTTTGGCTTATT
7	1699255	G	A	0/0/0/0/0/1	0/0/0/0/0/0	0	0	0	429	429	AAAATTAGGCTACAA[G/A]ACGAAATGTGATAT
7	1699259	A	G	0/0/0/0/0/1	0/0/0/0/0/0	435	0	0	0	435	TTAGGCTACAAGACG[A/G]AATGTGATATAAAT
7	1699440	A	C	0/0/0/0/0/1	0/0/0/0/0/0	622	1	0	1	624	GCTGCTTGTTCTGAA[A/C]TACGCGATAGCCTC
7	1699509*	C	T	0/0/0/0/0/1	0/0/0/0/0/0	0	0	633	0	633	GATCCTTAGCCCCTA[C/T]GACACCTCCGCTTC
7	1700046*	A	G	0/0/0/0/0/1	0/0/0/0/0/0	341	0	1	0	342	AAATGGTCATTTAGA[A/G]ATGGATTTAAATGC
7	1700440*	G	A	0/0/0/0/0/1	0/0/0/0/0/0	0	0	0	434	434	GTTGTGAAGGACTCA[G/A]AGCTAGCACTGAGA

* Polymorphic marker that amplified during KASP genotyping

Table 2. Primer details for the KASP markers used to genotype 122 TB progenies and 82 other diverse lines for *Meloidogyne enterolobii* and *M. incognita* resistance with high accuracy (>80%).

LG	Marker BP	Primer Allele X	Primer Allele Y	Primer Common	Allele X	Allele Y	Sequence
4	7038930	CAAATAAACTAGTGAATAAACAATTAAAGCAG	GCAAATAAACTAGTGAATAAACAATTAAAGCAA	CCTATCTTTTGGAGTTATTGACAACAGAAA	G	A	GAGATGAGGGGGAAAAAGGCAAATAAACTAGTGAATAAACAATTAAAGCA[G/A]AAATTTCTGTTGTCAATAACTCCAAAAGATAGGTGATTATGTAGAATCTT
4	7039636	CACTGACGCAGACAGGTTTACA	CACTGACGCAGACAGGTTTACG	AAGGATCAATGCCAGATCCAAACTGATTA	T	C	TTTTGGAAAGATAAAGTGACAAAGGATCAATGCCAGATCCAAACTGATTA[T/C]GTAAACCTGTCTGCGTCAGTGAGCTACCCATTTGAGCAGCTCCAAGAGCT
7	1699509	GTTTATGGAAGCGGAGGTGTCG	GGTTTATGGAAGCGGAGGTGTCA	CTTAATACTTCTCTGGAGATGGATCCTTA	C	T	CTTCTTCTGATTTTCTTAATACTTCTCTGGAGATGGATCCTTAGCCCCTA[C/T]GACACCTCCGCTTCCATAAACCCCAGCTGCCTCCCTTCAGAATGGAATTC
7	1700440	ATTGAAGCTCTCAGTGCTAGCTC	GTATTGAAGCTCTCAGTGCTAGCTT	GCAGGCTTTCTGCAGTTGTTGTGAA	G	A	TTCTCTGTTCATCTGTTTGCAGGCTTTCTGCAGTTGTTGTGAAGGACTCA[G/A]AGCTAGCACTGAGAGCTTCAATACTGTTCTGCTTTAATCCTGCTTCCATA

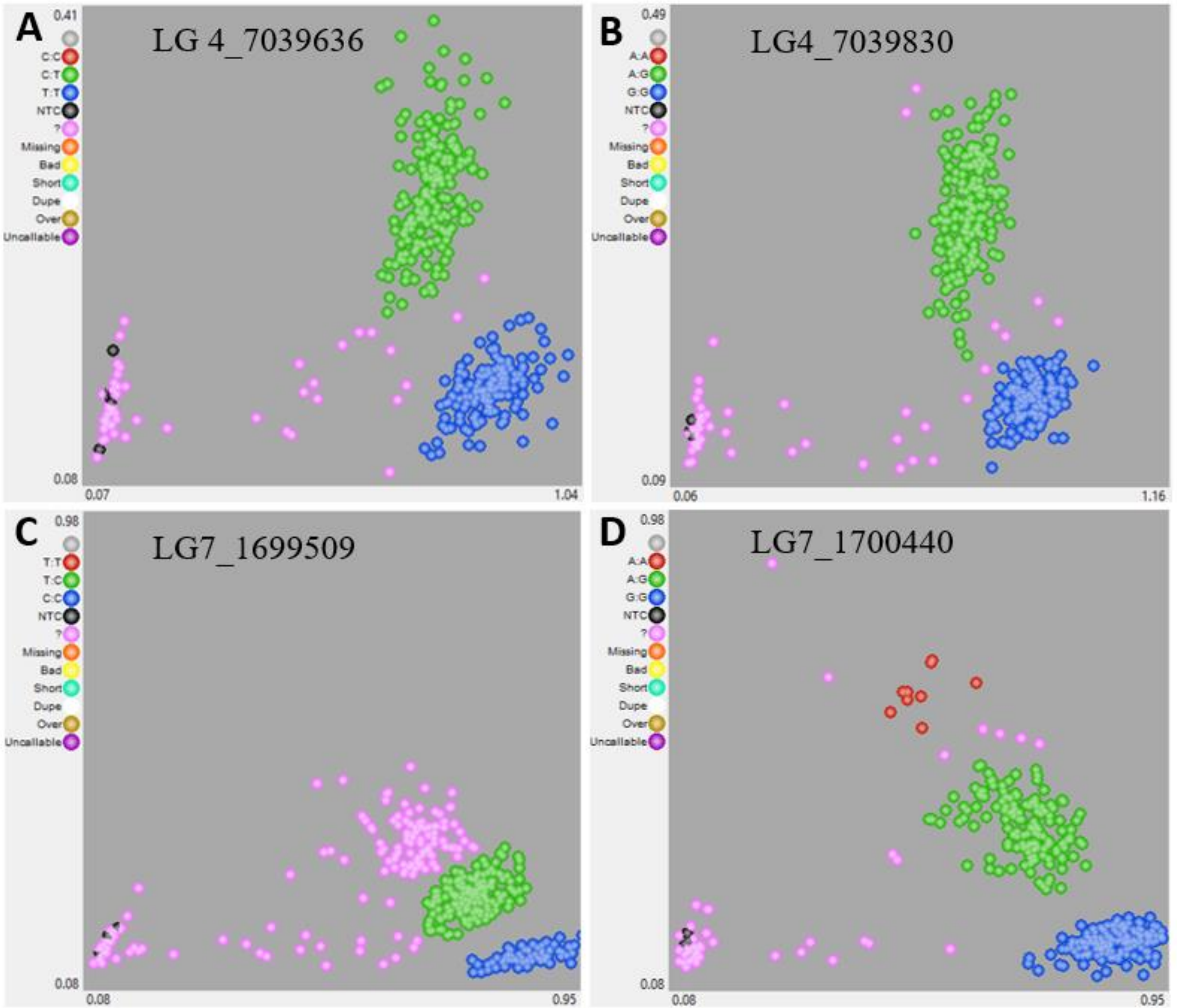

Figure 1. SNPViewer cluster plots of four polymorphic markers in a panel of 204 samples. The axes refer to intensity of fluorescence for the X and Y forms of fluorescent labels. **A** Marker LG4_7039636 associated with *Meloidogyne enterolobii* resistance. Genotypes in the C:T heterozygous state (associated with resistance) are aligned in the center of the plot, while homozygous T:T genotypes (susceptible) are clustered near the X-axis. **B** Marker LG4_7039830. A:G predicted resistance and G:G susceptibility. **C** Marker LG4_1699509 for *M. incognita* resistance. T:C predicted resistance and C:C susceptibility. **D** Marker LG4_1700440 for *M. incognita* resistance. A:G predicted resistance and G:G susceptibility.

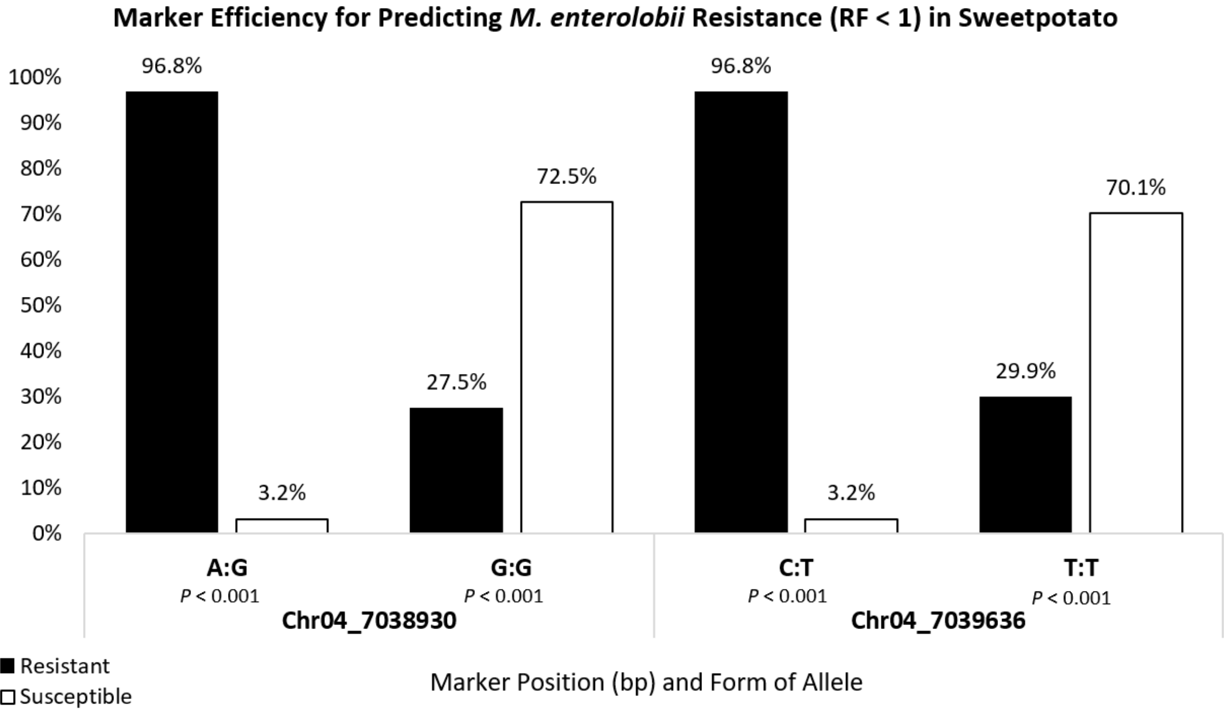

Figure 2. Two markers (LG4_7038930, A:G; LG4_7039636, C:T) with 96.8% accuracy predicting resistance (RF<1) to *Meloidogyne enterolobii* in a diverse panel of sweetpotato germplasm, including 122 segregating progeny of the cross 'Tanzania' (resistant) by 'Beauregard' (susceptible). An additional 82 genotypes were tested, representing sweetpotato lines from Africa, Asia, South America, as well as the USDA and other US public breeding programs. A chi-squared goodness of fit test determined both markers to be highly significant ($P<0.001$). Six other markers were polymorphic, but less than 80% accurate or not above significance thresholds.

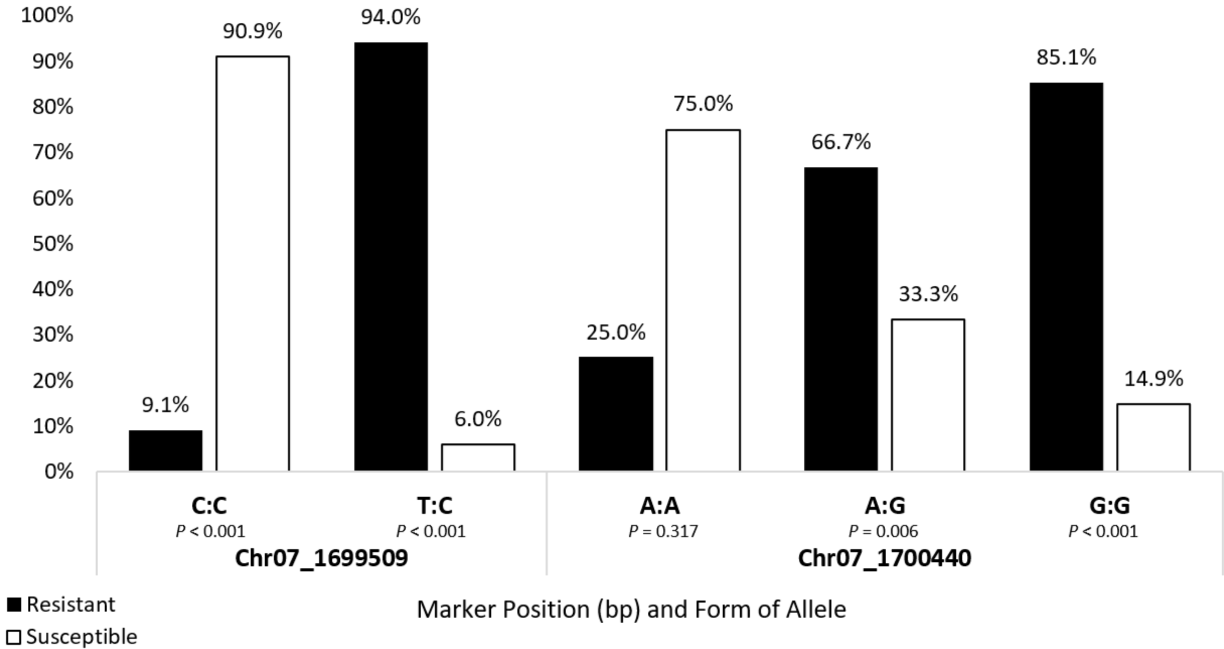

Figure 3. Two markers with >80% accuracy predicting resistance (gall count ≤10) to *Meloidogyne incognita* in a diverse panel of sweetpotato germplasm, including 122 segregating progeny of the cross 'Tanzania' (resistant) by 'Beauregard' (susceptible). An additional 82 genotypes were tested, representing sweetpotato lines from Africa, Asia, South America, as well as the USDA and other US public breeding programs. A chi-squared goodness of fit test determined two of these markers to exceed 80% accuracy ($P<0.001$). These were LG7_1699509 (C:C, susceptible; T:C, resistant) and LG7_1700440 (G:G, resistant). Other forms of LG7_1700440 did not exceed accuracy or significance thresholds and as thus were not of practical use. One other marker was polymorphic, but less than 80% accurate and not above significance thresholds.

APPENDICES

Appendix 1. Mean resistance scores of greenhouse-grown ‘Tanzania’ x ‘Beauregard’ (TB) progenies inoculated with 10,000 eggs of *Meloidogyne enterolobii*, and rated at 60 days. Inoculated (+) and uninoculated (-) check lines were included. ‘Tanzania’ and ‘Jewel’ were resistant, while ‘Beauregard’ and ‘Covington’ were susceptible. For *M. enterolobii*, resistance is measured primarily as reproductive factor (RF) equal to or less than 1. We measured resistance by percent of total root mass with galls (% galling), RF (final eggs/10,000), and eggs per gram of root tissue (total final eggs/root mass). Two replicates were included, and means are reported. Note that one replicate of Jewel (-) was contaminated, biasing the mean.

Clone	Mean % Galling	Mean RF	Mean Eggs/g. Root
Tanzania(-)	0.0%	0.00	0.0
Jewel(-)	1.0%	0.03	4.5
Beauregard(-)	0.0%	0.00	0.0
Covington(-)	0.0%	0.00	0.0
Tanzania(+)	0.0%	0.03	7.5
Jewel(+)	2.3%	0.03	6.0
Beauregard(+)	14.5%	13.69	3912.5
Covington(+)	12.5%	6.84	2190.8
TB001	0.5%	0.21	47.4
TB002	5.5%	4.20	1462.5
TB003	0.0%	0.29	78.7
TB004	0.0%	0.06	10.9
TB005	13.5%	38.44	12703.5
TB006	4.0%	4.13	794.6
TB007	1.0%	0.10	26.5
TB008	6.5%	2.44	715.0
TB009	5.0%	9.63	3909.3
TB010	0.0%	0.04	7.6
TB011	10.0%	12.69	4108.1
TB012	0.0%	0.01	2.3
TB013	15.0%	21.63	5889.1
TB014	9.0%	11.00	2864.3
TB015	0.0%	0.23	68.2
TB016	1.0%	1.14	280.4
TB017	0.5%	0.00	0.9
TB018	1.0%	0.23	36.4
TB019	0.0%	0.05	9.2
TB020	0.0%	0.02	3.3
TB021	0.0%	0.07	14.0
TB022	0.0%	0.01	3.0
TB023	0.0%	0.01	4.4
TB024	0.5%	1.58	253.6
TB025	10.0%	8.25	2703.1
TB026	11.5%	23.88	5401.3
TB027	0.5%	0.01	2.2
TB028	9.0%	9.81	2549.9
TB029	6.5%	8.75	3187.0
TB030	9.0%	22.44	6563.9
TB031	0.0%	0.03	5.9
TB032	2.5%	2.69	640.6
TB033	12.5%	9.44	1727.8
TB034	0.0%	0.22	44.5
TB035	0.0%	0.05	14.2

Appendix 1. (Cont.)

TB036	0.5%	0.02	3.5
TB037	0.0%	0.15	50.9
TB038	0.0%	0.07	12.1
TB039	0.5%	0.33	67.9
TB040	0.0%	0.02	3.2
TB041	4.0%	8.44	1683.0
TB042	0.0%	0.01	3.0
TB043	7.5%	13.75	5831.4
TB044	0.0%	0.01	5.9
TB045	7.5%	9.25	3523.6
TB046	0.0%	0.30	71.2
TB047	0.0%	0.10	25.0
TB049	0.5%	0.06	24.7
TB050	0.0%	0.00	0.7
TB051	22.5%	10.44	2478.8
TB052	0.0%	0.05	11.7
TB053	15.0%	8.56	2685.2
TB054	2.0%	0.04	7.4
TB055	0.0%	0.07	14.1
TB056	0.0%	0.01	4.7
TB057	0.0%	0.11	24.6
TB058	0.0%	0.02	4.7
TB060	0.5%	0.01	2.2
TB061	0.0%	0.15	63.4
TB062	1.0%	0.15	30.6
TB063	1.0%	0.06	12.3
TB064	0.5%	0.30	40.8
TB065	2.5%	14.17	4780.8
TB066	1.5%	0.02	4.3
TB067	6.5%	10.56	3633.2
TB068	0.0%	0.08	16.1
TB069	4.5%	17.88	7609.1
TB070	3.0%	0.69	124.1
TB071	1.0%	0.01	1.8
TB072	6.5%	5.56	3220.7
TB073	0.0%	0.01	1.3
TB074	0.5%	0.04	15.4
TB075	5.0%	11.06	4804.4
TB076	1.5%	0.00	0.9
TB077	0.0%	0.04	5.4
TB078	3.0%	6.79	2069.8
TB079	0.0%	0.08	25.2
TB080	0.5%	0.01	0.8
TB081	0.0%	0.28	90.3
TB082	5.0%	13.19	3073.5
TB083	10.5%	5.81	1625.8
TB084	1.0%	0.13	25.0
TB085	3.5%	7.69	2450.4
TB086	0.5%	0.23	78.0
TB087	6.5%	5.06	2382.0
TB088	5.0%	15.13	4714.6
TB089	0.0%	0.12	22.7
TB090	0.5%	0.04	9.1
TB092	9.0%	16.19	5670.4
TB093	6.5%	35.69	7894.0

Appendix 1. (Cont.)

TB095	3.0%	0.03	4.8
TB096	0.0%	0.03	4.9
TB097	0.0%	0.06	23.7
TB098	0.5%	0.02	5.8
TB099	0.0%	0.02	5.5
TB100	12.5%	17.25	4241.8
TB101	0.0%	0.02	3.3
TB102	0.0%	0.32	131.0
TB103	9.0%	22.88	7036.5
TB104	5.5%	17.94	3320.3
TB105	0.0%	0.03	10.8
TB106	4.0%	12.44	2381.5
TB107	9.0%	8.00	2128.9
TB108	10.0%	6.69	1616.4
TB109	0.0%	0.17	65.6
TB110	0.5%	0.17	43.6
TB111	9.0%	11.13	4162.9
TB112	0.0%	1.24	480.6
TB113	0.0%	0.02	5.5
TB114	0.0%	0.88	339.9
TB115	0.0%	0.20	39.3
TB116	0.0%	0.04	9.9
TB117	0.0%	0.11	33.7
TB118	0.0%	0.13	29.7
TB119	0.0%	0.03	9.4
TB120	1.0%	0.08	20.2
TB121	9.0%	13.75	3994.9
TB122	4.0%	8.38	5419.7
TB123	1.0%	0.08	15.7
TB124	0.0%	0.30	117.3
TB125	6.5%	10.75	2166.8
TB126	0.0%	0.04	9.4
TB127	0.0%	0.07	19.5
TB128	9.0%	16.06	3570.8
TB129	15.0%	5.74	1087.3
TB130	6.5%	14.56	3366.2
TB131	4.0%	7.63	2057.2
TB132	7.5%	13.31	3365.2
TB133	0.0%	0.10	26.2
TB134	0.0%	0.02	5.1
TB135	30.0%	7.63	1913.8
TB136	20.0%	8.63	2664.6
TB137	4.0%	4.88	1782.8
TB138	0.0%	1.20	432.8
TB139	3.0%	17.69	5947.3
TB140	9.5%	6.63	1408.8
TB141	0.0%	0.13	31.0
TB142	0.0%	0.01	2.4
TB143	3.0%	0.08	16.0
TB144	1.0%	0.03	5.4
TB145	0.0%	0.02	6.0
TB146	0.5%	0.14	37.4
TB147	5.0%	13.63	4493.9
TB148	1.5%	0.03	10.4
TB149	5.0%	24.13	5162.6

Appendix 1. (Cont.)

TB150	3.0%	6.88	1750.8
TB151	10.0%	4.13	1570.8
TB152	0.0%	0.02	6.0
TB153	0.0%	0.05	13.3
TB154	5.0%	9.26	4344.1
TB155	0.5%	0.10	24.5
TB156	0.5%	1.46	373.6
TB157	5.0%	28.38	7842.4
TB159	7.5%	30.75	8153.7
TB160	10.5%	13.38	5259.3
TB161	11.5%	8.06	1692.9
TB162	9.0%	20.44	5889.6
TB163	0.0%	0.05	23.2
TB164	12.5%	14.44	3136.0
TB165	5.0%	4.57	1961.7
TB166	10.0%	11.81	3770.1
TB168	5.0%	15.94	4985.3
TB169	5.0%	5.81	1521.6
TB170	5.5%	11.25	2283.0
TB171	0.5%	0.15	56.4
TB172	9.0%	12.56	3281.4
TB173	12.5%	9.06	2086.8
TB174	1.5%	0.09	27.2
TB175	0.5%	0.27	84.9
TB176	7.5%	12.75	3041.8
TB178	17.5%	20.94	5217.3
TB179	6.5%	14.88	3719.2
TB180	0.0%	0.07	23.7
TB182	3.0%	19.69	10716.0
TB183	9.0%	13.50	3175.6
TB184	0.5%	0.06	15.8
TB185	5.0%	15.06	4416.0
TB187	3.5%	9.69	2645.8
TB188	10.5%	23.13	4266.4
TB189	8.0%	15.63	3081.7
TB190	3.0%	10.20	2174.5
TB191	0.0%	0.06	13.8
TB192	0.5%	0.03	20.2
TB193	6.0%	7.94	2586.3
TB194	0.5%	0.00	1.2
TB195	0.0%	0.03	9.1
TB196	15.0%	5.75	1541.9
TB197	14.0%	15.13	4064.2
TB198	7.0%	9.63	3368.0
TB199	4.0%	18.75	4512.7
TB200	0.5%	0.06	24.4
TB201	11.5%	13.19	3576.5
TB203	1.0%	0.03	8.4
TB204	0.0%	0.05	8.4
TB205	0.0%	0.01	2.7
TB206	12.5%	7.00	1761.0
TB207	0.0%	0.14	41.0
TB208	0.0%	0.02	10.2
TB209	0.0%	0.25	75.0
TB210	2.5%	14.69	2846.8

Appendix 1. (Cont.)

TB211	12.5%	13.25	3941.5
TB212	0.0%	0.19	47.5
TB213	4.0%	6.69	2083.1
TB214	0.0%	0.05	12.2
TB215	7.5%	3.64	1019.5
TB216	0.0%	0.31	111.5
TB217	0.0%	0.07	14.1
TB218	0.0%	0.09	31.5
TB219	4.0%	20.31	5634.7
TB220	1.0%	0.01	2.1
TB222	10.0%	18.50	6302.1
TB223	0.0%	0.14	40.4
TB224	10.0%	4.44	3587.0
TB225	2.0%	6.87	2346.5
TB226	0.0%	0.02	6.4
TB227	10.0%	12.31	3469.1
TB228	15.0%	21.06	4960.5
TB229	11.5%	24.94	7445.9
TB230	10.0%	16.00	2915.3
TB231	2.5%	13.25	4737.6
TB232	0.5%	0.10	32.0
TB233	0.0%	0.04	9.2
TB234	0.0%	0.08	18.9
TB235	8.0%	9.81	2575.1
TB236	17.5%	13.94	3169.6
TB237	7.5%	15.38	2823.2
TB238	0.0%	0.01	2.1
TB239	4.0%	19.56	8054.8
TB240	0.5%	0.03	4.8
TB241	0.0%	0.69	350.7
TB242	0.0%	0.04	14.4
TB243	0.0%	0.77	231.4
TB244	0.0%	0.04	10.5
TB245	3.5%	2.34	932.4
TB246	0.0%	0.03	11.7
TB247	12.5%	10.94	2018.1
TB249	7.5%	9.56	5248.2
TB250	5.0%	34.19	10592.6
TB251	5.5%	13.25	2881.4
TB252	6.0%	8.81	2040.6
TB253	4.5%	5.38	1422.9
TB254	6.5%	19.19	8830.8
TB255	6.5%	6.75	2662.2
TB256	7.5%	12.00	2346.6
TB257	2.0%	0.02	6.1
TB258	0.0%	0.24	38.5

Appendix 2. Genotype calls at accurate KASP marker positions for *Meloidogyne enterolobii* and *Meloidogyne incognita* resistance on various sweetpotato lines. Resistance was defined as RF≤1 for *M. enterolobii*, while gall index measured *M. incognita* resistance as ≤2 (Schwarz et al. 2021, Rutter et al. 2021, and unpublished data). Gall index refers to binned gall counts as determined by Oloka et al. 2021 and routine bioassays (unpublished), where: gall index 0=0galls, 1=1-3galls, 2=4-10galls, 3=11-30galls, 4=31-100galls, 5>100galls. Values highlighted in green indicate resistance, while red indicates susceptibility. Markers highlighted in yellow were not statistically significant and ambiguous. Greyed values were uncallable and/or lacked phenotype data.

	Chromosome 4 (*M.e.*) SNP Marker (BP)		**Chromosome 7 (*M.i.*) SNP Marker (BP)**			
Clone	**7038930**	**7039636**	**1699509**	**1700440**	***M.e.* RF**	***M.i.* Gall Index**
394	G:G	T:T	T:C	A:G	1.376	0.750
97A-04	G:G	T:T	?	G:G	0.254	#N/A
Averre	G:G	T:T	T:C	G:G	25.133	#N/A
BayouBelle	G:G	T:T	?	A:G	25.633	#N/A
Beauregard	G:G	T:T	C:C	G:G	13.691	4.133
Bellevue	G:G	T:T	C:C	A:G	22.800	#N/A
BenikoMachi	A:G	C:T	T:C	G:G	0.276	#N/A
Bienville	G:G	T:T	T:C	A:G	0.205	#N/A
Bonita	G:G	T:T	C:C	A:G	16.833	#N/A
Caromex	A:G	C:T	?	G:G	0.004	#N/A
Carver	A:G	C:T	?	G:G	0.018	#N/A
Centennial	A:G	C:T	?	G:G	0.011	3.000
Covington	G:G	T:T	T:C	G:G	6.844	0.750
DM04-0001	G:G	T:T	T:C	G:G	65.383	4.083
Evangeline	G:G	T:T	T:C	G:G	14.800	0.150
Excel	G:G	T:T	T:C	A:G	38.150	#N/A
Gem	A:G	C:T	?	G:G	0.011	#N/A
Hernandez	G:G	T:T	C:C	A:G	16.548	2.266
Jewel	A:G	C:T	?	G:G	0.027	1.888
Koganesengan	?	T:T	T:C	A:G	0.933	#N/A
L14-31	A:G	C:T	C:C	G:G	0.105	1.500
L14-41P	A:G	C:T	?	A:G	0.038	1.250
L50	A:G	C:T	C:C	A:A	0.032	#N/A
L96-117	A:G	C:T	T:C	G:G	0.744	#N/A
MC14-0363	G:G	T:T	T:C	A:G	22.183	0.714
MC15-0032	Uncallable	T:T	Uncallable	Uncallable	11.535	0.250
MC16-0062	G:G	T:T	T:C	G:G	0.095	1.000
MD320	G:G	T:T	T:C	Uncallable	0.451	#N/A
MD88-116	A:G	C:T	?	G:G	0.108	#N/A
Mojave	A:G	C:T	?	A:G	0.017	#N/A
Mugande	G:G	T:T	Uncallable	G:G	0.310	#N/A
Murasaki-29	A:G	C:T	T:C	A:A	0.016	0.900
NancyHall	A:G	C:T	?	G:G	0.002	#N/A
NC02-0350	G:G	T:T	T:C	G:G	0.159	1.000
NC02-0423	Uncallable	Uncallable	Uncallable	A:G	2.327	0.500
NC04-0531	G:G	T:T	?	G:G	17.233	0.444
NC05-0408	G:G	T:T	T:C	G:G	0.103	0.900
NC07-0364	Uncallable	T:T	Uncallable	Uncallable	0.183	2.583
NC07-0745	G:G	T:T	?	G:G	0.223	2.000
NC07-0847	G:G	T:T	T:C	G:G	44.483	1.083
NC08-0036	G:G	T:T	?	G:G	0.097	3.750
NC08-0553	G:G	T:T	T:C	A:G	9.267	0.625

Appendix 2. (Cont.)

NC09-0122	G:G	T:T	T:C	A:G	40.317	0.750
NC09-0136	G:G	T:T	T:C	A:G	0.055	0.625
NC09-0188	G:G	T:T	T:C	G:G	0.017	0.500
NC09-0912	G:G	T:T	?	A:G	10.717	0.500
NC09-1105	A:G	C:T	T:C	G:G	0.088	0.000
NC10-0357	G:G	T:T	?	G:G	0.451	#N/A
NC11-0234	G:G	T:T	C:C	A:G	33.025	2.909
NC11-0632	G:G	T:T	T:C	G:G	0.325	#N/A
NC12-0219	G:G	T:T	T:C	A:G	7.788	#N/A
NC12-1149	Uncallable	T:T	T:C	G:G	0.152	3.000
NC14-0129	G:G	T:T	C:C	G:G	3.591	0.250
NC14-0353	G:G	T:T	?	G:G	0.142	0.857
NC15-0185	G:G	T:T	C:C	A:G	0.235	3.500
NC17-0308	G:G	T:T	T:C	A:G	0.176	0.800
NC413	G:G	T:T	T:C	A:G	33.933	0.250
NCP06-0020	G:G	T:T	?	A:G	25.350	3.100
NCP13-0030	G:G	T:T	T:C	A:G	#N/A	0.571
NCP13-0057	Uncallable	T:T	T:C	A:G	0.136	1.143
NCP13-0073	G:G	T:T	T:C	G:G	0.304	#N/A
NCP13-0285	G:G	T:T	Uncallable	G:G	14.767	3.933
NCP13-0315	G:G	T:T	C:C	A:G	20.333	3.429
Norton	A:G	C:T	?	G:G	0.012	#N/A
Okinawa100	G:G	T:T	?	A:G	0.030	#N/A
Patriot	?	T:T	T:C	G:G	27.467	#N/A
PelicanProcessor	A:G	C:T	T:C	A:G	0.025	#N/A
PortoRico	A:G	C:T	?	?	#N/A	4.599
RedResisto	A:G	C:T	?	A:A	0.038	#N/A
Regal	G:G	T:T	T:C	G:G	0.477	#N/A
Resisto	G:G	T:T	T:C	A:G	0.050	#N/A
Ruddy	G:G	T:T	T:C	A:G	11.670	#N/A
SouthernDelite	A:G	C:T	?	A:G	0.036	#N/A
Tanzania	A:G	C:T	T:C	A:G	0.027	0.222
TB001	?	?	?	?	0.208	0.200
TB002	?	?	?	?	4.201	1.600
TB003	?	?	?	?	0.285	2.000
TB004	A:G	C:T	C:C	A:G	0.058	3.200
TB006	G:G	T:T	T:C	A:G	4.125	0.600
TB008	G:G	T:T	C:C	G:G	2.435	4.200
TB010	A:G	C:T	?	G:G	0.040	0.400
TB012	A:G	C:T	?	G:G	0.011	1.200
TB016	A:G	C:T	T:C	A:G	1.144	1.200
TB017	?	?	?	A:G	0.004	1.600
TB018	A:G	C:T	?	G:G	0.226	0.400
TB019	A:G	C:T	?	A:G	0.048	0.800
TB019	A:G	C:T	?	G:G	0.048	0.800
TB022	?	?	?	G:G	0.013	1.000
TB023	A:G	C:T	T:C	A:G	0.011	0.400
TB024	?	?	?	?	1.576	1.250
TB027	A:G	C:T	T:C	A:G	0.008	1.600
TB031	A:G	C:T	T:C	G:G	0.028	1.600
TB033	G:G	T:T	T:C	A:G	9.438	2.000
TB035	A:G	C:T	T:C	G:G	0.045	0.600
TB038	A:G	C:T	T:C	G:G	0.074	1.600
TB039	A:G	C:T	T:C	G:G	0.328	1.000
TB041	G:G	T:T	T:C	G:G	8.438	1.000

Appendix 2. (Cont.)

TB043	G:G	T:T	C:C	A:G	13.750	4.600
TB046	A:G	C:T	T:C	A:G	0.300	1.600
TB047	A:G	C:T	?	G:G	0.095	0.400
TB049	A:G	C:T	T:C	G:G	0.064	2.000
TB050	?	?	?	G:G	0.002	1.400
TB052	A:G	C:T	C:C	A:G	0.053	3.800
TB054	A:G	C:T	C:C	?	0.042	4.400
TB056	A:G	C:T	C:C	A:G	0.012	3.600
TB057	A:G	C:T	T:C	G:G	0.112	1.400
TB058	A:G	C:T	T:C	G:G	0.019	1.200
TB060	A:G	C:T	T:C	G:G	0.012	1.800
TB062	A:G	C:T	T:C	A:G	0.153	0.600
TB063	A:G	C:T	?	G:G	0.063	1.200
TB065	G:G	T:T	C:C	G:G	14.168	3.600
TB066	A:G	C:T	C:C	A:G	0.020	3.800
TB068	A:G	C:T	T:C	G:G	0.076	1.600
TB070	G:G	T:T	T:C	G:G	0.689	0.800
TB073	A:G	C:T	C:C	A:G	0.006	5.000
TB076	A:G	C:T	T:C	A:G	0.004	1.000
TB077	A:G	C:T	T:C	G:G	0.039	1.400
TB079	A:G	C:T	T:C	G:G	0.081	1.000
TB080	A:G	C:T	T:C	G:G	0.005	1.200
TB083	G:G	T:T	?	A:G	5.813	0.800
TB084	A:G	C:T	T:C	G:G	0.126	1.200
TB087	G:G	T:T	C:C	A:A	5.063	4.800
TB089	A:G	C:T	T:C	G:G	0.116	1.000
TB090	A:G	C:T	T:C	A:G	0.043	1.000
TB095	A:G	C:T	T:C	A:G	0.029	1.400
TB096	A:G	C:T	T:C	A:G	0.032	1.000
TB097	A:G	C:T	T:C	A:G	0.056	0.600
TB098	A:G	C:T	T:C	G:G	0.016	1.200
TB102	A:G	C:T	T:C	A:G	0.318	0.600
TB105	A:G	C:T	T:C	A:G	0.028	1.200
TB107	?	T:T	C:C	A:G	8.000	3.600
TB108	G:G	T:T	T:C	A:G	6.688	2.000
TB109	A:G	C:T	?	G:G	0.170	0.600
TB110	A:G	C:T	T:C	G:G	0.174	2.000
TB112	A:G	C:T	C:C	A:A	1.236	4.600
TB113	A:G	C:T	T:C	A:G	0.015	0.800
TB114	A:G	C:T	T:C	A:G	0.884	0.400
TB119	A:G	C:T	T:C	A:G	0.031	1.000
TB122	G:G	T:T	C:C	A:G	8.375	3.400
TB123	A:G	C:T	T:C	A:G	0.082	1.200
TB131	?	?	?	?	7.625	3.000
TB133	A:G	C:T	T:C	G:G	0.103	2.200
TB135	G:G	T:T	C:C	A:G	7.625	5.000
TB137	G:G	T:T	T:C	A:G	4.875	1.800
TB140	G:G	T:T	C:C	A:G	6.625	0.600
TB141	A:G	C:T	T:C	G:G	0.129	0.800
TB143	A:G	C:T	T:C	G:G	0.083	1.200
TB150	G:G	T:T	T:C	A:G	6.875	1.200
TB151	G:G	T:T	T:C	G:G	4.125	1.000
TB155	A:G	C:T	C:C	A:G	0.098	5.000
TB156	A:G	C:T	C:C	G:G	1.456	3.200
TB161	G:G	T:T	?	G:G	8.063	1.000

Appendix 2. (Cont.)

TB163	A:G	C:T	?	G:G	0.047	0.500
TB169	G:G	T:T	T:C	G:G	5.813	0.400
TB170	G:G	T:T	C:C	A:G	11.250	3.400
TB173	G:G	T:T	C:C	A:G	9.063	4.400
TB178	G:G	T:T	T:C	G:G	20.938	2.000
TB180	A:G	C:T	T:C	G:G	0.066	1.200
TB190	G:G	T:T	C:C	A:G	10.199	4.800
TB191	A:G	C:T	T:C	G:G	0.058	0.600
TB193	G:G	T:T	C:C	A:G	7.938	2.600
TB195	A:G	C:T	T:C	G:G	0.031	0.000
TB196	G:G	T:T	T:C	A:G	5.750	1.800
TB200	?	?	?	G:G	0.057	1.000
TB203	A:G	C:T	?	G:G	0.033	1.200
TB204	A:G	C:T	?	A:G	0.050	0.000
TB205	A:G	C:T	T:C	G:G	0.011	0.600
TB206	G:G	T:T	C:C	A:G	7.000	3.800
TB207	A:G	C:T	T:C	A:G	0.136	0.600
TB208	A:G	C:T	T:C	G:G	0.023	1.200
TB212	A:G	C:T	?	A:G	0.192	1.600
TB214	A:G	C:T	T:C	A:G	0.052	1.200
TB215	G:G	T:T	T:C	G:G	3.636	1.400
TB216	A:G	C:T	T:C	G:G	0.308	1.400
TB217	A:G	C:T	?	G:G	0.074	1.000
TB218	A:G	C:T	T:C	G:G	0.088	1.400
TB219	G:G	T:T	C:C	A:A	20.313	3.800
TB222	G:G	T:T	C:C	A:G	18.500	3.400
TB226	A:G	C:T	T:C	G:G	0.018	1.400
TB233	A:G	C:T	T:C	G:G	0.036	1.400
TB239	G:G	T:T	C:C	G:G	19.563	3.200
TB242	A:G	C:T	T:C	A:G	0.041	0.800
TB243	A:G	C:T	T:C	A:G	0.771	1.400
TB245	G:G	T:T	T:C	A:G	2.343	2.000
TB247	G:G	T:T	T:C	A:G	10.938	2.400
TB250	G:G	T:T	C:C	G:G	34.188	3.400
TB253	G:G	T:T	?	G:G	5.375	1.400
TB257	A:G	C:T	T:C	A:G	0.019	2.200
TC2	G:G	T:T	T:C	A:G	0.059	#N/A
Tib11	A:G	C:T	?	G:G	0.024	#N/A
Tib4	G:G	T:T	?	G:G	5.709	#N/A
Whitestar	A:G	C:T	T:C	A:G	0.029	#N/A